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Qualified scientific work (Manuscript)

DISSERTATION

BREEDING AND GENETIC BASES OF WINTER WHEAT
EAR TRAITS

Specialty: 201 - Agronomy

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Submitted for a scientific degree of Doctor of philosophy

The dissertation contains the results of own research. The use of ideas,

results and texts of other authors have references to the relevant source

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ANNOTATION

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The dissertation presents data on the results of research on the genetic mechanism of ear traits such as the number of grains and the weight of one thousand grains in wheat, the use of SSR molecular markers to construct a genetic linkage map and QTL analysis related to ear traits, the results of F2 analysis with 145 individual plants created from "Mexico Very Large Colossus/Bainong 419" as a mapping population. According to the research results, a genetic linkage map of wheat was created, which contains 143 loci of molecular markers and covers 19 wheat chromosomes with a total genome length of 3128.17 cM, an average distance between markers of 25.23 cM, and a minimum genetic distance of 3.57 cM. The analysis was carried out using the method of full composite mapping of intervals based on the linear stepwise regression model of the IciMapping 4.0 software. The results showed that in chromosomes 1B, 2B, 2D, 3B, and 6B, a total of nine additive loci related to spike number were scanned, and their contribution to the genetic variability of phenotypic traits ranged from 4.922% to 21.1044%; among them, QGNS~1B and QGNS~1B and QGNS~3B2 were major loci with large genetic effects, explaining 21.1044% and 15.8886% of the phenotypic variation. One additional QTL locus was detected on QTGW~3B, which could explain 11.4727% of the phenotypic variation. Identification and analysis of epistatic QTL loci for spikelet number and 1000-grain weight showed that nine epistatic QTL loci were

associated with spikelet number and 1000-grain weight. Among them, there were six epistatic QTL loci associated with grain number per ear, which were located on chromosomes 2B, 2D, 3B, and 6B, respectively, and the effect values of the QGNS~2B, QGNS~2D, and QGNS~3B1 loci were different. is less than zero. The genetic effect values of all loci were greater than zero. This suggests that there are random loci on chromosomes that do not directly affect the phenotype, but these loci can affect phenotypic traits through interactions with each other. The genetic contribution of these loci to 1000-grain weight phenotypic variation also shows a strong effect of epistasis.

Grain development is important for the production of wheat (Triticum aestivum L.), which is one of the major food crops worldwide. MicroRNAs (miRNAs), being a type of small regulatory RNAs, play an important role in plant growth and development. Although plant grain/seed development is widely studied, information on miRNA regulation of early wheat grain development is limited. Based on the results of our research, miRNAs and their targets were investigated in the early stages of grain development of wheat cultivar 'Bainong 4199' at 7 DAP (days after pollination) and 14 DAP using high-throughput small RNA and degradome sequencing. 105 known and 79 novel miRNAs were identified, including 46 known and 32 novel miRNAs from the 7 DAP library and 87 known and 78 novel miRNAs from the 14 DAP library, respectively. Analysis of miRNA expression found that 39 of these identified miRNAs, including 19 known and 20 novel miRNAs, were differentially expressed between 7 DAP and 14 DAP. A total of 266 targets were predicted for 40 known wheat miRNAs, 152 targets for 13 other known plant miRNAs, and 258 targets for 25 novel miRNAs in small RNA and degradome analyses. Twenty-three targets were predicted

for 7 differentially expressed miRNAs, including 3 known and 4 novel miRNAs. Most of these miRNAs potentially regulate multiple targets, while others act on only one target. Functional analysis suggested that miRNAs and their targets are widely involved in the regulation of early wheat grain development and metabolism. The expression patterns of randomly selected miRNAs and targets were confirmed by real-time quantitative polymerase chain reaction with consistent and reliable results. This indicates that many known and novel miRNAs and their targets play a significant role during the early development of common wheat grain. Understanding the function of novel miRNAs and their targets, as well as miRNA-mediated regulatory networks involved in wheat grain development, has led us to elucidate the molecular mechanisms underlying wheat grain development and to make ingenious molecular improvements in wheat breeding.

Keywords: winter wheat (triticum aestivum L.),QTL localization,grain number spike ,thousand grain weight,yield , molecular mechanism, molecular markers,microRNAs, degradome, target genes, early developing grains.

АНОТАЦІЯ

Чєнь Цяоянь. «Селекційно - генетичні основи ознак колосу пшениці озимої».- Рукопис дисертації на здобуття наукового ступеня доктора філософії (PhD): Спеціальність 201 «Агрономія». - Сумський національний аграрний університет, Суми, 2023 р.

У дисертаційній роботі представлено дані про результати досліджень стосовно генетичного механізму таких ознак колоска, як кількість зерен і маса тисячі зерен у пшениці, використання молекулярних маркерів SSR для побудови карти генетичного зв'язку та аналізу QTL, пов'язаного з ознаками колоса, результати аналізу F2 зі 145 окремих рослини, створеними з "Мексико дуже великого колоса/Bainong 419" як картографічної популяції. За результатами досліджень було створено карту генетичного зчеплення пшениці, яка містить 143 локуси молекулярних маркерів і охоплює 19 хромосом пшениці із загальною довжиною геному 3128,17 cM, середньою відстанню між маркерами 25,23 cM і мінімальною генетичною відстанню 3,57 cM. Аналіз проводився за допомогою методу повного композитного відображення інтервалів на основі лінійної моделі покрокової регресії програмного забезпечення IciMapping 4.0. Результати показали, що в хромосомах 1B, 2B, 2D, 3B і 6B загалом було відскановано дев'ять адитивних локусів, пов'язаних із числом шипів, і їхній внесок у генетичну варіативність фенотипових ознак коливався від 4,922% до 21,1044%; серед них QGNS~1B і QGNS~1B і QGNS~3B2 були основними локусами з великими генетичними ефектами, пояснюючи 21,1044% і 15,8886% фенотипової варіації. Один додатковий локус QTL був виявлений на QTGW~3B, що могло пояснити 11,4727% фенотипічної варіації. Виявлення та аналіз

епістатичних локусів QTL для кількості колосків і маси тисячі зерен показали, що дев'ять епістатичних локусів QTL були пов'язані з кількістю колосків і масою тисячі зерен. Серед них було шість епістатичних локусів QTL, пов'язаних із кількістю зерен на колос, які були розташовані на хромосомах 2B, 2D, 3B та 6B відповідно, а значення ефекту локусів QGNS~2B, QGNS~2D та QGNS~3B1 були різними. менше нуля. Значення генетичного ефекту всіх локусів були більшими за нуль. Це свідчить про те, що в хромосомах є випадкові локуси, які безпосередньо не впливають на фенотип, але ці локуси можуть впливати на фенотипові ознаки через взаємодію один з одним. Генетичний внесок цих локусів у фенотипічні варіації маси тисячі зерен також демонструє сильний вплив епістазу.

Розвиток зерна є важливим для виробництва пшениці (Triticum aestivum L.), яка є однією з основних продовольчих культур у всьому світі. МікроРНК (міРНК), будучи різновидом малих регуляторних РНК, відіграють важливу роль у рості та розвитку рослин. Незважаючи на те, що розвиток зерна/насіння рослин широко досліджується, відомості про регуляцію мікроРНК раннього розвитку зерен пшениці обмежені. За результатами наших досліджень мікроРНК та їхні мішені досліджувалися на ранніх стадіях розвитку зерна сорту пшениці «Bainong 4199» на 7 DAP (дні після запилення) та 14 DAP з використанням високопродуктивної малої РНК і секвенування деградома. Було ідентифіковано 105 відомих і 79 нових мікроРНК, у тому числі 46 відомих і 32 нові мікроРНК з бібліотеки 7 DAP і 87 відомих і 78 нових мікроРНК з бібліотеки 14 DAP відповідно. Аналіз експресії мікроРНК встановив, що 39 із цих ідентифікованих мікроРНК, включаючи 19 відомих і 20 нових мікроРНК, диференційовано експресувалися між 7 DAP і 14

DAP. Загалом було передбачено 266 мішеней для 40 відомих мікроРНК пшениці, 152 мішені для 13 інших відомих мікроРНК рослин і 258 мішеней для 25 нових мікроРНК у аналізах малих РНК і деградомних аналізів. Було передбачено двадцять три мішені для 7 мікроРНК з диференціальною експресією, включаючи 3 відомі та 4 нові мікроРНК. Більшість цих мікроРНК потенційно регулюють кілька мішеней, тоді як інші діють лише на одну мішень. Функціональний аналіз передбачав, що мікроРНК та їхні мішені широко беруть участь у регуляції розвитку раннього зерна пшениці та метаболізму. Патерни експресії випадково вибраних мікроРНК і мішеней були підтверджені за допомогою кількісної полімеразної ланцюгової реакції в реальному часі з послідовними та надійними результатами. Це вказує, що чимало відомих і нових мікроРНК та їх мішеней відіграють значну роль під час раннього розвитку зерна м'якої пшениці. Розуміння функції нових мікроРНК та їх мішеней, а також опосередкованих мікроРНК регуляторних мереж, залучених до розвитку зерна пшениці, допоїло нам з'ясувати молекулярні механізми, що лежать в основі розвитку зерна пшениці, і здійснити геніальні молекулярні вдосконалення в селекції пшениці.

Ключові слова: пшениця озима (triticum aestivum L.), локалізація QTL, зернистість колосу, маса тис. зерен, урожайність, молекулярний механізм, молекулярні маркери, мікроРНК, деградом, цільові гени, ранній розвиток зерна.

LIST OF PUBLISHED WORKS ON THE TOPIC OF THE DISSERTATION

Articles in scientific professional journals citing Scopus

(1). **W.; Yongang Y.; Ye T.; Halyna Zhatova; Puwen S.; Dongxiao L.;Yuanyuan G.; Huanting G.; Trotsenko Volodymyr; Qiaoyan C.; Haiyan H.; Chengwei L Liuliu**. A novel wheat β-amylase gene *TaBMY1* reduces Cd accumulation in common wheat grains. www.elsevier.com/locate/envexpbot, (2022) .

(2**). Chen, L.; Lv, Q.; Yang,W.; Yang, H.; Chen, Q.; Wang, B.; Lei,Y.; Xie, Y.** TaNBR1, a Novel Wheat NBR1-like Domain Gene Negatively Regulates Drought Stress Tolerance in Transgenic Arabidopsis. International Journal of Molecular Sciences 23, 4519. (2022).

Articles in scientific professional publications of Ukraine

(3). **Qiaoyan Chen, Kandyba Nataliya, Xingqi Ou, Xinhua Li, Wenhui Wei.** Influence of the sowing period and denesityon yield and yield components of three semi – winter wheat varieties. *Селекція та насінництво.* 2021. Вип. 120. С. 79 – 87. https://doi.org/10.30835/2413-7510.2021.251041

(4). **Qiaoyan Chen, Kandyba Nataliya, Xingqi Ou, Xinhua Li, Wenhui Wei.** Effect of correlation analysis of date, planting density and main agronomic traits in winter wheat variety Bainong 207/ Bulletin of

the Sumy National Agricultural University of *Agronomy and Biology*. Sumy, 2021. Issue. 23 (45). P. 71–77. https://doi.org/10.32845/agrobio.2021.3.9

(5). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Effect of low temperature on growth and development ofwheat and response mechanism. *Breeding and seedproduction.* Kharkiv: Institute of Plant Breeding named after V. Ya. Yuriev of the Ukrainian Academy of Agrarian Sciences. Issue 118 (2020). P. 149 -153.

Articles in scientific journals of other countries

(6). **Qiaoyan Chen, Kandyba Nataliya.** Study of wheat resistance to low temperatures. *Goncharov readings*: a collection of abstracts of the International scientific-practical conference (May 24 -25, 2019). Sumy: Sumy National Agrarian University, 2019. P. 12

(7). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Research progress of cold resistance genes in winter wheat. Interacion de las ciencias fundamentales yaplicadas en el paradigmadela sociedad post – industrial: Coleccion de documentos cientificos « ЛОГОΣ» con astas de la Conferencia Internacional Cientifica y Practica (Vol. 1), 24 de abril de 2020. Barscelona, Espana. Platforma Europea de la Ciencia. URL: http://sci-conf.com.ua. DOI 10.36074/24.04.2020.V1.30. Pp. 88 – 90.

(8). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Effect of low

temperature anti-oxidation system. The 9th International scientific and practical conference «Scientific achievements of modern society» (April 28 -30, 2020) Cognum Publishing House, Liverpool , United Kingdom. 2020. Pp.270 – 272.
URL:http://sci-conf.com.ua.

(9). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Research Progress in Transcriptomics. The 9th International scientific and practical conference “Dynamics of the development of world science” (May 13-15, 2020) Perfect Publishing, Vancouver, Canada. 2020. Pp. 144 – 149. URL: http://sci-conf.com.ua.

(10). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Effects of Low Temperature on wheat growth and development. Міжнарод. наук. – практ. конф. присвячена 91-річчю з дня народження доктора с. - г. н, наук, проф. Гончарова М. Д. (25 - 26 травня 2020). – Суми : Сумський НАУ, 2020.– С.105 - 106.

(11). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Research status of QTL mapping of grain number per spike in winter wheat. Abstracts of the IV International Scientific and Practical Conference (Budapest, February 9 – 12, 2021). Hungary 2021. 706 p. Pp. 25–30. Available at : DOI: 10.46299/ISG.2021.I.IV URL:https://isg-konf.com
https://isg-konf.com/uk/prospects-and-achievements-in-applied-and-basic-sciences-ua/

(12). **Qiaoyan Chen, Kandyba Nataliya, Wenhui Wei.** Advances in micro RNA research of winter wheat. The V International Science Conference «Theoretical and scientific bases ofdevelopment of scientific thought», February 16 – 19, 2021, Rome, Italy. 685 p. Pp. 24-29. URL: https://isg-konf.com. Available at : DOI: 10.46299/ISG.2021.I.V. https://isg-konf.com/uk/theoretical-and-scientific-bases-of-development-of-scientific-thought-ua/

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ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AP	Ammonium Persurface
ARF	Auxin-responsive factor
BC	backcross
Bis	N,N-methylene-bis-acrylamide
Bp	Base pair
BSS	Basal spikelet sterility number per spike
CIM	Complex amplified polymorphic sequence
COG	Cluster of orthologous groups
CTAB	cetyltriammonium bromide
ddH2O	Distilled and deionized water
DH	Double haploid
DAP	Days after pollination
EDTA	Ethylenediaminetetra acetic acid
FSS	Fertile spikelet number per spike
GO	Gene ontology
GNS	Grain number per spike
ICIM	Inclusive composite interval mapping
KEGG	Kyoto Encyclopedia of Genes and Genomes
MAS	Marker-assisted selection

NIL	Near isogenic lines
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PH	Plant height
PVE	Phenotypic variation explained
qRT-PCR	Quantitative real-time polymerase chain reaction
QTL	Quantitative trait loci
RIL	Recombination inbred line
SBP	SQUAMOSA promoter-binding protein
SL	Spike length
SPL	SQUAMOSA promoter-binding protein-like
sRNAs	Small RNAs
SSR	Simple sequence repeat
TEMED	N,N,N’N’-Tetramethylethylene-diamine
TSS	Total spikelet number per spike

INTRODUCTION

Relevance of the topic. Winter wheat is one of the world's three major grain crops, and China ranks first in the world in terms of both area planted and total wheat production. According to the prediction, by 2050, the world population will increase from the current 7 billion to 9 billion, and the food production will have to be increased by more than 30% in order to meet the increasing consumption demand of human beings. Therefore, the cultivation of new varieties of the three major food crops, namely, high yielding and stable yielding wheat, rice and maize, is an effective way to satisfy the future demand of human beings for food. Yield is a quantitative trait controlled by multiple genes, with a complex genetic basis, and is easily influenced by the environment. Utilizing traditional breeding methods is time- and resource-consuming. However, with the development of molecular biology and biostatistics, QTL mapping of quantitative trait loci based on genetic mapping of molecular markers provides an effective technical means to study the genetics of quantitative traits.

Actuality of theme. Yield is composed of the spike number per unit area, grain number per spike and thousand grain weight. When the spike number per unit area and thousand grain weight are constrained, increasing the number of grains is the key to improving yield. Therefore, in crop improvement, molecular marker-assisted selection does not require many

years of breeding experience, not depending on the environment and the developmental stage of the crop, and the monitoring of good alleles effectively reduces or avoids the introduction of unfavorable genes.

The purpose and objectives of the study. The aim is to reveal the genetic mechanism and genetic composition of wheat multi-spike grain number, and provide theoretical basis for subsequent wheat germplasm innovation and wheat high-yield breeding.. The goal was to solve the following tasks:

1. The genetic loci of the key genes of multiple panicle number were determined by F2 population, and then the key genes from " Mexican Large Spike " were cloned by mapping cloning method by constructing near-isogeniclines of each genetic locus.

2.Understandings of the functions of novel-miRNA and their targets and the miRNA-mediated regulatory networks involved in wheat grain development, will help us to elucidate the molecular mechanisms underlying wheat grain development and carry out ingenious molecular improvements in wheat breeding.

Object of study. Genetic composition of three factors of winter wheat yield and regulation of miRNA in wheat grain development.

Subject of study. The genetic loci regulating panicle number were found to provide theoretical basis for wheat breeding for high yield. Mechanism of miRNA regulating grain growth and development.

Research methods. General scientific methods: field trials, random sampling, laboratory tests, problem analysis, problem solving. The measurement of biometric parameters of plant growth and development, in particular the number of grains per spike and the weight of 1,000 grains, using relevant software to analyze data results and reliability.

The scientific novelty. The genetic mechanism of grain number per spike of wheat was studied by using the extreme difference ingrain number per spike of two parents (Mexico Large Spike and Bainong 419), and on the basis of eliminating the influence of acre spike number and 1000-grain weight on grain number per spike of parents and the targeted population, the research results would be more scientific and reliable.

The personal contribution of the applicant is to plan and conduct research, summarize scientific data of references (literature) on the topic of the dissertation, analysis of experimental data, formation of conclusions and recommendations for selection, preparation and writing of scientific papers. Scientific articles have been published both independently and in co - authorship.

The structure and scope of the dissertation. The dissertation structure contains introduction, literature review, two sections, conclusions, discussions , breeding Proposals and a list of references, with seven parts.

CHAPTER 1 LITERATURE REVIEW

Section 1 Preliminary localization of QTL for grain number per spike and thousand grain weight

The domestication of wheat occurred 10,000 years ago in the Fertile Crescent[1, 2], and since then, wheat has become one of the largest and most important food crops in the world, it provides about 30% of the food production and more than 20% of the calories and protein in the daily diet of humans today[3, 4]. According to the forecast, the world population will increase from the current 7 billion to 9 billion by 2050, and the grain yield must be increased by more than 30% to meet the increasing human consumption demand, therefore, breeding new high-yielding and stable wheat varieties is an effective way to meet the future human food demand[5]. Yield mainly depends on three factors: spike number per unit area, grain number per spike and thousand grain weight, among spike number per unit area is determined by the tiller characteristics of the variety itself, spike grain number and thousand grain weight depend on late spike development, and spike grain number and thousand grain weight are negatively correlated [6, 7], and at the physiological maturity stage, yield increase ultimately depends on spike grain number [8- 10], which has been confirmed in the practice of breeding high-yielding wheat varieties in recent years.

Yield is a quantitative trait controlled by multiple genes, with a

complex genetic basis, and is susceptible to environmental influences [11]. The traditional breeding methods is time and resource consuming. However, with the development of molecular biology and biostatistics, quantitative trait locus (QTL)mapping techniques for quantitative trait loci based on molecular marker genetic mapping have provided an effective technical tool to study the genetics of quantitative traits [12]. With the help of DNA markers and QTL mapping, complex quantitative traits can be located on chromosomes by means of forward genetics [13].

This study intends to analyze the genetic characteristics of wheat yield components at the QTL level, to provide a basis for genetic improvement of yield traits.

1.1 DNA molecular markers

The application of molecular markers can be traced back to the early 1990s, at that time, restriction fragment length polymorphism (RFLP) markers were applied for gene localization, variety identification, identification of wheat-rye recombinants and identification of homologous chromosome arms in wheat. In wheat breeding, PCR-based molecular markers, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and functional diagnostic markers based on gene sequences, have been widely used and have made important contributions to wheat molecular breeding and genomics strategies [14-16].

Although the use of RFLP markers for constructing wheat linkage groups has been studied earlier, it failed to become an ideal marker due to its low frequency in bread wheat, high cost and time-consuming method. This has led researchers to use PCR-based molecular markers; two major categories of which include random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR). Only a few examples of RAPD markers being used to locate important QTL and being converted into more authentic sequence tag sites (STS) or sequence signature amplification region (SCAR) markers. For example, including the QTL for Russian wheat aphid (Dn), Lr24 and Lr28. PCR-RFLPisa simple and rapid method for detecting codon mutations based on another restriction endonuclease site caused by codon mutations [17]. Identification of DNA sequences encoding γ-alcoholysin and non-encoding γ-alcoholysin of different alleles of common wheat 15 using restriction fragment length polymorphism (RFLP) revealed seven allelic variants of the encoded γ-alcoholysin Gli B1 [18].

Genotyping using simple sequence repeat (SSR) markers has been one of the most widely used methods for identifying individuals for decades. Because SSR markers are highly informative, co-dominant and multi-allelic, they are experimentally reproducible within and between laboratories and can often be transferred between related species[19]. Although SSRs are often used for gene mapping and markers, they have

limited potential for use in actual plant breeding for the following four reasons. First, obtaining precise information on multiple alleles per locus is a challenge; Second, it is difficult to integrate or compare SSR data from different platforms or groups; Third, the number of SSR motifs in the genome is limited and unevenly distributed; Fourth, gel-based SSR analysis is low-cost and time-consuming for genotyping. Therefore, it is crucial to establish simple, accurate and high-throughput molecular marker-assisted breeding platforms[20]. Salt-responsive gene-based SSRs were developed for wheat, and phylogenetic analysis revealed four taxa. Salt-sensitive genotypes were mainly distributed in taxa I and III, while the remaining two taxa had medium and high salt tolerance genotypes [21]. A new apple genotyping kit (named Ap17) was developed using simple sequence repeats (SSR) for rapid, convenient and accurate genotype identification of apples [22].

SNPs are abundant in crop genomes and are ideal markers for genetic discovery studies and molecular breeding. Similarly, SNPs from cloned genes and genome-wide linkage and association analysis, using an array-based platform complemented by sequencing genotyping, can develop a powerful toolkit for breeding. Future practical breeding platforms should employ automated genotyping based on array or sequencing, target function polymorphism based on economic traits, and provides desirable prediction accuracy for quantitative traits and is commonly used in a wide

range of genetic backgrounds in crops. The development of such a platform faces both serious challenges at the technical level due to the ineffective cost, it also faces a serious challenge to the level of knowledge due to the large genotype-phenotypic gap in crops. So far, a series of high density SNP genotyping arrays have been designed and used in marker-assisted breeding of common wheat, such as the Illumina Wheat 9K iSelect SNP array、the Illumina Wheat 90KiSelect SNP genotyping array 、the Wheat 15K SNP array、the Axiom® Wheat 660K SNP array, the Wheat 55K SNP array, the Axiom® HD Wheat genotyping (820K) array 、the Wheat Breeders' 35K Axiom array and the Wheat 50K Triticum TraitBreed array[16, 23-27]. The wheat 660K SNP array has the highest percentage (99.05%) of genome-specific SNP, and its physical location is reliable. SNP density analysis showed that SNPs were almost uniformly distributed throughout the genome. In addition, 229,266 SNPS in wheat 660K SNP array were located in 66,834 annotated genes or promoters[28].

1.2 Principles and methods of QTL mapping

QTL isthe location on a chromosome of a gene for a trait that varies continuously for inherited traits in biology. Since QTL localization was used in tomato for the first time, There are increasing reports on QTL localization[29]. Molecular markers were used for QTL mapping, detecting the linkage between molecular markers and QTL, determining the location of QTL by calculating the exchange rate between unknown

and known markers and estimating the effect of QTL, to perform QTL localization, the following conditions need to be met.

1.2.1 Construct the mapping population

Firstly, we need to determine the segregating population based on the phenotypic data of the quantitative traits of QTL localization. When constructing the segregating population, we need to consider both the parental selection, population type and population size.

Parental selection

The key to the success of QTL localization lies in the parents selection. The parents selection should be based on the following principles.

(1)Genetic differences between parents. Constructing the segregate population, the genetic differences between the two parents should be neither too large nor too small. The evolutionary relationships, phenotypic differences, and geographical differences in the distribution of the parental were used as criteria to determine the affinity. Polymorphism is high in heterozygous crops and low in self-cross crops. For example, the polymorphism of maize is good, and ideal populations can be constructed generally between inbred lines, while the polymorphism of tomato is extremely poor and requires the use of progeny from different species to construct populations [30, 31].

(2)Extremely high purity requirements between parents

(3)Due consideration should be given to whether their hybrid

offspring can reproduce normally. If the parents are distantly related, most of the pairs and recombination between chromosomes do not proceed properly for hybrid individuals in meiosis, resulting in a low rate of recombination between linked loci and biased segregation. In more serious cases, the phenomenon of incompatibility of crosses can occur, and the fertility of the offspring can be reduced, even leading to defeat and inability to continue the reproduction of hybrid offspring.

(4)Since chromosome exchange, translocation and other variation problems can occur, the parental material and its hybrid individuals should also be identified and analyzed at the cellular level, to avoid affecting the effectiveness of genetic linkage mapping construction.

Selection of Separate Population Types

Based on their genetic stability, QTL localization populations can be divided into two categories. (1) Temporary separation of groups. It mainly includes F2 and F3 populations and backcross (BC)populations, etc. The advantages of this type of population are: simple population construction, rich genetic information provided, and additive and dominant effects can be estimated; The disadvantage is that there is separation between individuals, which does not allow for long term preservation and multi-generational use. (2) Permanently separated populations. such as recombinant inbred line (RIL), doubled haploid (DH) and near-isogenic line (NIL) population. The population are classified by strain and can be

subjected to replicated block trial, distinguishing between block effects, repeat effects and random errors, thereby to increase the accuracy of QTL detection; However, it takes a long time to build a population, and is vulnerable to environmental and human factors.

F2 population. The F2 population is one of the earliest and most widely used mapping populations in quantitative trait locus[32-34]. It is obtained by crossing the parents to obtain F1 and then self-crossing. F2 populations are easy to construct, to have a diversity of segregation types, to provide abundant genetic information, and can estimate both additive and dominant effects. There are also two shortcomings, one of which is the diversity of segregation types and the existence of heterozygous genotypes, resulting in the inability to identify dominant pure and heterozygous genotypes by phenotype. The second is that it is not easy to preserve for a longtime, because the genetic structure of the population changes after one generation of sexual reproduction.

Backcross population. Backcross population are produced from multiple crosses between F1 and one of its parents. The population have only two genotypes in the locus, directly to reflect segregate the proportion of F1 generation gametes. Therefore, the localization accuracy is higher than that of the F2 population, and it is not easily preserved for along time as the F2 population. In addition, it is not easy to establish larger populations for some crops that are difficult to hybridize manually, and

they are prone to false hybrids.

Recombinant inbred line population. RIL population are generated by self-crossing multiple generations of hybrid progeny and are generally established from F2 by replacing the single grain transmission method. Since the population are multigenerational self-cross and the plant line in the population are purity, the RIL population is a permanent segregating population that can be used and retested for a long time. The population also has shortcomings in that there is self-recession in heterogeneously pollinated plants, which makes it difficult to construct RIL populations; The RIL population took longer time to construct compared to the F2 population [35, 36].

Double haploid population. The DH population is first formed by chromosome doubling ofF1 heterozygotes, and the formation of haploid plants is induced using anther culture method, and then the DH strain is formed by chromosome double haploid plants. DH plants are homozygous lines produced by self-crossing and area permanent segregating population. Some plants have very difficult anther culture to obtain DH population, causing limitation in the applicability of DH population; Secondly, the floricultural ability of plants is related to genotypes, and the floricultural process can produce selection effects on pollen of different genotypes, which can destroy the genetic structure ofDH populations and cause severe segregation bias, thus it affects the accuracy of QTL localization[37, 38].

Near-isogenic line population. NIL is obtained by repeated backcrossing, Strains with donor target introduced chromosome fragments, and other genomic components consistent with the reincarnated parents. NIL is the breakdown of multiple QTL into individual G.J.Mendel factors in the same genetic background to accomplish the transformation of quantitative traits to qualitative traits, allowing for fine localization and map cloning[39]. Compared with other segregating population, multiple genetic factors of the whole genome segregate at the sametime, leading to the interference of QTL localization results by genetic background and influence the localization accuracy.

In addition to the above listed mapping populations, there are also Nested association mapping population (NAM) and Multi-parents advanced generation intercross (MAGIC)[40, 41]. The following is a comparison of various commonly used QTL localization population：

Table 1-1 Comparison of several populations in common use for QTL mapping

Population type	F2	BC	RIL	DH	NIL
Accuracy	Low	Low	Medium	Medium	High
Population size	Big	Big	Medium	Small	Small
Permanent	-	-	+	+	+
Cost	Low	Low	Medium	Medium	High
Time	Short	Short	Long	Long	Long

Separation ratio	1:2:1	1: 1	1:1	1:1	1:2:1
Additive effect	+	+	+	+	+
Dominant effect	+	+	-	-	-
Interference of genetic background	Big	Big	Big	Big	Small
Amount of information	Richest	Rich	Rich	Rich	Rich

Note: +: Yes, - : No. Same below.

Determination of population size

The accuracy of QTL localization, in addition to the population type, and also depends largely on the population size. The larger the population, the higher the accuracy of localization. However, the population is too large, which not only increases the experimental workload, but also increases the cost. Therefore, it is essential to determine the optimal population size. In actual research, the size of the population is determined by the purpose of the experiment, and a small population may be selected if only a molecular genetic map is constructed; For QTL localization of agronomic traits (quantitative traits), larger populations need to be selected. The size of the selection populations also differ between population types. For example, F2 segregating populations have more types of genotypes in separating progeny than other mapping populations, and it is necessary to obtain a

large enough population sample size, so that all genotypes are likely to occur. Generally, when the size of the F2 population is more than double the size of the backcross population, in order to make the mapping accuracy of the two comparable. The order of the required population size is F2, RIL, BC1, and DH for comparable mapping accuracy.

1.2.2 QTL localization methods

QTL localization methods are divided into two main categories, depending on the basis of individual grouping. (1) The grouping of phenotypic data based on quantitative traits is called Trait-based analysis (TBA), It is the mixing of DNA from two extreme individuals to detect genetic polymorphism between the two DNA pools [42]. The molecular markers that show differences between the two pools are considered to be linked to the QTL, which is also called "Based Segregate Analysis" (BSA). This method is widely used in qualitative traits [43, 44]. It can significantly reduce the number of DNA samples, reduce the cost of marker analysis, that is suitable for the localization of some resistance genes, however, it can only be used for QTL localization of single traits, and the sensitivity and accuracy are low, and only QTL with large effects can be detected. So, this type of method is rarely applied in QTL localization at present. They are not described in detail in this paper.

The other type of grouping is based on marker genotypes, called Marker-based ananysios (MBA)[45], This includes Single Marker Analysis (SMA), Interval Mapping (IM), Composite Interval Mapping (CIM), Inclusive Composite Interval Mapping (ICIM) and Mixedomposite Interval Mapping (MCIM). Interval Mapping, ICIM) and Mixedomposite Interval Mapping (MCIM) based on the hybrid linear model. A comparison of the characteristics of five different commonly used QTL localization methods was summarized (Table 1-2).

(1) Single Marker Mapping (SMA)

SMA is a method of marking the mean difference of phenotypic traits corresponding to different marker genotypes. If a marker is linked to a quantitative trait, the prerequisite is that there must be a significant difference in the mean value. Since this method does not require the construction of a complete genetic linkage map, it is widely used in early QTL localization [46-48]. In addition, there are many shortcomings in this method: ① It is not possible to determine whether a marker is linked to one trait or to multiple traits. ② It can only determine the linkage to that specific marker, but cannot know the exact location on the chromosome; ③ Genetic effects can be underestimated and some false positives can be easily detected. ④ The detection efficiency is lower, but a larger population sample size is required.

(2) Interval Mapping (IM)

Based on the principle of single marker mapping, it was proposed that an interval mapping method with two adjacent molecular markers [49]. The main statistical methods and mathematical models used are regression analysis, likelihood ratio or least squares methods. The IM method has distinct advantages over the SMA method: ①This method can be positioned within two known marker intervals, so that the approximate position on the chromosome is known; ② in the presence of only one marker on a chromosome, estimates of position and effect values tend to be asymptotically unbiased; ③The detection efficiency is improved compared to single marker assays, but the required population sample size is reduced. As this method compensates some of the defects and deficiencies of SMA method to some extent, it has been used more in the genetic study of quantitative traits.

(3) Complex Interval Mapping (CIM)

A composite interval mapping method that can combine multiple linear regression with interval mapping was first proposed in 1994[50]. A model is detected for a particular marker interval and fitted together with all markers linked to other QTL, which in turn controls the genetic effect of the background. If all QTL loci are not subject to epistatic effects and genotypes and environments also interact, the maximum likelihood estimates of each parameter are obtained by interval mapping methods, likelihood profiles are drawn, and QTL are located in possible marker

intervals based on the significant differences between likelihood ratio statistics. Its main advantages are: ① although only one interval is detected at a time, it can turn a multidimensional scan of multiple into a one-dimensional scan; ② the marker information of the whole genome can be reused; ③ the influence of genetic background on the detection results can be minimized to a large extent, thus improving the accuracy and efficiency of mapping. However, this method cannot analyze complex genetic issues such as epistasis and interactions with the environment, and is prone tobias in localization and effect estimation.

(4) Inclusive Composite Interval Mapping (ICIM)

The complete interval mapping is derived from the composite interval mapping [51]. There are two main processes: Firstly, based on the information of known molecular markers, Some important marker variables were selected by stepwise regression analysis method and their genetic effect values were estimated. The corresponding phenotypic data values were then corrected using the linear model obtained by stepwise regression, and one- and multidimensional scans were performed in the whole genome using the data obtained after correction. In addition, analysis software based on complete interval mapping has been successfully applied in the detection and analysis of nested association mapping populations[52].

(5) Mixed Composite Interval Mapping (MCIM)

To evaluate the epistasis between QTL localizations and the interaction effects with the environment, a composite interval mapping method was proposed based on a mixed linear model [53]. This method allows analysis of additive QTL loci, different types of epistatic QTL, and genotype-environment interaction effects to be analyzed. Compared with the composite interval mapping method, this method can avoid the influence of selected markers on QTL effect analysis, and also can unbiasedly analyze the interaction effect between QTL and environment. The composite interval mapping method based on the mixed linear model can be extended to analyze QTLs with additive × additive, additive × dominant, dominant × dominant epistasis and their interaction effects with the environment. These effect estimates can be used to predict common heterosis based on QTL main effects, reciprocal heterosis based on QTL-environment interactions, and to directly estimate the breeding values of individuals, thus improving the efficiency of breeding.

Table 1-2 Comparison of the five analytical methods for QTL mapping

Analytical methods	SMA	IM	CIM	ICIM	MCIM
Number of markers	1	2	Multiple	Multiple	Multiple
Accuracy	Low		High	High	High
Additive effect	+	+	+	+	+
Dominant effect	+	+	+	+	+

Epistssis effect	-	-	-	+	+
Interaction with environment	-	-	-	+	+
Efficiency	Low	Medium	Medium-High	High	High
Model and method	Variance regression likelihood	regression likelihood	likelihood	likelihood	Mixed linear model

1.2.3 Common analysis software for QTL localization

The statistical analysis software widely used for QTL localization include Excel, SAS, DPS, SPSS, etc.; QTL analysis software include MAPMAKER/QTL1.1, QTL cartgrapher2.5, QTLNetwork 2.0, Ici Mapping 4.0, JoinMap 4.0, etc. Among them, QTLNetwork 2.0 and Ici Mapping 4.0 can analyze the interactions between genes and environment. The threshold values used for QTL localization were mostly between 2.0 and 3.0 (P=0.01, P=0.005, P=0.001), but the optimal LOD values were different for different population types, population sizes and different degrees ofphenotypic variation. The ideal LOD values were obtained using a permutation test, which was calculated using the QTL cartgrapher 2.5 software [54].

1.3 Domestic and international research progress

1.3.1 Grain number per spike

The inflorescence of wheat is the spike, which is a non-branched inflorescence, and the axillary meristem differentiated from the inflorescence meristem develops directly into a spikelet, each spikelet meristem producing multiple florets. Inflorescence meristems cannot proliferate indefinitely, to generate a limited number of lateral spikelet meristems tissue and a terminal spikelet meristem. The grain number per spike trait is determined by traits, such as spike length, number of spikelets per spike, number of fertile spikelets, number of sterile spikelets and spikelet bearing density, and is a complex quantitative trait[55, 56]. Spike structure has a considerable influence on florets development and consequently on the final grain number per spike, which in turn affects yield [57].

A recombinant inbred line (RIL) population combining HG28 and HG67 was used to locate the major quantitative trait locus qGN4.1 for grain number per spike in indica rice genotype Pusa 1266. Overexpression of the nal1(LOC_Os04g52479) gene is unlikely to induce high grain number per spike spike in these QTL-NILs. In contrast, another closely linked gene nal1 (LOC_Os04g52590) encoding a protein kinase structural domain, is consistently overexpressed in high grain number per spike NILs [58]. A high-density genetic map was constructed for the double haploid population of Kechengmai 1/Chuanmai 42, and a total of 27 qtl associated with total spikelet number per spike (TSN) and fertile spikelet number per

spike (FSN) were detected on chromosomes 1B, 1D, 2B, 2D, 3D, 4A, 4D, 5A, 5B, 5D, 6A, 6B, and 7D. QTsn/Fsn.cib-3D dramatically increased TSN and FSN, which were highly significantly correlated with grain number (GNS) per spike [59]. A total of 27 SNS QTL were detected in a recombinant inbred line (RIL) population of a normal spikelet cultivar and multiple-spikelet wheat line (with a longer spike with more canonically oriented apical spikelets)). sau-2D is considered to be a major QTL, by correlating spikelet number with other yield traits, spikelet number per spike (SNS) was positively correlated with spike length(SL), anthesis date (AD), and grain number per spike (GNS) [60]. There was no QTL detected on chromosome 2A for the florets number of total spikelets and fertile spikelets, Among them, the GNI-A1 gene has been identified, which increases grain number by increasing fertile florets per spikelet [61]. A high-resolution genetic map was developed using a promoter capture assay with exon combination assumptions in 2019, a QTL was localized for reliable spikelet (SNS) on the long arm of chromosome 7, This gene is homologous to the APO1 gene of rice and could be the best candidate for SNS [62].

1.3.2 Thousand grain weight

Thousand grain weight is also a component of yield and is controlled by multiple genes, including environmental and genetic factors. DH linkage mapping was constructed using Spark x Rialto, A QTL locus for

thousand grain weight was localized on chromosome 6A [7]. Construction of hybrid recombinant inbred lines between common wheat Nongda 3331 and Tibetan semi-wild wheat accession Zang 1817, A total of 15 qtl on TGW were identified, distributed on 11 chromosomes; Among them, the main QTL (QTgw-4D) located in the Xbarc1118-Xbarc105 locus interval of chromosome 4D explained 25.08% of the phenotypic variation[63]. Using the Nongda 3338 (ND3338)/Jingdong 6 (JD6) double haploid population, the major QTL ofTGW were identified in the range of 15.7 cM (92.7-108.4 cM) on chromosome 4AL; Field trials with different environments showed that JD6 increased thousand grain weight by 5.16-27.48% compared to ND3338 [64]. QTL localization of 60 RILs (Zhongmai 871 and Zhongmai 895) using 660 K SNP array, four genetic regions on chromosomes 1AL, 2BS, 3AL and 5B were found to have significant effects on TGW-related traits[65]. Rht-B1 is a plant height semi-dwarf gene on chromosome 4B and is a possible candidate gene for TGW[66]. ND3338 and JD6 crossed to obtain 203 DH populations by in vitro culture and thirteen stability TGW QTL localized on chromosomes 2A, 2D, 4A, 4B, 5A, 6A and 7A, the favorable alleles were derived from the biparental ND3338 and JD6[67].

1.3.3 Yield

Some scholars have started their related studies directly from yield traits and also detected many QTL loci related to yield. Three yield QTL

were detected on chromosome 2H, and one QTL on 7H.2 and 3H.1, respectively, with genetic contributions ranging from 8.82-43.16% [68]. QTL controlling yield were detected on 6D and 2A, respectively by constructing the RIL population of the HD 2808/ HUW 510 cross combination , Chromosome 6D from HUW 510 and 2A from HD 2808 with a genetic contribution of 9.46-16.26% [69].568 spring wheat germplasm resources from 36 countries were evaluated, QTL controlling yield were detected on chromosomes 2D, 4B, 6B, and 5A, respectively, one of them also has an unknown QTL [70][56]. The F1-DH lines of the cross between "UI Platinum" and "LCS Star" were used for QTL detection, Only one QTL locus, QGy.uia2-7D to control yield traits was detected, its genetic contribution was 14-17% [71]. Genetic analysis of yield traits was carried out using 114 recombinant inbred lines of "Belikh2/Omrabi5", Localized on chromosome 2A, 3A, 3B, 4B, 5A, 6B, 7A, Xwmc182, Xwmc388, Xwmc398, Xbarc182 and Xwmc177 were closely linked to yield traits [72].

1.4 Application of QTL localization in crop genetic breeding

The application of molecular marker technology to assist in the genetic improvement of crop cultivars is called marker assisted selection (MAS). The principle is to discover molecular markers that are closely linked to the target QTL, and then simply detect the linked markers at the molecular level to find out whether they carry the target QTL. Compared

to conventional breeding methods for crop improvement, MAS does not require years of breeding experience, is not influenced by environmental constraints and crop developmental stages, Monitoring for good alleles effectively reduces or avoids the introduction of unfavorable genes[73, 74]. In recent years, research on QTLin common wheat has developed rapidly, and QTL localization methods have played a great role in the mining of new gene sources and crop genetic breeding. MAS first came from Tanksley for tomatoes, In contrast to conventional breeding methods, which require more than a hundred single plants backcrossed for more than six generations, the selection was successful by utilizing a molecular marker strategy using only a few dozen single plants backcrossed for three generations[75]. Introduction of the master QTL q HSR1 for resistance to head smut using MAS into highly susceptible material, the resistance of the improved selfed lines was significantly improved and the combinations were still highly resistant to the disease[76]. The Opaque2 gene significantly increased the lysine content in maize endosperm protein, The successful introduction of the opaque2 gene into a superior maize variety based on the MAS strategy resulted in the successive production of high quality protein maize (QPM) [77]. KRN4, an important regulator controlling the maize female spike development gene UB3, was successfully cloned using a Genome-wide association study (GWAS) combined with map cloning [78].

The first genetic linkage map of wheat is an RFLP marker-based genetic linkage map constructed using the "International Wheat Mapping Initiative" ("ITMI"),which has been widely used for QTL analysis of yield traits as well as disease resistance in wheat [79]. From the mutant library of Kronos and Cadenza, mutants of VRT2 and SVP1 were identified, which could increase the spikelet number, and the mutant of Cadenza was transferred to Kronos, and the study was carried out after several backcrosses and crosses, by transgenic overexpression of VRT2, the lemma and palea of transgenic lines with high VRT2 expression became longer, and the spikelet meristem was transformed into inflorescence meristem. VRT2 was crossed with vrn/ful2 and the young spikes of vrt2/vrn1/ful2, VRT2/vrn1/ful2 at the same period were compared by scanning electron microscopy (SEM), We could clearly observe that the spikelet phenotype of vrt2/Vrn1/ful2 tended to be normal compared to VRT2/Vrn1/ful2 plants, with significantly shorter spikelet lemmas and palea and a large reduction in the number of spikelet florets [80]. Two recombinant inbred lines (RIL) populations were crossed between the highly resistant and tolerant varieties 'Blizzard' and 'Bonneville'and the disease susceptible variety 'Rainer'. The two recombinant self-incompatible (RIL) populations were tested for resistance to common black spike and dwarf stalk diseases in both field and greenhouse growing seasons, the QTL for head smut resistance were localized on chromosomes 1AL, 1BS, 7AL and 7DS [81]. The PM

resistance loci in the F2 population of the Korean cucumber cross using the inbred lines powdery mildew resistance and powdery mildew susceptible were identified, and two QTL, named pm5.2 and pm6.1, were found on chromosomes 5 and 6, respectively [82]. Three QTLs, qlb-czas1, qlb-czas2 and qlb-cazas8 were detected to be associated with rice blast in the F6 recombinant inbred line (RIL) population from the cross Yugu 5 and Jigu 31 [83].

1.5 Purpose and significance of the study

Wheat is known as the "world's grain" and is widely distributed around the world. Winter wheat does not compete with rice and corn and other summer grains for land and has its own unique advantages in meeting the world's future food needs. Henan is the core production area of the national grain strategic project, Wheat is being planted on more than 1/5 of the country's area, accounting for 1/4 of the country's total production and 1/3 of the country's commercial amount. From 2003 to 2015, the total wheat production in Henan has achieved "thirteen consecutive increases", behind the continuous increase is aslight increase in the sown area, the use of good varieties, policy support and the increase in inputs. At present, the wheat planting area in Henan Province has been maintained at the level of 550 ha for five consecutive years, and there is very little room to continue to expand its planting area. To ensure that the total wheat yield in Henan Province continues to increase, in addition to continuing to improve the

low-yielding fields, breeding new high-yielding and stable wheat varieties is an effective way to ensure a sustainable increase in wheat production, and the elucidation of the genetic mechanism of high-yielding and stable wheat yield is a prerequisite for further breeding a new generation of high-yielding wheat varieties.

High crop yields and stable yields that can withstand adverse environmental conditions are necessary to meet farmers' motivation and national food security needs. Spike number per unit area, grain number per spike and thousand grain weight are the three components of yield, Crop stability mainly includes resistance to pests and diseases, adverse climate, soil and other environmental tolerances. Throughout the past and present of wheat breeding in Henan Province, the improvement of its yield is the result of the coordinated improvement of three factors of yield, among which the improvement of spike number per unit area is the most obvious. The new high-efficiency wheat variety "Bainong 419", which has been approved, has 7.2-7.8 million spike number per hectare, 36-42 grain number per spike, 43-48 grams per thousand grain weight, and a yield of more than 12,000 kg per hectare. Spike number per unit area of "Bainong 419" tends to be saturated, and there is limited room for further improvement in thousand grain weight. The most likely effective way to continue to improve wheat yields in Henan Province based on "Bainong 419" is to increase the grain number per spike. "Mexican Super Spike" is a

large spike type variety with large spikes and many grain number, the average grain number of its main spike is more than 85, and the thousand grain weight is smaller than that of "Bainong 419". It can be seen that the wheat grain number per spike regulatory genes should have their own independent genetic control pathways, in addition to the interactions with other genetic factors of yield elements. In this paper, we used these two varieties as parents to construct F2 mapping population to carry out a systematic study, aiming to deeply reveal the mechanism of multiple grain number per spike in wheat, and provide a theoretical basis for the subsequent innovation of new wheat germplasm and high yield breeding of wheat.

Section 2 Small RNA and degradome analyses uncover the extensive effects of miRNAs and targets in early developing grains of common wheat

1.6Origin and effect mechanism of miRNA

1.6.1Origin of miRNA

miRNAs are a class of small non-coding RNAs with important regulatory functions that are commonly found in eukaryotes, its mature sequence is usually only 20-24 nucleotides in size [84]. Mature miRNAs are produced by processing their primary transcripts through a series of nuclease shears, followed by binding to the RNA silencing complex, Regulation of gene expression by directing the shearing or repression of translation of target m RNA through the principle of base complementary pairing. The functions of mi RNA are wide-ranging, which contain plant development, protein degradation, signaling, response to adversity stress, and regulation of its own metabolism [85-87]. Currently, research on mi RNA has become increasingly popular worldwide.

In 1993, a professor and his team studying the genetic analysis of the nematode Caenorhabdities elegans discovered a miRNA that they named Line-4[88]. It was shown that it does not encode any protein and regulates nematode cell development by base-complementary pairing of Line-4 and Line-14 to complete the developmental transition. In 2000, researchers identified another gene similar to Line-4 from nematodes-let-7, regulating

nematode development and completing the larval to adult transition [89]. In the same year, researchers identified homologs of let-7 in humans, Drosophila and other animals[90]. Since then, the study of miRNAs has become a hot topic. In 2001, Bartel, Tuschl and Ambros laboratories identified a series of small molecule RNAs with potential regulatory functions from Cryptobacterium showyeri, invertebrates, and vertebrates, respectively, and officially named them mi RNA [91-93].

In 2002, there was a breakthrough in miRNA research in plants, obtained information on plant miRNA biosynthesis, mechanisms of action, and biological functions[94, 95]. At present, a large number of mi RNAs have been found, not only in model plants such as Arabidopsis and rice but also a considerable number of mi RNAs have been found and validated in other plants[96-98]. Based on the large number and diversity of plant mi RNA, plant mi RNA may not only be involved in its own temporal developmental regulatory processes, but its functions may involve all aspects of plant growth and development and play an important role in many physiological activities. As the number of discovered miRNAs increased, miRNA libraries were established internationally (http://www.mirbase.org/). As of December 2021, mi RBASE 22 has been released and a total of 24521 mi RNA precursor sequences and 30424 mature miRNA sequences are registered in it. These include 6150 miRNA precursor sequences and 7390 mi RNA mature sequences in plants.

1.6.2 miRNA effect mechanism

The mature chains of mi RNA are selected for integration into RISC to form silencing complexes, The entire cell is scanned to select complementary nucleic acids in parallel to make them functional. Due to the difference in the complementation degree between miRNA and target gene m RNA and the different binding regions, they also work in different ways. There are three main types of mi RNA effect modes based on their complementary relationships：（1）mi RNAs are fully or nearly fully complementary paired with target gene mRNAs, and most mi RNAs exert regulatory functions by shearing target genes [99]; in general, mi RNA cleaves the target gene mRNA at the 10th-11th base [100, 101]. (2) mi RNA-mediated inhibition of mRNA translation. miRNA is usually capable of incomplete complementation with multiple recognition sites at the 3' end of the untranslated region of the target gene m RNA, This hinders the translation of ribosomes, which in turn can inhibit their protein expression levels [102]. Although the detailed process of this mode of action is not yet fully understood, the vast majority of animal mi RNA functions are achieved through this mode of action [103]. (3) In yeast and plants, very few mi RNAs were found to repress the transcription of target genes by triggering their methylation on the genome [104]. DNA methylation is one of the first discovered pathways of genetic modification, Numerous studies have shown that DNA methylation can cause changes in DNA

conformation, DNA stability, DNA-protein interaction patterns, and chromatin structure, thus regulating gene expression[105, 106].

1.7 Progress of miRNA biological function research

Many studies have shown that the synthesis of mi RNA in plants is strictly regulated, otherwise, various developmental defects will occur, such as plant size, anthesis date, and fertility, etc. It shows that mi RNA plays an important role in plant development [107]. In addition, during the growth and development of plants, miRNAs play a pivotal role in a variety of adversity stresses [99].

1.7.1 miRNAs are involved in plant growth and development

Plants go through multiple growth and developmental processes throughout their lives, it mainly contains conidia forming embryos, seed germination, morphogenesis, and flower, fruit, and seed formation[108]. These stages have distinct morphological features or new organ development, and one of the stage transitions is the transition from nutritional to reproductive growth. Plants need the proper conditions and time to complete anthesis and seed set, so stage transition at the appropriate time is critical for plants [109]. The normal expression of mi RNA is essential for normal plant growth and development, and they directly or indirectly regulate key transcription factors related to cell growth and differentiation involved in the plant growth and development process. For example, young leaves turn to mature leaves, nutritional growth changes

to reproductive growth, inflorescence differentiation turns to floral organ growth and the establishment of organ polarity. Presently miRNA has been reported extensively in rice, maize, barley, and Brassicanapus[110-113].

miRNA regulates the grain growth and development

We used high-throughput sequencing at 5, 15, 25, and 30 DAP to analyze and identify a large number of miRNAs associated with grain filling, some of them may be involved in the regulation of starch accumulation or grain formation based on the target genes predicted[114]. The abundant mi RNAs in middle-late-stage target genes are mainly involved in carbohydrate and nitrogen metabolism, translocation, and kinase activity, which maybe related to seed filling [115]. The expression of miR396, miR164, miR156, and miR319 targets is relatively high at early stages of seed development but sharply down-regulated at later stages, representing a miRNA-mediated transition from rapid cell proliferation to grain filling in wheat seeds [116]. High-throughput sequencing was used to obtain 605 conserved miRNAs and 268 new miRNAs in developing wheat grains, and it was hypothesized that 86 of these conserved miRNAs might be involved in the regulation of wheat grain filling and 18 new miRNAs might have important roles in the maturation of wheat grains[114]. Nine varieties were selected and developing seeds were collected at 10, 20 and 30 days after anthesisto verify the gene expression of miR396 during grain development. Eleven of the 18 target genes were found to be growth

regulatory factor (GRF) genes, and miR396 was involved in seed development by regulating the expression of GRF genes (GRF1, GRF6 and GRF9) during wheat grain filling [117]. The fruit enlargement is an important and complex biological process. Through high-throughput sequencing, the author identified 1253 known mi RNAs and 1269 new mi RNAs from 9 small RNA libraries of cucumber fruits. With pollination RNA libraries, a total of 105 highly differentially expressed miRNAs were identified in fruits at 5 days post flowering. Based on the functional prediction of miRNAs and target genes, our findings suggest that miRNAs have potential regulatory roles in cucumber fruit enlargement by focusing their target genes[118].

Plant mi RNA is involved in regulating embryo development and root growth

Lateral root formation, meristem development, apical organ separation, silique and vascular cell development, and cell wall synthesis and cellulose development in Arabidopsis are all associated with mi RNA [119, 120]. Small RNAs and their targets were identified during embryogenesis in cotton somatic cells, and the hypocotyls and embryogenic callus of land cotton YZ1 seedlings were compared. A total of 36 known mi RNA families were found to be differentially expressed, with 19 mi RNA families represented by 29 precursors. The expression profiles of miR156, miR167 and miR3476 were up-regulated from

globular-stage somatic embryo to embryogenic callus. While miR164 was down-regulated in embryogenic callus to cotyledon-stage somatic embryo (CE), suggesting that these five mi RNAs may be involved in the re-differentiation process of Somatic embryogenesis in cotton[121].

mi RNAs are involved in the regulation of plant stem and leaf development

The expression ofmiRNA and its potential target genes was evaluated on the stem tips of "Changfu 2" and the spiny shoot mutation "Yanfu 6"[122]. A total of 700 mature miRNAs were identified, including 202 known apple mi RNAs and 498 potential new mi RNA candidates. miRNA164, miRNA166, miRNA171 and their potential targets and associated phytohormones appear to regulate the growth shootapical meristem. miRNA159, miRNA167, miRNA396 and their potential targets, as well as related phytohormones appear to regulate cell division and internode length. In Arabidopsis, miR171c affects stem branching through negative regulation[123] . mi R396 slows down cell proliferation by inhibiting the activity of growth regulatory factor (GRF), thereby regulating the number of cells in leaves and the size of leaves [124, 125].

mi RNAs are involved in the formation and development of floral organs

Among the known and conserved mi RNAs, a number of mi RNAs commonly associated with flower morphogenesis and development were

identified, which belong to the MIR156/157, MIR159, MIR165/166, MIR167, and MIR172 families. The MIR167 family members that accumulate in large numbers during flower development are Ll-miR280, Ll-miR281 and Ll-miR285, which may target tarf6 and arf8. In Arabidopsis, the synergistic action of three mi RNAs, miR159/MYB, miR167/ARF6/ARF8 and miR319/TCP4, and their target genes is a prerequisite for the developmental maturation of calyx, petals and anthers [126]. Overexpression of mi R172 resulted in reduced petals and conversion of sepals to carpels, while overexpression of target genes resistant to miR172 resulted in plants with late anthesis and increased petal and stamen phenotypes [127, 128]. It was found that mi R444 is also involved in the morphogenesis of floral organs[129] .

1.7.2 mi RNA response in plant adversity

The former mainly includes pests and diseases, while the latter mainly includes high temperature, hot and dry wind, drought, low temperature, salt stress, cadmium stress, etc. We identified 186 known miRNA in cultivated grapes and 427 known miRNA in Beibinghong 59 of the identified mirna were conserved mirna homologous to Cabernet Sauvignon and Beibinghong. In addition, 105 and 129 new mi RNAs were identified in Cabernet Sauvignon and Beibinghong, respectively. expression of some mi RNAs in Cabernet Sauvignon and Beibinghong was associated with low temperature stress[130]. To better understand the molecular mechanisms

of Powdery mildew（PM） resistance in cucumber, we sequencedsRNA and degradase libraries constructed from PM-infected leaves 48 h after treatment with D8 and SSSL508-28 and the corresponding uninfected controls. By comparative analysis, 32 and 6 PM-responsive differentially expressed miRNAs were identified in D8 and SSSL508-28, respectively. More differential mi RNAs were identified in the comparison between ID (PM-inoculated D8) and NID (non-inoculatedD8), while the comparison between IS (PM-inoculated SSSL508-28) and NIS (non-inoculatedSSSL508-28) showed that miRNA expression levels may vary considerably depending on the strain/variety [131]. The expression of seven mi RNA, miR156a, 159b, 166e-3p, 394, 396c-3p, 812 and 827, was decreased in rice disease resistance. These seven mi RNA have previously been shown to respond to pathogen infection with incompatible interactions that may lead to activation of multiple defense responses. Our data suggest that these seven differentially expressed mi RNAs may be involved in the regulation of rice blast in rice[132].

Four treatment groups were designed: control group, water deficit stress, heat stress and water deficit stress plus heat stress, and samples were collected at five developmental time points: 5, 15, 25, 35 and 45 days post anthesis (DPA). A multi-omics analysis of the sRNAome, transcriptome and degradome was performed to construct a miRNA-mRNA network affecting starch synthesis, protein metabolism and other seed

characteristics[133]. Expression of mi R165/166, mi R169, mi R172, mi R393, miR396, miR397 and miR408 was found to be up-regulated, while mi R156/157, mi R159/319, mi R164, mi R394 and mi R398 were down-regulated under low temperature stress in Arabidopsis [134]. mi R414, mi R415, mi R837-5p, and mi R10546-akr were up-regulated in expression under high temperature stress [135]. mi RNA is also involved in stress response to trace elements. Studies have shown that mi R398, miR397, mi R408, and mi R857 are induced to be expressed under low copper stress and maintain copper homeostasis in plants[136, 137].

1.8 Research methods of plant miRNAs

1.8.1 High-throughput sequencing method

High-throughput sequencing, also known as "next-generation" sequencing technology, is based on Sanger sequencing and deep sequencing of single molecules [138, 139]. High-throughput sequencing has now become one of the most efficient and accurate methods for the identification of plant mi RNA, and many articles on the identification of plant mi RNA are reported every year [140, 141]. This technology has the advantages of large amount of sequencing data, high accuracy and low cost, therefore, it is widely used in life science, agricultural science and medical field, etc. To identify mi RNAs of two maize hybrid lines（PH6WC and PH4CV）and their targets of action in seed development, two sRNA and two degradase libraries were constructed, and the results indicated that

miRNAs associated with reproductive development (miR156, miR171, miR396, and miR444) may be differentially expressed in seed development [142]. The expression of 19 conserved mi RNAs and 13 new candidate mi RNAs were analyzed by high-throughput sequencing. mi RNA expression varied greatly in young leaves, stems and flowers. The expression of eight conserved mi RNAs (miR166, miR168, miR319, miR394, miR399, miR827, miR894 and miR5139) and six novel miRNAs (miRC1, miRC14, miRC16, miRC112, miRC179 and miRC181) did not change significantly in different tissues[143]. By the principle of small RNA high-throughput sequencing, 30 and 29 miRNAs were detected at 5°Cand - 10°C, respectively, which were mainly involved in transcriptional regulation, metabolism, stress response and signal transduction based on target gene prediction and functional analysis[116]. A total of 54 new conserved mi RNAs and 8 new mi RNAs were identified in the pollen of Brassica campestris ssp. Chinensis, eighteen of these mi RNAs differed in expression between the buds of male sterile and fertile lines by more than 2-fold, and q RT-PCR analysis showed that most differentially expressed mi RNAs were preferentially expressed on the buds of male sterile lines [144].

1.8.2 Degradome Sequencing

Degradome Sequencing is a newly emerged method for mi RNA target gene detection [145]. By high-throughput sequencing of degraded

fragments generated by mi RNAs-mediated shearing of target m RNAs in cells or tissues, combined with bioinformatics analysis, the target genes of mi RNAs are accurately and efficiently screened and the functions of the corresponding target genes are annotated. This method has the characteristics of high sequencing efficiency, low sequencing cost and large sequencing quantity compared with previous methods for mi RNA target gene identification, and has the advantages of three of high-throughput sequencing technology, bioinformatics analysis and rapid amplification of c DNA ends validation. We have identified for the first time a miRNA-like long hairpin locus that can produce 21~22nt phase siRNAs that can translocate and cleave target mRNAs, The predicted and validated targets of these development-related miRNAs are involved in different cellular responses and metabolic processes, including cell proliferation, growth hormone signaling, nutrient metabolism, and gene expression[116]. Sequencing analysis of mi RNA degrading enzymes revealed significant changes in the expression of miR-164d, which regulates genes encoding antioxidant enzymes, in tetraploid rice, resulting in reduced miRNA-mediated target gene cleavage [146]. By analyzing degradome data, we identified 41, 65 and 12 target genes associated with differentially expressed miRNAs in ovule development. We found that changes in the expression of phytohormone-related miRNAs may play an important role in ovule development, providing evidence for cross-

communication between sporophytic tissues and female gametophytes [147]. By degradome sequencing analysis, some target genes interacting with the erythropathogen were identified, candidate miRNAs were identified by qRT-PCR, indicating that the expression of most miRNA was negatively correlated with the expression of their targets[96].

1.9 Purpose and significance of the research

Wheat is one of the world's most important food crops. As the global population continues to grow, the problem of food shortage will become increasingly critical. However, wheat breeding has reached a new stage with the current level, To a large extent depends on people's understanding of the key genes and molecular regulation mechanisms that control wheat growth and development [148]. mi RNA is a class of small endogenous non-coding single-stranded RNA molecules that are widely present in animals and plants, it affects almost all biological processes and is a new level of gene expression regulation. Therefore, to investigate the regulatory role of mi RNA in wheat growth and development will greatly contribute to wheat genetic improvement research.

The early stage of grain development is an important period that determines grain yield and quality characteristics for wheat harvest. Grain development indicates that wheat enters the stage of reproductive growth, which is the period that determines the grain weight, and miRNA has become a hot research field in grain development. To identify miRNAs

involved in wheat grain development, in the present study, wheat grains at 7 days after pollination (DAP) and 14 DAP, which represent two transitional early developmental phases of wheat grains, were isolated. High-throughput small RNA and degradome sequencing were performed to explore the miRNAs and their target genes that possibly participate in the regulation of early grain development.

CHAPTER 2 PREMIMINARY LOCALIZATION OF QTL FOR GRAIN NUMBER PER SPIKE AND THOUSAND GRAIN WEIGHT

In the Yellow river winter wheat region, Henan Province has more than 80 million mu of wheat, accounting for nearly a quarter of the country's area, and is the largest wheat-growing province in China. According to projections, the world's population will increase from the current 7 billion to 9 billion by 2050, and food production will have to increase by more than 30% to meet growing human consumption demand[5]. As the world's arable land per capita is decreasing, increasing yield potential has been one of the key objectives of wheat breeding. Spike traits are closely related to yield traits and therefore genetic studies of spike traits are of great relevance.

In this study, we used the mapping population to construct a genetic linkage map and to locate QTL for some spike related traits in order to resolve their genetic mechanisms.

2.1 Experimental materials

2.1.1 Trial plant materials

Parental material: Mexican Large Spike (MLS),Bainong419(BN419); F2 population constructed by crossing Mexican Large Spike with Bainong 419, which contains 145 single plants. All the above materials are kept and created in our laboratory. The morphological characteristics and grain traits of the whole plants and spikes of the parents are shown in Figure 2-1.

Figure 2- 1 From left to right: A: Mexican Large Spike, Bainong 419

2.1.2 Molecular markers and sources

The SSR markers used were from previous studies and have been published publicly on the GrainGenes2.0 (http://wheat.pw.usda.gov/GG2/index.shtml) website. The wheat SSR primer sequences were evenly distributed across the three chromosome clusters of wheat A, B, and D. A total of 200 pairs of SSR primers were collected and synthesized by Shanghai Biotechnology Synthesis (Additional Table 2- 1).

2.2 Experimental method

2.2.1 Field trials

The F2 population and parents were planted in 2018-2019 in the experimental field of Henan Institute of Science and Technology with 2 m row length and 0.2 m row spacing, 20 rows of the F2 population and 5 rows of each parent, with conventional cultivation management.

2.2.2 Investigation of agronomic traits

At maturity, 10 randomly selected plants of parental material and single plants ofF2 population were investigated for agronomic traits such as spike length, spikelet number, spikelet grain number and spikelet bearing density with reference to the method[149], in which the average value of each trait of parental material was taken.

2.2.3 DNA extraction and reagent configuration

DNA extraction

The parents (Mexican Large Spike and Bainong 419) and 145 single seedling leaves were taken at the seedling stage and the genome was extracted by CTAB method[150] . The specific procedure was performed as follows.

(1) Take proper number of leaves into 2mL centrifuge tubes and grind them into powder with liquid nitrogen in the refrigerator to be used.

(2) Preheat the CTAB solution in a water bath at 65 ℃ for 30 min, and precool the isopropanol at 4 ℃.

(3) After the CTAB solution is preheated, add 800 uL in 2mL centrifuge tubes in turn, then put them into a 65℃ water bath and keep them warm for 30-60min, shaking them gently for several times during the period (about 20minin turn, you can shake them slightly and quickly when you first put them into the water bath, and then the shaking must be gentle)

(4) Add 800 uL of chloroform:isoamyl alcohol (24:1), operate in a fume hood and shake gently 100-200 times to turn milky white, do not shake vigorously, otherwise the DNA will be mechanically cut.

(5) Centrifuge at 15-20°C, 12000rpm for 10min.

(6) After centrifugation, aspirate the supernatant (use a clipped tip to do this, too sharp a tip will break the DNA strand) into a new centrifuge tube, then add 0.6 times (480uL) the volume of supernatantin isopropanol (4℃ refrigerator) and shake well (ifflocculent material precipitates, it can be put in 4℃ refrigerator overnight).

(7) Centrifuge at 15-20℃, 12000rpm for 5min, pour off the supernatant, and wash the precipitate left with 500uL 70% ethanol twice, place it on the ultra-clean table and blow dry.

(8) Add 200uL ddH2O to dissolve and store at -4℃.

After quantification of DNA concentrations using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), samples were diluted to 50 ngµl-1 with sterile water. DNA-labeled SSRs were evaluated.

Configuration of related reagents

(1) CTAB extraction solution configuration

CTAB 20g/L

NaCl (58.44) 1.4mol/L (81.816g)

EDTA (292.25) 10mmol/L (2.9225g)

Tris (121.14) 100mml/L (12.114g) PH=8.0

(2) Chloroform: isopropyl alcohol = 24:1

2.2.4 Analysis of molecular markers based on PCR principles PCR reaction volume

A 10 uL volume was used for PCR reactions, and the specific configuration of the reaction volume is shown (Table 2-1).

Table 2-1 PCR reaction volume

Reaction	Volume（uL）
Former primer	0.5
Reverse primer	0.5
DNA	1
2×Es Taq MasterMix (Dye)	5
ddH_2O	3

Amplification procedure

Pre-denaturation at 95 ℃ for 5 min, denaturation at 95 ℃ for 30 s, annealing at 60 ℃ for 30 s (annealing temperature depends on primers), extension at 72 ℃ for 30 s, termination at 72 ℃ for 7 min, and finally storage at 4 ℃.

Detection of DNA staining in polyacrylamide gel electrophoresis

(1) Preparation of the glass plate:Scrub the glass plate repeatedly with

detergent and rinse it with clean water. After the glass plate is dry, cover the concave plate on the flat plate with clamps on both sides.

(2) Configuration of polyacrylamide gel: take 40 mL of configured 8% acrylamide, add 400 uL of 10% ammonium persulfate and 40 uLTEMED procoagulant in turn, and gently shake the three solutions well.

(3) filling glue: fill the configured glue slowly along the concave plate, in order to prevent bubbles, the process of filling glue should keep tapping the glass plate, after the glue is filled, quickly insert a comb between the plate and the concave plate, 15min after the glue coalesces, then pull out the comb slowly.

(4) Assembly of electrophoresis tank: put the plates filled with glue on each side of the electrophoresis tank, add 1×TBE buffer, not exceeding the maximum limit, and clamp it well with clamps, then add 1×TBE buffer to submerge 1/3 of the recess.

(5) Pre-electrophoresis: insert the electrode, 40W power pre-electrophoresis for more than 20minto clear the air bubbles.

(6) Spot sample: 2 uL of PCR product was added to each well and electrophoresis was performed at 180 Wpower for 90 min (electrophoresis time can be adjusted according to molecular quantity amount).

(7) After electrophoresis, pour the electrophoresis buffer (which can be recycled for secondary use) into the container, remove the glue plate from the electrophoresis tank, and then separate the concave plate from the

plate, with the glue located on top of the plate.

(8) Silver staining of amplification products for color development [151]

Staining: The unloaded gel was placed in a prepared 0.1% silver nitrate solution and shaken for 15 min on a shaker. The silver nitrate solution can be reused 2-4 times, and the staining time needs to be extended by 2-3 min for each repetition.

Rinse: After the silver dyeing, pour off the silver nitrate and quickly rinse the gel with distilled water 2-3 times.

Formation: Put the gel into the developing solution, add the appropriate amount of formaldehyde, and end the development when the DNA bands appear (on a shaker).

Washing: Pour off the developing solution and rinse the glue with distilled water, 2-3 times.

Photographing: Rinse the clean gum on top of the LED light box for photographing and saving.

(9) Band type records: maternal band type is recorded as A, paternal band type is recorded as B, heterozygous band type is recorded as H, missing or undetected band type is recorded as X.

Reagent configuration

(1) 10% Ammonium persulfate: weigh 1g of ammonium persulfate on an electronic balance, put it into a glass beaker, add 10mL of ultrapure

water to dissolve, and store it at -20 ℃ after dissolution. (Note: the amount of reagent configured at one time should preferably be used up within two weeks)

(2) 5 × TBE: weigh 54 g Tris, 27.5 g boric acid, dissolve in an appropriate amount of distilled water, add 7.44 g EDTA-Na2 to dissolve, and finally use distilled water to fix the volume to 1L.

(3) Configure 1L of 8% acrylamide gel storage solution: weigh 78g of acrylamide, 2g of methyl fork, measure 200mL of 5 × TBE, and finally fix the volume to 1L with distilled water.

(4) Silver staining solution: weigh 1g of silver nitrate, add 1000mL of distilled water to prepare 0.1% AgNO3 solution.

(5) Developer (configuration 1L): 20g sodium hydroxide and 0.36g anhydrous sodium carbonate, 4 mL formaldehyde, add 1L distilled water, shake and mix well (formaldehyde must be added now).

2.2.5 Commonly used instruments and equipment

(1) Micro pipettes：Eppendorf

0.1-2.5 uL 0.5-10 2.0-20 20-200 100-1000

(2) Digital display thermostatic water bath Shanghai Weicheng Instrument Company Limited

(3) JA2003A Electronic Balance Shanghai Jingtian Electronic Instrument Company Limited (4) QT-2A Vortex Mixer Shanghai Qite Analytical Instruments Company Limited

(5) TGL-16G Desktop centrifuge　Made by Shanghai Anting Scientific Instrument Factory (6)2720 Thermal Cycler (Thermo fisher 2720)

(7) JY600HC Electrophoresis instrument　Beijing Liuyi Biotechnology Company Limited

(8) Orbital Shaker TS-1000　Haimen Qilinbeier Instrument Manufacturing Company Limited

(9)Labpure water system　AIKE

2.2.6 Construction of genetic linkage map

Genetic linkage mapping was performed using the Mapping function of QTL ICIMapping Version 4.2 (https://www.isbreeding.net/) software. Based on the distribution information of SSR marker loci on chromosomes published in GrainGenes 2.0 (http://wheat.pw.usda.gov), the polymorphic markers were first sorted with the command "ANCHOR" so that each locus could be located on the corresponding chromosome. The "Grouping" command was used to assign all of the classified markers to the appropriate chromosome positions by setting the LOD value to 2.5; then the default values and algorithms were used for Ordering and Ripping, and the window size was set to 5; finally, the results were output using the Outputting command to output the results.

2.2.7 Analysis of phenotypic data

The analysis of normality, ANOVA and correlation between different

traits was performed for parental and F2 population spike traits using Excel 2021 and SPSS 22.0 software.

2.2.8 Additive QTL detection and analysis

QTL analysis was performed using the complete composite interval mapping method based on the stepwise regression linear model of QTL ICIMapping Version 4.2 software[152].The LOD threshold was set at 2.5, the progress interval was 1.0 cM, and the stepwise regression probability $P<0.001$ for QTL detection[153]. The favorable alleles were judged based on the coding of the parents at the time of QTL mapping, and if the additive effect was positive, it indicated that the potentiation effect originated from the parent with coding 2; if it was negative, it originated from the parent with coding 0[150].

2.2.9 Superordination QTL detection and analysis

The QTL ICIMapping Version 4.2 software was also used for the supernatural sex detection. The specific parameters were set as LOD threshold 5, progression interval 5.0 cM, and stepwise regression probability $P<0.0001$. The magnitude of epistatic effect between parental and recombinant types could be judged according to the positive or negative effect value of epistatic loci on phenotypic traits. If the effect value is greater than zero, it indicates that the epistatic loci belonging to the parental type have a higher effect than those of the recombinant type; conversely, it indicates that the recombinant type has a higher effect than

the parental type.

2.2.10 Naming of QTL

The naming of QTL was done by QTL + trait + chromosome name[154]. QTL are denoted by Q, traits are denoted by their abbreviations, chromosomes are denoted by the name of the wheat chromosome, and QTL for the same trait located on the same chromosome are distinguished by 1, 2, etc. after the chromosome.

2.3 Results and analysis

2.3.1Variation in two spike-related traits between grain number per spike and thousand grain weight

The ANOVA results showed that the two parents differed to a highly significant level in the two spike phenotypic traits of spike number and thousand grain weight (Table 2-2 and Figure 2-2), which was consistent with the principle of parental selection when constructing the QTL mapping population. The number of spike grains of the maternal Mexican Large Spike was phenotypically a high-value parent, while the thousand grain weight of the paternal Bainong 419 was phenotypically a high-value parent. The results of population normality analysis showed that the two traits were basically normally distributed, and there was bi-directional superparental segregation, indicating that both were quantitative traits inherited[155] . Correlation analysis showed that spike number and thousand grain weight were highly significantly positively correlated with

a correlation coefficient of 0.953.

Table 2-2 Variation in two spike-related traits between grain number per spike and thousand grain weight

Trait	Parent		F$_2$ Population						Correlation analysis
	MTD	AiKang58	Mean	Maximum	Minimun	SD	Skewness	Kurtosis	TGW
GNS	103.3	51.3**	69.32	121	21	25.95	0.22	-1.82	1
TGW	42.85	49.15**	47.31	63.18	28.19	5.39	0.047	0.94	0.953**

Note: One-way ANOVA was used to analyze the significance of differences (*, p< 0.05; **, p<0.01).

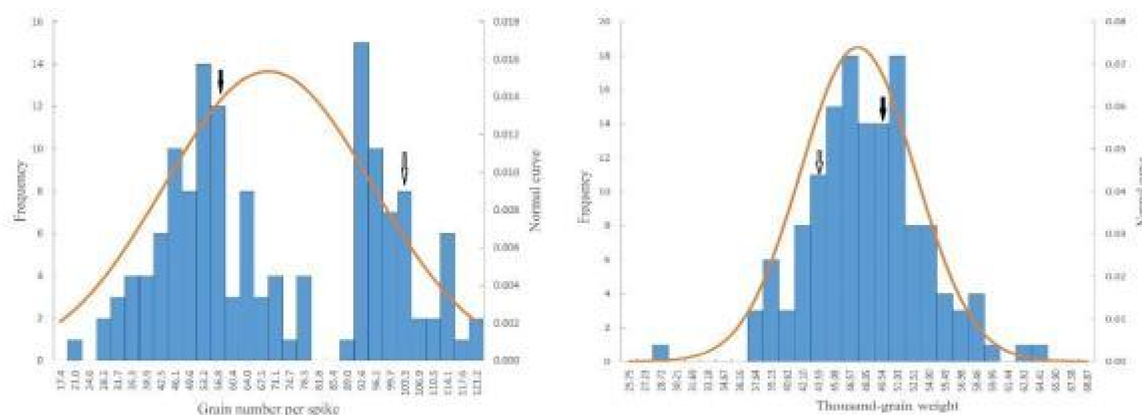

Fig 2-2 Varivation distribution of two spike related traits in 145 per plant derived from the cross Mexican Large Spike ×Bainong 419

⇩ indicates Mexican Large Spike，⬇ indicates Bainong 419.

2.3.2Polymorphism screening of SSR markers and amplification of polymorphism in F2 population

Atotal of 143 pairs of polymorphic markers with distinct differences

and clear bands were screened for molecular marker polymorphism using 300 pairs of SSR primers for the F1 cross between the parents Mexican Large Spike and Bainong 419 and the two parents. The 143 pairs of polymorphic markers were further genotyped and genetic maps were constructed for 145 individual plants of the F2 population. The amplified bands of some polymorphic markers in the parental and F2 populations are shown in Figures 2-3 and 2-4.

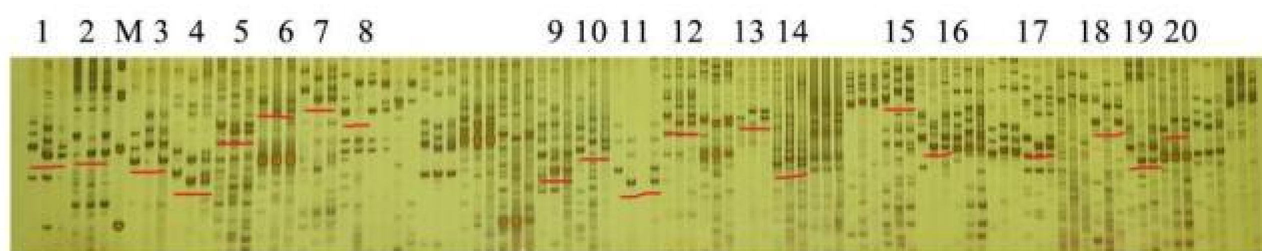

Figure 2-3 Amplification results of some SSR polymorphic markers between parents and F1

(M: D1500;1: yzu010590;2: yzu014970; 3: yzu015521;4: yzu022773;5: yzu026778;6: yzu028352;7: yzu030251;8: yzu031469;9: yzu035060;10: yzu1036610;11: yzu038419;12: yzu042815;13: yzu044501;14: yzu045544;15: yzu048245;16: yzu048727;17: yzu069171;18: yzu069938;19: yzu096666;20: yzu098401)

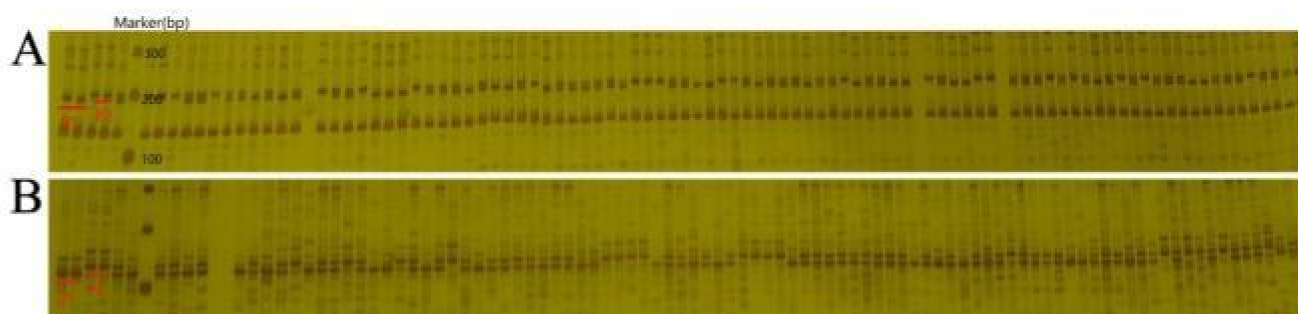

Figure 2-4 Segregation of some polymorphic molecular markers in the F2 population

(A: yzu113903; B: yzu129560; P1:BN419; P2: MLS)

2.3.3 Construction of genetic linkage maps

In this study, we used 145 SSR polymorphic marker loci covering 19 chromosomes of wheat to construct a preliminary genetic linkage map (Figure 2-5) with a total length of 3128.17 cM using the F2 population of "Mexican Large Spike/Bainong 419" as the mapping population, and the average distance between markers was 25.23 cM. The average distance between markers was 25.23 cM, the maximum genetic distance between markers was 113.85 cM, and the minimum genetic distance was 3.57 cM, and the genetic density between some markers was greater than 50 cM, which was mainly due to the small density of molecular markers, and more markers should be added in the next study to increase the density of the map.

The distribution of 145 polymorphic marker loci on chromosome groups A, B and D was uneven, among which 77 marker loci existed on chromosome group B, the largest number, accounting for 54.22% of the total number of marker loci; 41 marker loci existed on chromosome group A, the second largest number, accounting for 28.87% of the total number of marker loci; only 24 marker loci existed on chromosome group D, the smallest number, accounting for 16.9% of the total number of marker loci.

Only 24 marker loci were found on chromosome D, which was the least number of marker loci, accounting for 16.9% of the total number of marker loci.

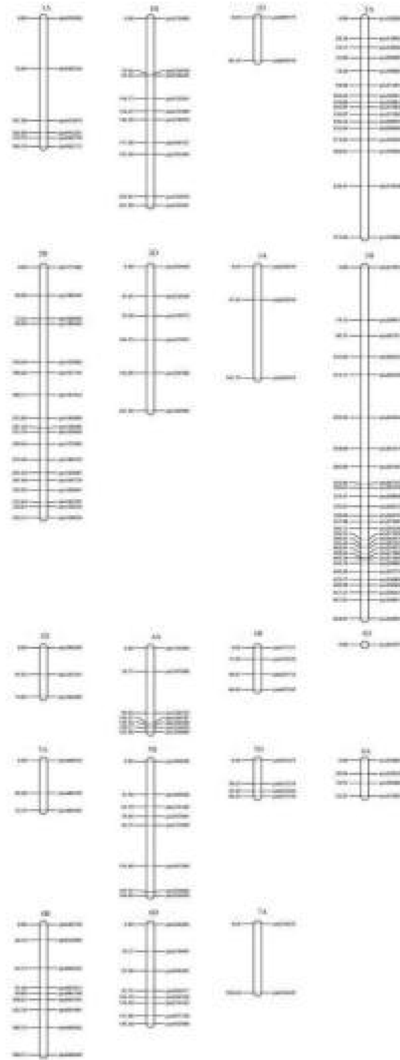

Figure 2-5 Genetic linkage map constructed for the F2 population based on MLS/BN419

2.3.4 Additive QTL analysis of grain number per spike and thousand grain weight

The additive analysis of the two spike related traits of grain number per spike and thousand grain weight was performed using the complete

composite interval mapping method. As shown in Table 2-3 and Figure 2-6, atotal of 10 additive QTL related to grain number per spike and thousand grain weight were detected, which could explain 4.922%~21.1044% ofthe phenotypic variation.

Table 2-3 Estimated additive(A) of QTLs for grain number per spike and three spike related traits

Trait	QTL	Flanking marker	LOD value	Additive effect	PVE (%)
Grain number per spike	QGNS- 1B	yzu0350060~yzu036610	2.7921	- 1.8891	21.10
	QGNS-2B	yzu137802~yzu148335	6.76	-2.1323	6.98
	QGNS-2D	yzu217893~yzu220186	6.8806	-6.3469	5.67
	QGNS-3B1	yzu263179~yzu287403	3.824	0.8613	4.92
	QGNS-3B2	yzu260727~yzu284764	13.8011	0.2541	15.89
	QGNS-3B3	yzu273259~yzu262178	5.8658	1.0579	6.14
	QGNS-3B4	yzu297374~yzu256856	7.2595	2.2648	7.27
	QGNS-6B1	yzu646758~yzu654906	4.2824	0.5321	5.77
	QGNS-6B2	yzu662234~yzu663011	8.9629	7.0816	7.70
Thousand-grain weight	QTGW-3B	yzu263179~yzu287403	3.6517	- 1.328	11.47

Grain number per spike

A total of nine additive QTL loci associated with grain number per spike were detected, which were distributed on chromosomes 1B, 2B, 2D, 3B and 6B, among which four associated QTL loci existed on chromosome

3B, two associated QTL loci existed on chromosome 6B, and one associated locus each existed on chromosomes 1B, 2B and 2D. It could explain 4.922%~21.1044% of the phenotypic variation ingrain number per spike. On three loci QGNS~1B, QGNS~2B and QGNS~2D, Bainong 419 increased the number of spike grains and Mexican large spike decreased grain number per spike; on nine loci QGNS~3B1, QGNS~3B2, QGNS~3B3, QGNS~3B4, QGNS~6B1 and QGNS~6B2, Mexican large spike increased grain number per spike and Bainong 419 decreased grain number per spike. QGNS~1B and QGNS~3B2 had large genetic effects and were the main loci, explaining 21.1044% and 15.8886% of the phenotypic variation.

Thousand grain weight

One additive QTL locus controlling thousand grain weight was detected on QTGW~3B, which could explain 11.4727% of the phenotypic variation, QTGW~3B locus Bainong 419 increased thousand grain weight and Mexican large spike decreased thousand grain weight.

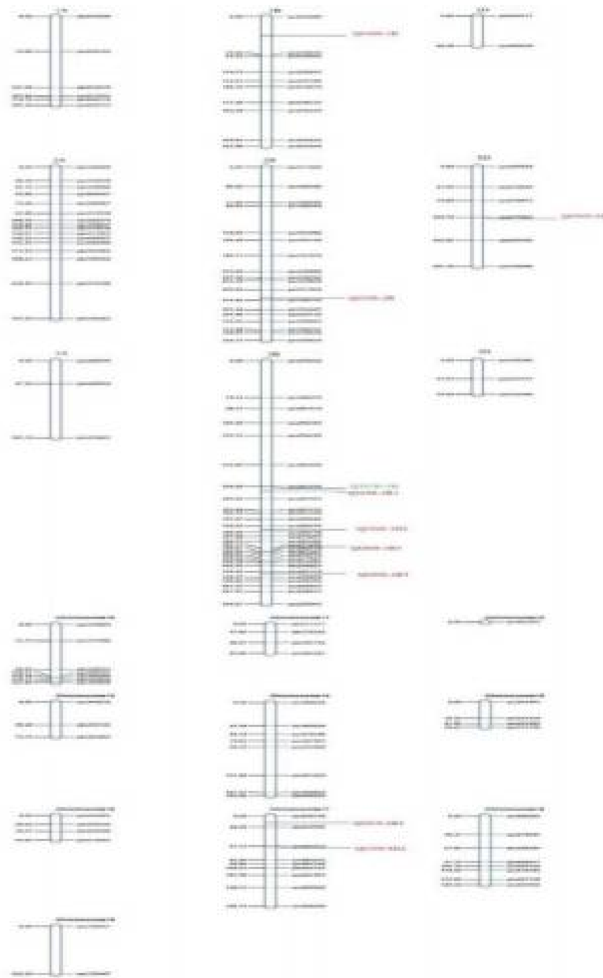

Fig. 2-6 Position of additive grain number per spike and thousand-grain weight related traits in wheat

2.3.5 QTL analysis for epistasis of two spike-related trait loci, grain number per spike and thousand grain weight

The detection and analysis of the epistatic QTL loci for grain number per spike and thousand grain weight showed that a total of nine epistatic QTL loci related to grain number per spike and thousand grain weight were identified. Among them, six epistatic QTL loci related to grain number per spike were found on chromosomes 2B, 2D, 3B and 6B, and the effect values at QGNS~2B, QGNS~2D and QGNS~3B1 loci were all less than

zero, therefore, the genetic effect of epistatic QTL loci belonging to recombinant type was greater than that of epistatic QTL loci belonging to parental type, which could explain 41.91% of the total phenotypic variation. The effect values at the QGNS~3B2, QGNS~6B1 and QGNS~6B2 loci were all greater than zero for the parental type epistatic QTL loci, indicating that the parental type epistatic loci had a higher effect than the recombinant type epistatic loci, which could explain 37.23% of the total phenotypic variation. The above data suggest that the effect of epistasis has a greater effect on the phenotypic variation of grain number per spike (Table 2-4).

A total of three epistatic QTL loci related to thousand grain weight were detected, mainly on chromosomes 3B and 5A, among which, the genetic effect values of QTGW~3B1 and QTGW~3B2 loci were greater than zero, which belonged to parental type of epistatic QTL loci, and the genetic effect values of QTGW~5A loci were less than zero, which belonged to recombinant type of epistatic QTL loci. 17.4% of the total variation in phenotype. This indicates that there are some random loci on the chromosome that do not directly affect the phenotype, but these loci can affect the phenotypic traits by interacting with each other. The genetic contribution showed that epistasis also had a large effect on the phenotypic variation in thousand grain weight.

Table 2-4 Epistatic effects of two spike-related traits, spike grain number and thousand grain weight

Trait	QTL	Flanking marker	LOD value	Additive effect	PVE(%)
Grain number per spike	QGN-2B	yzu137802~yzu148335	6.8794	-2.5142	15.66
	QGN-2D	yzu217893~yzu220186	7.1146	-7.0915	14.71
	QGN-3B1	yzu260727~yzu284764	4.5774	-0.3971	11.54
	QGN-3B2	yzu256282~yzu297374	6.3598	1.6685	16.44
	QGN-6B1	yzu646758~yzu654906	4.0094	5.0042	8.81
	QGN-6B2	yzu662234~yzu663011	5.5779	6.1593	11.98
Thousand-grain weight	QTGW-3B1	yzu264542~yzu287403	2.8059	-1.4509	8.17
	QTGW-3B2	yzu263179~yzu279201	3.7847	-1.4647	5.96
	QTGW-5A	yzu446725~yzu460465	2.528	1.5204	3.26

2.4 Conclusion and Discussion

The factors influencing the QTL localization results are mainly the selection of segregating population, population size, molecular marker type and number, environmental conditions, statistical methods and marker density of genetic linkage mapping. The above factors can be considered together to locate the QTL loci associated with the target traits more

accurately and precisely.

2.4.1 Mapping population and molecular markers

The first prerequisite for QTL localization is to construct a genetic linkage map, and to construct a genetic linkage map, it is necessary to select suitable parental combinations to create mapping populations. In the early days of quantitative trait research, temporary segregating populations such as backcross populations and F2 populations were usually used, and with the development of molecular biotechnology, permanent segregating populations such as DH populations and RIL populations replaced temporary segregating populations [156, 157]. Permanent segregating populations can be grown in trials in the same year and different locations or in different years and locations for certain traits that are susceptible to environmental conditions to study the effects of environmental conditions and genotype interactions. Compared to temporary segregating populations, permanent segregating populations are genetically stable and can be used for a long time. Temporary segregating populations are still widely used because of the simplicity of their creation and the abundance of genetic types[37, 158].

In crop QTL localization studies, some of them use interspecific hybrid populations [159], which are more distantly related, have high parental polymorphism, and can provide rich differences when constructing genetic linkage maps, but still use interspecific hybrid

populations in order to be close to breeding objectives [160]. In this study, the F2 population constructed between Mexican extra-large spike and Bainong 419, which differed greatly in spike traits, was used as a mapping population for the initial localization of QTL for spike number and thousand grain weight. The population was suitable for QTL analysis by statistical analysis of spike number and thousand grain weight of its parents and F2 population.

Common wheat is a heterozygous hexaploid species (2n=6x=42; AABBDD) with a genome size of 17,000 Mb (Megabase), which is quite large and complex, and more than 80% of the DNA is repetitive sequences with a narrow genetic base, resulting in low polymorphism of various genetic markers among different materials. SSR markers are basic repetitive units in the genome consisting of 1-6 nucleotides The basic repetitive units of the The same type of microsatellite can be distributed in different positions throughout the genome. The polymorphism of seats is formed by the different number of basic unit repeats. Compared with other types of molecular markers, the primers have the advantages of co-dominance, high polymorphism, good stability, simple operation, low dosage, random distribution in the genome, etc., especially in wheat. Therefore, SSR markers are considered as an ideal tool for genetic studies in wheat [161]. In this study, using 300 primer pairs distributed on chromosomes A, B, and D, the parents and their segregating populations

were analyzed and 143 primer pairs were polymorphic among the parents, accounting for 47.67%, and the SSR markers were highly polymorphic among the parents, which was due to the large genetic differences between the two parents[162].

2.4.2 Construction of genetic linkage maps

In this study, the F2 population of the Mexican extra-large spike, long spike and high grain number variety was selected as the mapping population, and the population sample size was 145. 143 SSR molecular markers with polymorphism among the parents were used for the construction of the wheat genetic linkage map, from the results, these molecular markers were unevenly distributed on wheat chromosome groups A, B and D. Among them, chromosome group B had the largest number of marker loci with 77 loci, accounting for 54.22% of the total number of marker loci. The B chromosome group had the highest number of markers, with 77 markers, accounting for 54.22% of the total number of markers; followed by the A chromosome group, with 41 markers, accounting for 28.87% of the total number of markers; and the D chromosome group had the least number of markers, with only 24 markers, accounting for 16.9% of the total number of markers.

In the present study, SSR markers had the highest polymorphism on chromosome group B and the lowest polymorphism on chromosome group D. This result is consistent with previous studies [163-165]. When

constructing genetic linkage maps, the problem of uneven distribution of molecular marker loci on chromosomes often occurs, which maybe due to the absence or low polymorphism of individual chromosomal segments of both parents and the difference in the probability of exchange between different segments of chromosomes. For example, regions at the ends of the chromosomes have a higher frequency of exchange than near the mitotic sites. This problem can be solved by choosing different combinations. There are also large gaps between multiple markers with distances greater than 50 cM, mainly due to the low density of molecular markers.

2.4.3 Localization of QTL for related traits

In this study, QTL ICIMapping V4.0 software with the complete interval complex mapping method was used to detect and analyze the additive and epistatic QTL loci associated with different traits (Table 3-2 and Figure 3-5). A total of nine additive QTL loci associated with spike number were detected on chromosomes 1B, 2B, 2D, 3B, and 6B, which could explain 4.922% to 21.1044% of the phenotypic variation in spike number. The results of the study did not agree with those of others, probably due to the small number of molecular markers used and the large genetic distance between markers; the small population sample, which reduced the efficiency of QTL detection; and the differences in environmental conditions. Therefore, the next study needs to increase the

number of molecular markers and population samples, encrypt the genetic linkage map, construct permanent segregating populations, conduct multi-year and multi-point trials, and conduct more in-depth genetic studies on QTL loci for spike traits.

Epistatic intercrossing refers to the fact that some random loci on the chromosome do not have a direct effect on the phenotype, but these loci can influence phenotypic traits by interacting with each other. There are three types of epistatic intercrossing: intercrossing between two additive QTL loci, intercrossing between additive loci and random loci, and intercrossing between random loci and random loci. The QTL analysis of the epistatic traits of spike length and some other spike traits showed that nine epistatic QTL loci related to spike number and thousand grain weight were detected in chromosome groups 2B, 2D, 3B, 5A, and 6B,which could explain 41.91% and 17.4% of the phenotypic variation, and the above data indicated that the epistatic effect on the phenotypic variation of both spike number and thousand grain weight The above data indicated that the effect of epistasis on the phenotypic variation of grain size and thousand grain weight was significant.

Five genomic regions affecting only TGW or GNS were identified using 191 recombinant self-incompatible lines on chromosomes 1B, 3A, 3B, 5Bor 7A, and chromosome 6A affecting both TGW and another region of GNS [166]. Thirteen TGW loci were localized on chromosomes 1AL,

2DL(2), 3DL, 4AL, 4BS, 5AL(2), 5AS, 5BL, 6A, 7AL, and 7BL, of which 11 loci increased TKW alleles from parental week 8425B, but Chinese spring contributed two positive alleles. Spike grain number identified 11 QTL for GNSon chromosomes 1BS, 2AL, 2B(2), 2D, 3AL, 3B, 4AL, 4BL, 6BL and 7BS. alleles for increasing KNS on chromosomes 2AL, 2B(2), 2D, 3B, 4AL and 4BL were contributed by Zhou 8425B, while chromosomes 1BS, 3AL, 6BL and 7BS alleles were from Chinese spring [167]. Nine stable QTL were identified located on chromosomes 2B, 4A, 5A, 6B, and 7A. The genomic region on chromosomes 2B and 4A covered two stable QTL for GNS (QGns.cau-2B.4 and QGns.cau-4A.4), of which the dominant alleles were all from ND3338; 13 stable QTL for TGW were localized on chromosomes 2A, 2D, 4A, 4B, 5A, 6A, and 7A, with favorable alleles being biparentally ND3338 and JD6 contributed [67]. Sixteen QTL for GNS were identified on chromosomes 1BS, 1BL (2), 3DL, 4AL, 4BS (3), 5BL, 5DL, 7AS, 7AL (2), 7BS, 7BL and 7DS, and on chromosomes 2AS, 2BS, 3AL, 3B, 3DL, 4AL, 4BS (2), 4DS (2), 5AL (2), 5DL, 6AL , 6BL, 7AL and 7BL were detected on 17 QTL for TGW[168]. Twenty-one QTL for TKW were detected on chromosomes 1D, 2D, 3A, 3D, 4A, 4B, 4D, 5A, 5B, 6A, 6D, 7B, and 7D, and they explained 1.1%-32.3% of the phenotypic variation in TGW, respectively. Chromosomes 1D (Xwhs179-Xmwg938), 2D (Xbarc297-Xbcd718 and Xbcd102-Xbcd262), 4B, 5A (Xbarc360-Xmwg624), 7B and 7D (Xrz2-Xbarc126), seven alleles of

"Opata 85" on TGW contributed positively to TGW. The other 14 alleles that contributed positively to TGW were from "W7984" [169]. The results of the above studies are not consistent with the results of the present study, which may be due to the different selection populations and molecular markers.

CHAPTER 3 SMALL RNA AND DEGRADOME ANALYSES UNCOVER THE EXTENSIVE EFFECT OF MIRNAS AND TARGE IN EARLY DEVELOPING GRAINS OF COMMON WHEAT

3.1 Materials and methods

3.1.1 Plant materials and sample preparation

The experiments were performed using the 7DAP and 14DAP grains of common wheat cultivar "Bainong 4199" (Triticum aestivum L.) bred and friendly provided by the Centre for Wheat Breeding, Henan Institute of Science and Technology. "Bainong 4199" is a high yield cultivar with plump grain and is widely planted in China. Seeds of "Bainong 4199" were firstly soaked in the tap water for 24 h and then disposed in 4°C dark chamber for one month. After vernalization, they were planted in the greenhouse maintaining 75% relative humidity, 26/20 °C day/night temperature, 12h light/dark photoperiod, and 10,000 lux light intensity. Day of pollination was recorded when half of the plants reached the flowering stage. For volume and fresh weight measurements, immature grains were collected for every 3-5 days, starting from 5 DAP to 34 DAP. And the grains were collected from the middle four rows of spikes at 7 and 14 DAP for deep sequencing and miRNAs analysis. Three biological replicates were made, and each consists of 100 grains. All samples were snap-frozen in liquid nitrogen and stored at −80°C for subsequent experiments.

3.1.2 methods

Small RNA and Degradome Sequencing

Total RNAs were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Qualified RNA samples must meet the condition of OD260/280 = 1.8–2.2

and RNA integrity number > 8.0. RNA samples in three biological replicates were equally to get respective RNA pool of 7 DAP and 14 DAP grains. These two RNA pools were used to construct small libraries by TruSeq Small RNA Library Prep Kit (Illumina, USA), according to the manufacturer's protocols. In brief, 700-μg RNA sample of each RNA pool was firstly fractionated on a 15% denaturing polyacrylamide gel, then the sRNAs with 18-30 nt were recovered. The 5' and 3' RNA adapters were ligated to the 5' and 3' ends of these sRNAs using T4 RNA ligase (Takara, Dalian, China). Purified ligation products were converted to cDNAs, which were used to construct the cDNA tag libraries by RT-PCR amplification. The size, purity and concentration of cDNA tag libraries were detected using an Agilent2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The cDNA tag libraries were sequenced using a HiSeq™ 2000 Sequencing System (Illumina, San Diego, CA, USA), according to the manufacturer's instructions.

The samples for miRNA sequencing were used to construct the degradome libraries. Degradome libraries were constructed by ligating polyA-enriched RNAs to the custom RNA adapter containing a 3' Mme I site. This is followed by reverse transcription, second-strand synthesis, Mme I digestion, ligation of 3' dsDNA adapter, gel-purification and PCR amplification. Amplified degradome tag libraries were then sequenced using a Solexa/Illumina genome analyzer [170].

MiRNAs identification and expression analysis

The reads from cDNA tag libraries sequencing were processed by Phred and Crossmatch (http://www.phrap.org/phredphrapconsed.html)[171]. Clean reads were obtained after low-quality reads and adapter sequences were removed. The 18–30 nt clean reads (tags) were matched to the sequences of Rfam[172], GenBank, and RepBase[173] databases to distinguish rRNA, scRNA, snoRNA, snRNA and tRNA from clean reads. After those reads having more than 90% sequence similarity to above RNAs were removed, the remaining sequences were matched to wheat expressed sequence tag (EST) database (http://www.ncbi.nlm.nih.gov/nucest/?term=wheat) or wheat genome sequences (http://mips.helmholtzmuenchen.de/plant/wheat/uk454survey/index.jsp) [174]. Ultimately, the non-coding tags were used to identify the candidate miRNAs.

Known miRNAs and novel miRNAs were identified from above non-coding tags referring to the methods reported by Chu et al. [175], with some modifications. In brief, the non-coding tags were firstly matched to the sequences of miRBase 22.0, the perfectly matched tags were the known miRNAs, and the others were used to predict novel

miRNAs. The predicted miRNAs should have the potential forming a hairpin secondary structure when analyzed using RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi)[176] .

For eliminating the disturbing of gene length and sequencing deep to the count of miRNA actual expression level, the frequency of each read count was firstly normalized according to the formula TPMi = (Ni/Li) × 1,000,000/sum (Ni/Li +…….+ Nm/Lm); Ni is the number of reads mapped to gene i, and Li is the length sum of the exons of gene i. The fold-change of miRNA expression between the 14 DAP and 7DAP libraries was calculated as log2 (14DAP/7DAP). If the P-value was ≤ 0.01 and the normalized sequence count had a fold change of > 2 or < 0.5, the given miRNA was recognized to be differentially expressed.

3.2 miRNA target annotation

Prediction and annotation of miRNA targets were performed as the methods reported by Chu et al. [175]. Identified miRNAs were aligned against wheat sequence databases. MiRNA targets were predicted according to the Target Finder (https://github.com/carringtonlab/TargetFinder?). BLASTN hits with less than four mismatches were chosen as candidate targets; for the non-target predicted miRNA, the psRNA Target software (http://plantgrn.noble.org/psRNATarget/) (version 12) was used to predict

the targets in wheat transcripts with prediction score cutoff value = 3.0, length for complementarity scoring = 20, and target accessibility = 25.

For the degradome sequencing data, 20-21 nt sequences of high quality were collected for subsequent analysis. The unique reads that perfectly matched wheat expressed sequence tag (EST) database from NCBI or the contig sequences from WGS assembly were retained. Approximately 15 nt upstream and downstream of 5' wheat EST sequences, mapped by degradome reads, were extracted to generate 31 nt target signatures as 't-signatures' [177]. The CleaveL and pipeline were used to identify miRNA targets [170]. Alignments with scores up to four where G:U pairs scored 0.5 and no mismatches were found at the site between the 10th and 11th nucleotides of the corresponding miRNAs were considered potential targets. Consistent mRNAs obtained from both methods were chosen as miRNA targets. To understand their functions, the putative target genes of the miRNAs were BLASTX and subjected to GO analysis.

3.3 MiRNA and Target validation by real-time PCR

QRT-PCR was performed to determine the validities of RNA Seq and target analysis. A total of 10 miRNAs and 8 target genes were selected randomly. RNA was extracted from three independent biological samples of 7 DAP and 14 DAP grains, individually, and used for transcription (RT) reactions. The One Step Primer Script miRNA cDNA Synthesis Kit (Takara) and PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time; Takara) were used for the RT reactions of miRNAs and targets following

the manufacturer's instructions, respectively. RT-PCR was performed using a Bio-Rad IQ5 Real-Time PCR Detection System (BIO-RAD, Hercules, CA, USA) with SYBR® Premix Ex Taq II™ (Takara). The total volume of each reaction is 25 μL [2.0 μL of diluted product, 2.0 μL of primers, 12.5 μL of SYBR® Premix Ex Taq™ (Perfect Real Time; Takara), and 8.5 μL of nuclease-free water]. All reactions were performed firstly at 95 °C for 30 s, then followed by 40 cycles (95 °C for 5 s, 61 °C for 30 s, and 72 °C for 30 s). All reactions were performed three times. Wheat U6 [GenBank: X63066] snRNA and the actin gene (GenBank: AB181991) were used as the endogenous control for miRNAs and target genes. The relative abundance of each miRNA was calculated by a comparative CT method (ΔΔCT) using the formula 2-ΔΔCT[178]. The miRNAs and target samples in the 0 DAP library with the CT value were selected as the calibrator, and the expression level was set as 1.0. The relative expression levels of the same miRNAs and targets were normalized through comparison. All primers used in this study arepresented in Table S15.

3.4 Results

3.4.1 Phenotypic analysis of developing grain

The development of wheat grain was evaluated by monitoring the pattern of increased grain weight and volume. Here, the growth of grain weight and volume was relatively slow at the early stage (before 11 DAP), increased sharply across 11 to 14 DAP and continued to rise until about 26 DAP (Fig. 3-1 A and 3-1 B). The appearances of developing grains at some representative time points are shown in Fig. 1C. Based on the pattern, we focused mainly on the transitional phases at 7 DAP and 14 DAP, the key periods for early grain development. To reveal the relevance between these changes and miRNAs abundance during early grain development, we then used the small RNA and degradome libraries from 7 DAP and 14 DAP

grains to compare the expression of miRNAs and to analyze the possible functions of their targets.

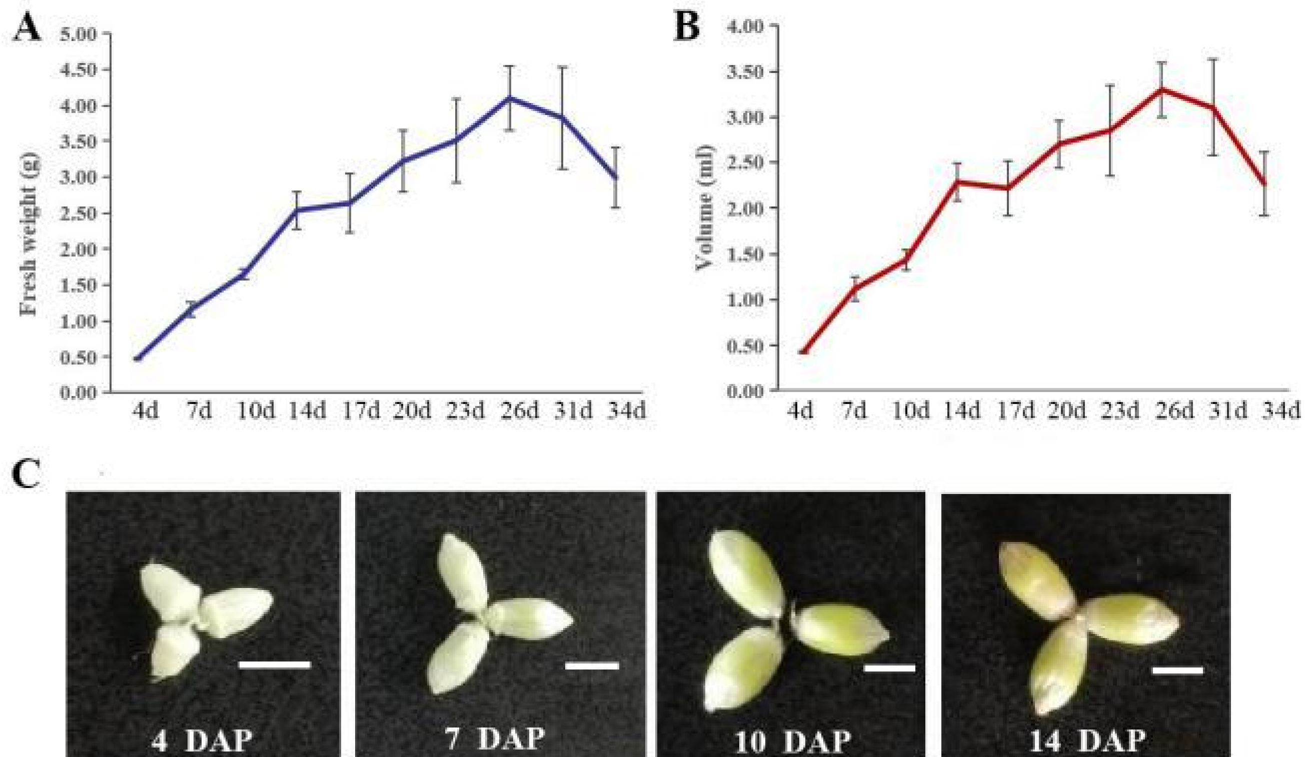

Figure 3- 1 Phenotypic changes of developing grains at different days after pollination

A: Fresh weight changes; B: Volume changes; C: Grain appearances at 0, 7, 10 and 14 DAP. Scale bars represent 1.0 cm. DAP: Days after pollination.

3.4.2 Deep-sequencing of sRNAs in early developing grains

To uncover the roles of miRNAs during wheat grain development at early stage after pollination, the grains of cultivar "Bainong 4199" at 7 DAP and 14 DAP were used to construct two small RNA libraries, which were then sequenced. The original sequencing fragments obtained by base identification analysis ofhigh-throughput sequencing results are Raw reads. Raw reads is filtered to remove low-quality, adapters, and N content greater than 10% of the sequencing fragments are Clean reads. A total of 43.18

million Raw reads and 24.4 Mb clean reads were obtained from these two libraries. A total of 14,020,684 (86.25%) and 14,212,862 (87.44%) sequences were obtained from the 7 DAP and 14 DAP libraries, respectively. These reads are equivalent to 1806597 (51.66%) and 1981099 (56.65%) unique sRNA sequences from the 7 DAP and 14 DAP libraries, respectively. As can be seen from the table, the 7 DAP and 14 DAP libraries have 73.69% (11978403) of the total sRNAs, but only 8.3% (290,265) unique sRNAs, and the total unique sequences in the 7 DAP library are less than the 14 DAP library (Table 3-1). The results show that, compared with 14 DAP, 7 DAP has a broader sRNA-mediated regulation of gene expression.

Table 3-1 Unique and total sRNA sequences between 7 DAP and 14 DAP

Category	Unique sRNAs	Percent(%)	Total sRNAs	Percent(%)
Total-sRNA	3,497,431	100	16,255,143	100
7 DAP&14 DAP	290,265	8.3	11,978,403	73.69
7 DAP-specific	1,516,332	43.36	2,042,281	12.56
7 DAP-total	1,806,597	51.66	14,020,684	86.25
14 DAP-specific	1,690,834	48.35	2,234,459	13.75
14 DAP-total	1,981,099	56.65	14212862	87.44

Non-coding RNAs, such as tRNA, rRNA, small nuclear RNA (snRNA), small nucleolarRNA (snoRNA) and small cytoplasmic(scRNA) were determined based on the databases Rfam (http://rfam.xfam.org) and

RepBase (http://www.girinst.org/repbase/). After removing the annotated RNAs (tRNA, rRNA, Repbase, and snoRNA), the number of the remaining unannotated sequences was 2,448,113 (accounted for 17.46%) in 7 DAP library and 3,456,209 (33.3%) in 14 DAP library of clean reads (Table 3-2). Of these unannotated sequences, 235,879 (10.64 %) reads in 7 DAP library and 1,458,680 (43.06 %) reads in 14 DAP library were matched perfectly to those from the whole genome shotgun (WGS) assembly (http://mips.helmholtzmuenchen.de/plant/wheat/uk454survey/index.jsp) and the National Center for Biotechnology Information (NCBI) (Table 3-3).

Table 3-2 Annotation and distribution of small RNAs in the libraries from 7 DAP and 14 DAP grains

Category	7 DAP		14 DAP	
	Number	Percentage(%)	Number	Percentage(%)
genome	241137	1.72	1488351	14.34
rRNA	11380128	81.17	6253147	60.25
scRNA	0	0	0	0
snRNA	2190	0.02	14543	0.14
snoRNA	122	0	296	0
tRNA	176952	1.26	620912	5.98
Repbase	13179	0.09	33897	0.33
Unannotated	2448113	17.46	3456209	33.3
clean reads	14020684	100	10379004	100

Table 3-3 Sequencing data of small RNA libraries derived from grains at 7 DAP and 14 DAP mapped to wheat reference genome

Samples	Total_Reads	Mapped_Reads	Mapped_reads(+)	Mapped_reads(-)
7 DAP	2,320,782	235,879 (10.64%)	165,101	70,778
14 DAP	3,387,722	1,458,680 (43.06%)	989,155	469,525

According to reports, sRNA length distribution generally reflects the specificity of a specific species or tissue[113, 179].The lengths of clean sRNAs ranged from 18 to 30 nt in both libraries,with the majority between 21 and 24 nt, the most common length is 21 nt in the 7 DAP library and 24 nt in the 14 DAP library (Fig. 3-2).In the two libraries, the length distribution of clean reads and miRNA is different, the distribution of sRNA length was respectively 61% and 73.89% between 20 ~ 24 nt, and the percentage of sRNA with 24 nt sequence (7 DAP was 26.79%, 14 DAP was 40.42%) was higher than other sequences, It shows that the most abundant sRNA is 24nt in length . In the 7 DAP library, the sRNA with 23 nt is second only to the 24 nt sRNA sequence, and the sRNA with 21 nt in the 14 DAP library (Table 3-4).

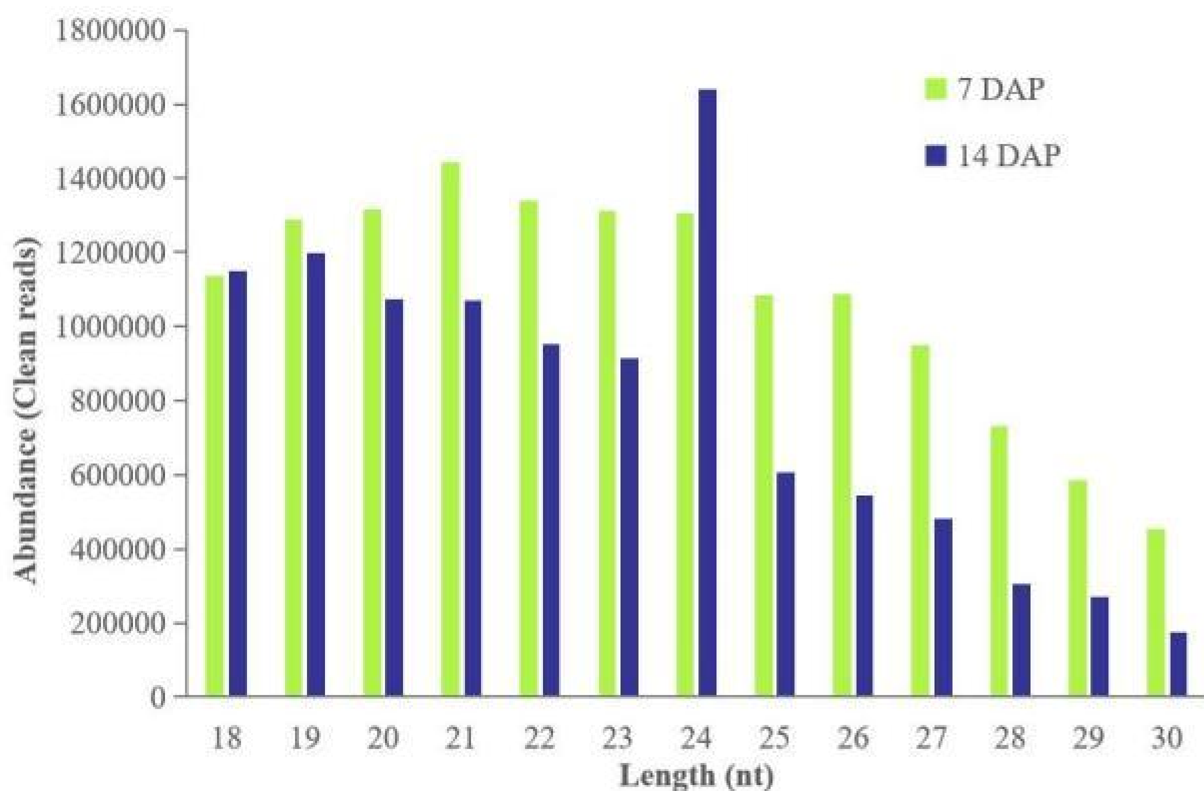

Fig 3-2 Length distributions of sRNAs in developing grains at 7 DAP and 14 DAP

Table 3-4 Summary of the length distribution of clean reads and miRNA by sequencing

Length	7 DAP		14 DAP	
	Clean(Percentage)	miRNA(Percentage)	Clean(Percentage)	miRNA(Percen
18	1135719(8.1)	19992(8.29)	1150609(11.09)	99454(6.68
19	1287699(9.18)	17843(7.4)	1196506(11.53)	99200(6.67
20	1313438(9.37)	19423(8.05)	1073720(10.35)	120417(8.09
21	1443091(10.29)	20704(8.59)	1068414(10.29)	142147(9.55
22	1337552(9.54)	18644(7.73)	952395(9.18)	117794(7.91)
23	1312859(9.36)	23733(9.84)	914729(8.81)	117916(7.92

24	1304959(9.31)	64608(26.79)	1641383(15.81)	601637(40.4
25	1083137(7.73)	14878(6.17)	605539(5.83)	61294(4.12
26	1086635(7.75)	11984(4.97)	543080(5.23)	39789(2.67
27	948284(6.76)	9195(3.81)	481484(4.64)	29206(1.96
28	729991(5.21)	6959(2.89)	305218(2.94)	18636(1.25
29	584165(4.17)	7677(3.18)	270065(2.60)	24545(1.65
30	453155(3.23)	5497(2.28)	175862(1.69)	16316(1.10
Total	14020684(100.00)	241137(100.00)	10379004(100.00)	1488351(100.

To find out whether base preference exists for small RNAs, the distribution of bases at each position in known small RNAs was counted. The results showed that the first base at the 5' end of MiRNAs is most abundant in Aand least abundant in G. This is inconsistent with the reported confirmed miRNA-specific sequences where the first base at the 5' end is most abundant in U and least abundant in G [180] (Fig S1). Deep sequencing of miRNA was analyzed with the miRDeep2 software v2.0.5[181], and the precursor miRNA sequences with hairpin-structure and the fold RNA structures of every candidate miRNA were listed in Table S1 and Fig S2.

3.4.3 Identification of known miRNAs and predicted novel miRNAs

To identify known miRNAs, we compared the unannotated sRNA sequences that match perfectly to the reference genome in miRBase 22.0 (http://www.mirbase.org) based on perfect match criterion. A total of 89

known miRNAs from wheat were detected in miRBase/*Triticumaestivum* in the two sRNA libraries, of which 46 were expressed in 7 DAP library and 87 were expressed in 14 DAP library (Table 3-5). Of the known miRNAs from other plant species, their secondary structure and miRNAs* were predicted based on RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi), and these miRNAs were predicted using MIREAP (http://sourceforge.net/projects/mireap/) by analyzing DCL1 cleavage sites and the minimum free energy. A total of 16 known miRNAs from other plant species were identified in the present two sRNA libraries, including 13 expressed in 7 DAP library and 16 expressed in 14 DAP library (Table3-5; S2 Table).

Table 3-5 The number of known miRNAs, novel miRNAs and targets identified from 7 DAP and 14 DAP grains

Library	Known miRNAs	Known other miRNAs	Novel miRNAs	Total
7 DAP	46	13	32	91
14 DAP	87	16	78	181
Total miRNAs	89	16	79	184
MiRNAs with target	41	13	25	79
Target genes	266	152	258	676

Regarding the expression of the known miRNA, including ata-miR9863a-3p, osa-miR396e-5p, tae-miR9670-3p and tae-miR7757-5p were most abundantly expressed, while some miRNAs other known miRNAs, such as tae-miR9672b, tae-miR9662a-3p, tae-miR167c-5p, tae-

miR156, tae-miR9777 and tae-miR9669-5p were moderately abundant (Fig.3-3). The majority of the abundantly expressed miRNAs were known miRNAs from the miRBase of wheat and conserved between plant species, such as miR156, and miR396e-5p (Table S2).

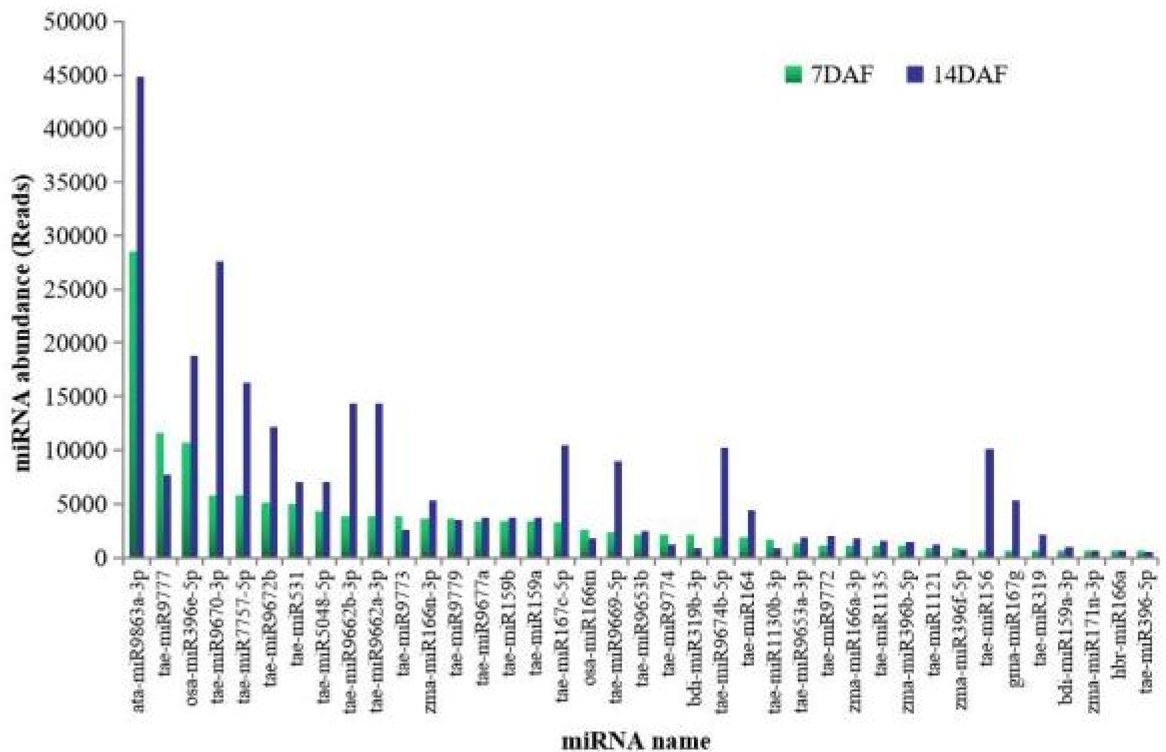

Fig. 3-3 Most abundantly expressed known miRNAs in 7 DAP and 14 DAP grains

Horizontal axis: The most abundant known miRNAs among all identified known miRNAs; Vertical axis: The reads abundances corresponding to miRNAs.

To predict novel miRNAs, the unannotated sRNAs were analyzed on the basis of pre-miRNA sequences that can form a canonical stem-loop hairpin structure [182]. A total of 79 novel miRNAs were predicted, of which 32 were expressed in 7 DAP library and 78 were expressed in 14 DAP library (Table 3-6). The minimal free energy of pre-miRNAs ranges

from -187.00 kcal mol^{-1} to -37.40 kcal mol^{-1}, with an average of about -96.83 kcal mol^{-1}. This indicates the high stability of the hairpin structures.

More novel miRNAs were expressed in 14 DAP than in 7 DAP, and 31 novel miRNAs were commonly expressed in two libraries, with 1 special in 7 DAP library and 47 specials in 14 DAP library. The majority of novel miRNAs presented a relatively low expression level. The most abundant novel miRNAs were novel-m0064_5p, novel-m0492_5p and novel-m0661_5p (Table S3).

Table 3-6 Summary of miRNA and target gene number from 7 DAP and 14 DAP

Types	All_miRNA	miRNA_with_Target	Target_gene
Known_miRNA	89	40	266
Known other_miRNA	16	13	152
Novel_miRNA	79	25	258
Total	184	78	676

3.4.4 Differentially expressed miRNAs between 7 DAP and 14 DAP grains

To identify miRNAs associated with early wheat grain development, the differentially expressed miRNAs were analyzed baesdon the statistical method reported by Audic and Claverie[183] . A total of 19 known and 20 novel miRNAs were differentially expressed ($r < 0.05$) between the 7 DAP and 14 DAP grains, among which 18 known miRNAs and 13 novel miRNAs were up-regulated in 14 DAP grains (S4 Table).

To verify the miRNA expression levels and the deep-sequencing results,

eight known and two novel miRNAs were randomly for quantitative real-time polymerase chain reaction (qRT-PCR). The results show that the expression patterns of these miRNAs are consistent with those from deep-sequencing,which indicate that oursRNA sequencing is reliable (Fig. 3-4).

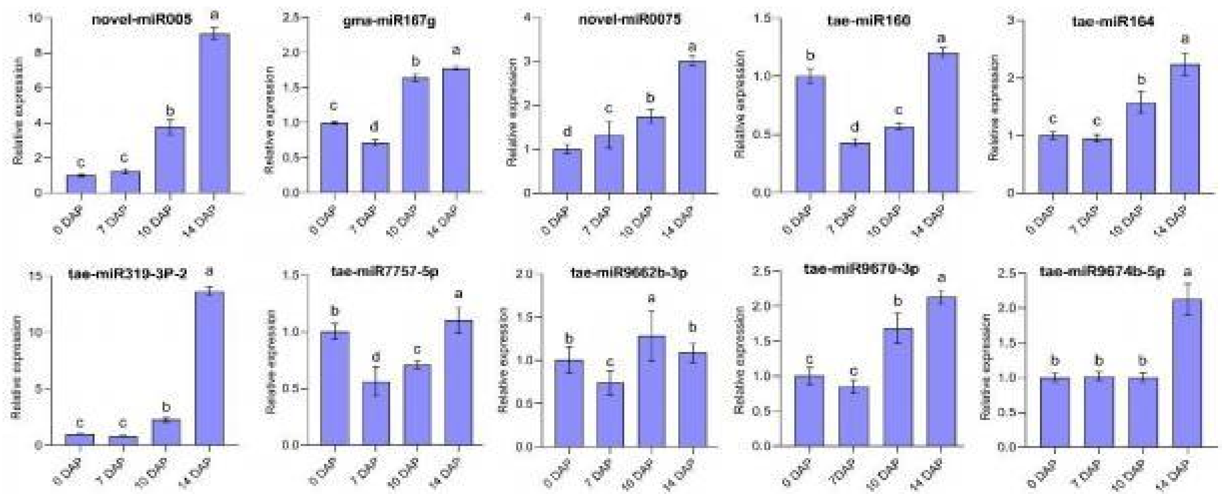

Fig. 3-4 Validating of partial differentially expressed miRNAs by qRT-PCR

Note: MiRNA expression data were normalized to wheat U6 small nuclear RNA gene [GenBank: X63066]. Experiments were performed in three biological replicates. Error bars represent one standard deviation (SD). Individual miRNA value is presented as fold-change (mean ± SD) compared to 0 DAP value set to 1.0. Horizontal axis: The stages of developmental grains; Vertical axis: The expression levels of randomly selected miRNAs (2-ΔΔct values).

3.4.5 Putative target genes involved in seed development

Plant miRNAs with their target genes have a nearly perfect pairing, and function through cleaving targets directly or, in some cases, by

translational repression. Therefore, identifying miRNA target genes is the key to understand their functions [183, 184]. Potential targets of miRNAs were computationally predicted using Target Finder software v1.6 script[185]. Starting with 184 total miRNA sequences, a set of 810 potential targets for 25 novel miRNAs, 13 conserved miRNAs and 40 known miRNAs were predicted (Table 3-6, Table S5 and S6). Among 88 DE miRNAs, 477 potential targets for 147 miRNAs were identified (S7 Table). The detailed sequence information of newly identified targets for all miRNAs and DE miRNAs arelisted in S5–S7 Tables.

Table 3-7 Summary of annotated all and differentially expressed miRNA targets

Annotated database	all miRNA targets	DE miRNA targets
COG	174	84
GO	605	341
KEGG	176	112
Swiss-Prot	632	365
NR	810	477
All	810	477

Since plant miRNAs usually have a strong favor for their target genes with important functions [186], the predicted target sequences of all miRNAs and DE miRNAs were subjected to blast search with GO, COG, KEGG, Swiss-Prot and NR databases, 810 targets from total miRNAs and 477 targets from DE miRNAs could successfully obtain annotated functional information (Table3-7, Fig 3-5 and S8–S9 Tables). Among all 810 miRNA targets, 174 (21.48%) could be annotated into COG database, 605 (74.69%)

into GO database, and 176 (21.73%) into KEGG database. However, for a total of 477 DE miRNA targets, only 84 obtained COG functional annotation. These targets were both found to be involved in general function prediction only and general function prediction only (17.46 %), replication, recombination and repair(11.9%), transcription and signal transduction mechanisms(8.73%), posttranslational modification, protein turnover, chaperones(5.56%), Cell wall/membrane/envelope biogenesis(4.76%),Translation, ribosomal structure and biogenesis(3.97%),Defense mechanisms, Amino acid transport and metabolism and Nucleotide transport and metabolism (3.17%),Lipid transport and metabolism(2.38%),Intracellular trafficking, secretion, and vesicular transport, Inorganic ion transport and metabolism and Secondary metabolites biosynthesis, transport and catabolism (1.59%), RNA processing and modification, Cell cycle control, cell division,chromosome partitioning and Energy production and conversion (0.79%) (Fig.3-6).In GO biological process enrichment analysis,84 DE miRNA targets were classified into cellular component (47.16%),molecular function (20.85%), and biological process (31.99%) (Fig. 3-6). In KEGG database, for 13 DE miRNA targets, each could be involved into different pathways including Starch and sucrose metabolism, Protein processing in endoplasmic reticulum, Porphyrin and chlorophyll metabolism, Purine metabolism, Cysteine and methionine metabolism, Histidine metabolism, mRNA surveillance pathway, Photosynthesis, Phenylpropanoid biosynthesis, Phenylalanine metabolism, RNA transport, Ribosome biogenesis in eukaryotes and Spliceosome (S10 Table and S3 Fig). The detailed annotation of each miRNA target is shown in S8 and S9 Tables. From annotation information above, it could indicate that these targets and their corresponding microribonucleic acids may play an important role in the

growth and development of wheat.

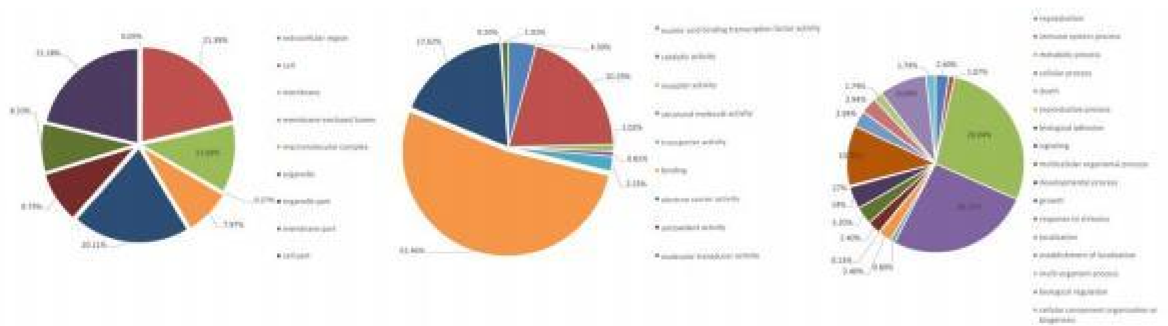

Fig.3-5 The major GO categories of“cellular component”, “biological process”, and “molecular function ” for the predicted genes of all the differentially expressed miRNAs during grain development

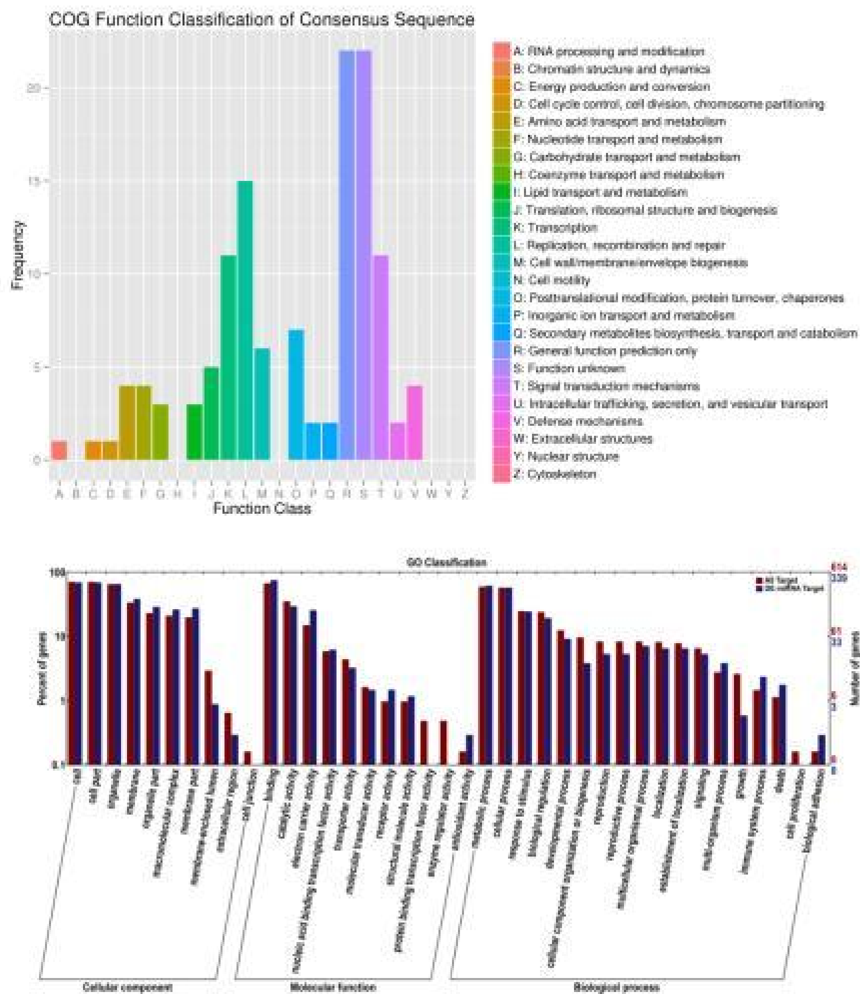

Fig.3-6 The COG function classification of consensus sequence and GO classification of all the differentially expressed miRNAs during grain

development

3.4.6 Degradome sequencing and data summary

After RNA was extracted and poly (A+) RNA was purified from the developing wheat grains at 7 DAP and 14 DAP, a degradome library was constructed and then subjected to degradome sequencing. A total of 10,620,205 clean reads including 4,612,965 unique reads were obtained (Table 3-8). A total of 2,776,485 (60.19%) unique reads were matched to the reference genome. Using BLASTN search against GenBank and Rfam databases, the structural RNAs (rRNAs, tRNAs, snRNAs, and snoRNAs) were removed, and the remaining reads were used to detect candidate targets of miRNAs.

Degradome analysis could provide experimental evidence for miRNA-mediated cleavage of target transcripts, so the target genes of miRNAs were analyzed by degradome sequencing. The results show that 23 predicted targets are cleaved by 7 miRNAs, including 3 known and 4 novel miRNAs. The 3 known miRNAs are tae-miR156, tae-miR160 and tae-miR1119, and the 4 novel miRNAs are novel-miR0011, novel-miR0012, novel-miR0036 and novel-miR0075 (Table 3-9, Table S11). Furthermore, most single miRNAs potentially regulate multiple targets, whereas some single miRNAs only acton one target gene.Tae-miR160 targets five genes including

Traes_ 1AL_ 147CF243C,Traes_ 1BL_54CD82AC3,Traes_7AL_E3ADC8C38,Traes_7BL_18D335F08 and Traes_7DL_55ADB3528; tae-miR1119 has three targets including Traes_6BS_04A3400AF, Traes_6BS_222CE7DA7 and Traes_6BS_4D03398A8; novel-miR0012 targets Traes_3DS_2F5F2C276, Traes_3B_8824DBB56 and Traes_3AS_7EEF1386F, and novel-miR0011 targets Traes_3DS_C6D17D438 and Traes_3B_E7D2E8720. Novel-miR0036,

novel-miR0075 and tae-miR156 target Traes_7AS_2084DE83B, Traes_5AL_147EA9565 and Traes_6BS_542961EA4, respectively. Of the 16 targets, 13 have functional annotations (Table 3-7, Fig S4).

Table 3-8 Sequencing data of degradome library derived from 7DAP and 14 DAP grains

Types	Number	Percent (%)
Clean number	10,620,205	100.00
Unique number	4,612,965	43.44

Table 3-9 miRNAs and targets identified by degradome sequencing

MiRNA name	Target genes
tae-miR160	Traes_1AL_147CF243C
tae-miR160	Traes_1BL_54CD82AC3
tae-miR160	Traes_7AL_E3ADC8C38
tae-miR160	Traes_7BL_18D335F08
tae-miR160	Traes_7DL_55ADB3528
tae-miR156	Traes_6BS_542961EA4
tae-miR1119	Traes_6BS_4D03398A8
tae-miR1119	Traes_6BS_222CE7DA7
tae-miR1119	Traes_6BS_04A3400AF
novel-miR0011	Traes_3B_E7D2E8720
novel-miR0011	Traes_3DS_C6D17D438
novel-miR0012	Traes_3DS_2F5F2C276
novel-miR0012	Traes_3B_8824DBB56
novel-miR0012	Traes_3AS_7EEF1386F
novel-miR0036	Traes_7AS_2084DE83B
novel-miR0075	Traes_5AL_147EA9565

3.4.7 Functions and expressions of miRNA targets

To understand the potential functions of miRNAs in the regulatory network of early wheat grain development, miRNA target genes were analyzed across small RNA and degradome libraries. A total of 266 targets for 40 known wheat miRNAs, 152 targets for 13 other known plant miRNAs and 258 targets for 25 novel miRNAs were predicted (Table S12).

Functional annotations for these target genes of differentially expressed miRNA were performed by BLAST analysis, and it was found that the targets included those genes encoding mitogen-activated protein kinase, the transcription factor GAMYB, WRKY transcription factor 33, F-box/kelch-repeat protein, the transcription factor PCF6, NAC domain-containing protein, ethylene-responsive transcription factor, SPX domain-containing protein, vegetative cell wall protein gp1, extensin precursor, cytokinin dehydrogenase 5, calcium-dependent protein kinase and squamosa promoter-binding-like protein (SPL), etc. (Table S13).

Further GO analysis revealed that some of the targets are involved in various biological processes such as mitotic cell cycle (GO:0000278), nuclear division (GO:0000280), cell morphogenesis (GO:0000902), seed development (GO:0048316), embryo development (GO:0009790) (4 targets for 2 miRNAs), meristem initiation (GO:0010014), carpel development (GO:0048440), cell differentiation (GO:0030154), cell division (GO:0051301), starch metabolic process (GO:0005982) and regulation of cell division (GO:0051302). These results imply that these

miRNAs may play important role in regulating grain development.

Then, GO analysis of the differentially expressed miRNAs between 7 DAP and 14 DAP grains was conducted to classify their target genes according to their cellular components, molecular function, and biological processes. A total of 10 molecular functions were identified, of which binding, catalytic activity, and nucleic acid transcription factor activity are the three most frequent functions. For biological processes, 20 categories were identified, of which cellular processes, metabolic processes and single organism processes are the three most frequent ones. For the cellular component, 11 categories were identified, of which cell part, cell and organelle were the three most frequent processes (Table S13, Fig. 3-7).

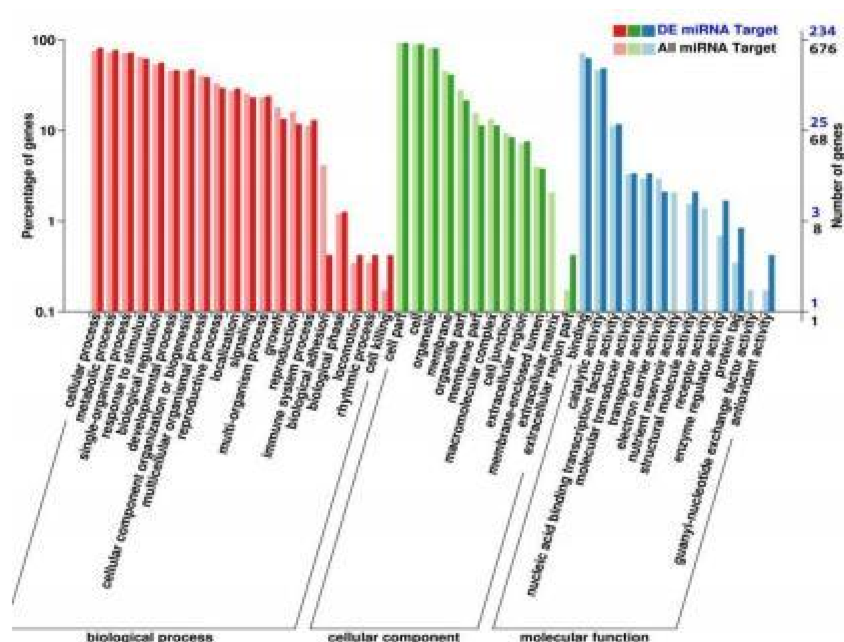

Fig. 3-7 GO analysis of the differentially expressed miRNAs targets between 7 DAP and 14 DAP grains

Furthermore, gene-specific qRT-PCR was check to validate the expression levels of potential targets. The 8 target genes of 8 miRNAs (five known and three novel) were selected randomly (Fig. 3-8). Combined with

the results from Fig. 3-4, the expression levels can be compared between five miRNAs (tae-miR160, tae-miR164, tae-miR7757-5p, tae-miR9662b-3p and tae-miR9674b-5p) and their targets. The compared results show that, at 7 DAP and 14 DAP, the expression of tae-miR160 and tae-miR164 are negatively correlated with the expression of their target genes, positively correlated for tae-miR9662b-3p and tae-miR9674b-5p and their targets, no clear correlation for tae-miR7757-5p and its target. It is likely that there is a complicated temporal and spatial regulation between miRNAs and their targets.

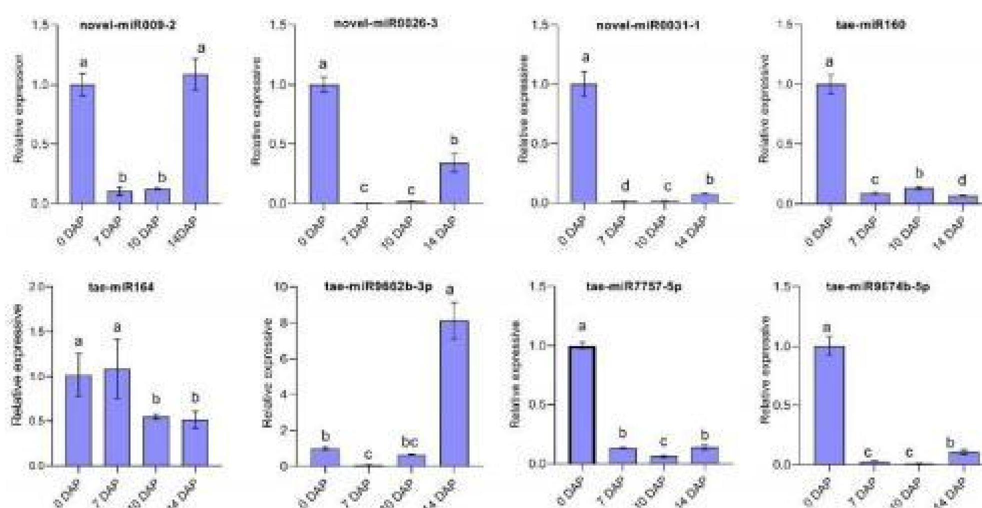

Fig 3-8 Confirmation of the expression patterns of partial miRNA targets by qRT-PCR

Note:Targets expression data were normalized to the actin gene (GenBank: AB181991). Experiments were repeated in triplicate. Error bars represent one standard deviation (SD). Individual target value is presented as fold-change (mean ±SD) compared to 0 DAP value set to 1.0. Horizontal axis: The stages of developmental grains; Vertical axis: The expression levels of randomly selected miRNA targets ($2^{-\Delta\Delta ct}$ values).

3.4.8 Conclusion and Discussion

This study suggests that quite a few known and novel miRNAs and their targets play extensive roles during the early development of common wheat grain. Understandings of the functions of novel-miRNA and their targets and the miRNA-mediated regulatory networks involved in wheat grain development, will help us to elucidate the molecular mechanisms underlying wheat grain development and carry out ingenious molecular improvements in wheat breeding.

It is known that miRNAs are involved in diverse developmental processes. Previous studies have shown that miRNAs were associated with development and stress response in wheat. Here, we profiled the miRNAs and their targets with the attempt to establish the potential association between miRNAs and early grain development of wheat, one of the most important crops for human foods.

In this study, 105 known and 79 novel miRNAs were identified from two libraries of grains at 7 DAP and 14 DAP. Expression analysis has shown that 19 known and 20 novel miRNAs were differentially expressed, and 18 known and 13 novel miRNAs were up-regulated at 14 DAP. During rice grain development, most miRNAs show increased expression from 5 to 10 DAP[187]. A few studies have been reported in wheat. Most of the differentially expressed miRNAs were up-regulated with grain and leaf development; and only small portion were down-regulated[116, 188] . These results suggest that most miRNAs are highly expressed during organ development in crops.

Of the differentially expressed known miRNAs, miR156 was up-regulated at 14 DAP compared to 7 DAP in the present study. Previous research has shown that miR156 directly represses the expression of SPL

transcription factor genes that play an important role in plant growth and development [189]. In rice, OsSPL16 controls grain size, shape and quality [186], and OsSPL13 positively regulates the cell size of grain hull and also controls grain size[190]. *TaSPL16*, a target of miR156 in wheat, is highly expressed in developing young panicles, and lowly expressed in developing grains, due to the high expression level of miR156[191]. Therefore, miR156 may as well play an important role in regulating wheat grain development.

It has been reported that miR164 targets the genes encoding for NAC (*NAM, ATAF,* and *CUC*) transcription factors that play major roles in the proper formation and separation of plant organs [192], auxin signaling and defense [193]. Auxin is crucial for pattern formation, cell division and cell expansion during the grain/seed development [194]. In wheat grains, NAC genes regulating senescence have the functions to improve protein, zinc and iron contents[195]. In this study, miR164 is up-regulated from 7 DAP to 14 DAP grains. It was also shown that miR164 was increased in abundance from 5 DAP to 20 DAP [188]. Furthermore, NAC transcription factors were predicted to be involved in regulating the timing of organ formation (GO:0048504), cell division and cell size, in response to temperature stimulus and light stimulus (Table S13). These results imply that miR164 takes part in the regulation of wheat grain development.

It has been shown that miR319 (bdi-miR319b-3p, tae-miR319) targets TCP (TEOSINTE BRANCHED 1, CYCLOIDEA and PCF) transcription factor genes in *Arabidopsis thaliana* [196]. This report has also shown that TCP 3 regulates the activity of miR164 during the differentiation of Arabidopsis leaves. In our study, we predicted that the targets of miR319 are involved in the regulation of diverse processes, including cell proliferation and differentiation, ovule development (GO:0048481),

mucilage biosynthesis involved in seed coat development, response to auxin and abscisic acid, and jasmonic acid-mediated and ethylene-activated signaling pathways (Table S13). A putative miRNA regulatory network in wheat grains showed that miR156, miR164, miR319 and miR396 play a role in cell proliferation [116]. In the present study, however, miR396 showed no difference during the early development of wheat grains.

MiR167 targets ARF (AUXIN RESPONSE FACTOR) transcription factor genes, which play critical roles in regulating plant growth and development[197] , ARF8 is a negative regulator of fruit initiation in Arabidopsis [198]. In this study, miR167 is up-regulated from 7 DAP to 14 DAP. In previous research, it was up-regulated from 5 DAP to 15 DAP [114], 7DAP to 14 DAP and down-regulated from 14 DAP to 28 DAP in wheat grain[116] . These results have shown that miR167 is important in wheat grain development. Research showed that ARF members are targeted by miR164, whereas many TFs such as TCP members are regulators of miR167[199] , indicating a mutual regulation between TFs and miRNAs.

The known miRNA tae-miR7757-5p targets the genes encoding WRKY DNA-binding domain and NB-ARC domain-containing proteins, which are involved in the regulation jasmonic acid-mediated signaling pathway, plant-type hypersensitive and hydrogen peroxide responses, and multi-organism process (Table S13). MiR9662 (tae-miR9662a-3p, tae-miR9662b-3p) targets the genes related to vegetative cell wall protein

synthesis, protein kinase activity (GO:0004672), protein phosphorylation (GO:0006468), plant-type cell wall organization (GO:0009664), multicellular organismal process (GO:0032501), and regulation of cellular process (GO:0050794). These show the extensive effects of miRNAs in early wheat grain development and might participate in regulating wheat grain development and metabolism.

The novel-miR0018 targets WRKY transcription factor genes. Previous research postulated that WRKY23 participates in the regulation of plant stem cell specification via auxin-dependent or auxin-independent signaling pathway [200]. WRKY23 could regulat auxin distribution patterns through controlling flavonol biosynthesis during Arabidopsis root development. [201]Perhaps novel-miR0018 plays important roles during the development of early wheat grain through its regulation of WRKY expression.

The novel-miR0019 targets MADS-box transcription factor genes. The MADS-domain regulator AGAMOUS-like 15 (AGL15) could enhance somatic embryo development in Arabidopsis and soybean when ectopically expressed [202], and AGL15 was accumulated to its highest amount during embryo development[203]. Meanwhile, we found that novel-miR0019 targets the genes related to cytokinin biosynthetic process, plant-type cell wall modification, post-embryonic morphogenesis, organ morphogenesis (GO:0009887), seed development (GO:0048316), ovule

development (GO:0048481) and meristem development (GO:0048507).

The novel-miR0036 was significantly down-regulated at 14 DAP compared to 7 DAP (Table S14), and it was predicted to target DHHC palmitoyl transferase gene. DHHC proteins regulats cell function and influences cell physiology and pathophysiology [204], indicating that novel-miR0036 plays an important role during wheat grain development. Other novel miRNAs target the genes that are involved in lipid transport and metabolism (novel miR0005), signal transduction mechanisms (novel miR0079), post-translational modification (novel miR0078), chromatin structure and carbohydrate transport (novel miR0034, novel miR0070), ribosomal structure and biogenesis (novel miR0075), replication, recombination and repair (novel miR0031) (Table S13). Perhaps these novel miRNAs widely participate in the regulations of early wheat grain development and metabolism, which need to be tested by genome editing technology in the future.

PROPOSALS FOR BREEDING

1. The aim of this project is to clone the major genes controlling the number of grains per spike in the multi-grain type "Mexican megaclus", and on this basis, to study the molecular regulatory pathways of the major genes causing the number of grains per spike, which will facilitate further research on the relevant mechanisms and regulatory networks, and provide theoretical guidance for future wheat germplasm innovation of multi-grain per spike and high-yield wheat breeding.

2. After cloning the target gene and verifying its function, combined with the vernalization chamber, artificial climate chamber, daylight intelligent greenhouse and large refrigeration greenhouse and other supporting facilities, it can ensure that at least three generations of wheat can be bred every year (12 months), which provides a guarantee for the addition of relevant experimental materials and the rapid construction of near-isogenic lines.

REFERENCE

1. Heun M, SchaferPregl R,KlawanD, Castagna R, AccerbiM, Borghi B, Salamini F: Site of einkorn wheat domestication identified by DNA fingerprinting. *Science* 1997, 278(5341):1312-1314.
2. Faris JD, Zhang ZC, Garvin DF, Xu SS: Molecular and comparative mapping of genes governing spike compactness from wild emmer wheat. *Mol Genet Genomics* 2014, 289(4):641-651.
3. Liu T, Wu LJ, Gan XL, Chen WJ, Liu BL, Fedak G, Cao WG, Chi D, Liu DC, Zhang HG *et al*: Mapping Quantitative Trait Loci for 1000-Grain Weight in a Double Haploid Population of Common Wheat. *IntJMol Sci* 2020, 21(11).
4. Yao HN, Xie Q, Xue SL, Luo J, Lu JK, Kong ZX, Wang YP, Zhai WL, LuN, Wei R *et al*: HL2 on chromosome 7D ofwheat (Triticum aestivum L.) regulates both head length and spikelet number. *Theor Appl Genet* 2019, 132(6):1789-1797.
5. Alexandratos N, Bruinsma J: World agriculture towards 2030/2050. *Esa Working Papers* 2012.
6. Griffiths S, Wingen L, Pietragalla J, Garcia G, Hasan A, Miralles D, Calderini DF, Ankleshwaria JB, Waite ML, Simmonds J *et al*: Genetic Dissection of Grain Size and Grain Number Trade-Offs in CIMMYT Wheat Germplasm. *Plos One* 2015, 10(3).

7. Simmonds J, Scott P, Leverington-Waite M, Turner AS, Brinton J, Korzun V, Snape J, Uauy C: Identification and independent validation of a stable yield and thousand grain weight QTL on chromosome 6A of hexaploid wheat (Triticum aestivum L.). *Bmc Plant Biol* 2014, 14.

8. Dolferus R, Ji XM, Richards RA: Abiotic stress and control of grain number in cereals. *Plant Sci* 2011, 181(4):331-341.

9. Powell N, Ji XM, RavashR, Edlington J, Dolferus R: Yield stability for cereals in a changing climate. *Funct Plant Biol* 2012, 39(7):539-552.

10. Guo ZF, Schnurbusch T: Variation of floret fertility in hexaploid wheat revealed by tiller removal. *JExp Bot* 2015, 66(19):5945-5958.

11. Kearsey MJ, Pooni HS: The Genetical Analysis of Quantitative Traits: The Genetical Analysis of Quantitative Traits; 1996.

12. Yang W, Wang L, Zhang G, Ma Z: Advances on Studies of Molecular Makers of Wheat QTLs. *Journal of Hebei Agricultural Sciences* 2002.

13. Mayer KFX, Rogers J, Dolezel J, Pozniak C, Eversole K, FeuilletC, Gill B,Friebe B, LukaszewskiAJ, Sourdille P *etal*: A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. *Science* 2014, 345(6194).

14. Somers DJ, Isaac P, Edwards K: A high-density microsatellite

consensus map for bread wheat (Triticumaestivum L.). *Theor Appl Genet* 2004, 109(6):1105-1114.

15. Liu YN, He ZH, Appels R, Xia XC: Functional markers in wheat: current status and future prospects. *Theor Appl Genet* 2012, 125(1):1-10.
16. Rasheed A, Xia XC: From markers to genome-based breeding in wheat. *Theor Appl Genet* 2019, 132(3):767-784.
17. Zhang Z, Chen ZH, Hou YP, Duan YB, Wang JX, Zhou MG, Chen CJ: PIRA-PCR for Detection ofFusarium fujikuroi Genotypes with Carbendazim-Resistance Alleles. *Plant Dis* 2015, 99(9):1241-1246.
18. Metakovsky E, Pascual L, Vaccino P, Melnik V, Rodriguez-Quijano M, Popovych Y, Chebotar S, Rogers WJ: Heteroalleles in Common Wheat: Multiple Differences between Allelic Variants of the Gli-B1 Locus. *IntJ Mol Sci* 2021, 22(4).
19. Vieira MLC, Santini L, Diniz AL, Munhoz CD: Microsatellite markers: what they mean and why they are so useful. *Genet Mol Biol* 2016, 39(3):312-328.
20. Rasheed A, Hao YF, Xia XC, Khan A, Xu YB, Varshney RK, He ZH: Crop Breeding Chips and Genotyping Platforms: Progress, Challenges, and Perspectives. *Mol Plant* 2017, 10(8):1047-1064.
21. Singh AK, Chaurasia S, Kumar S, Singh R, Kumari J, Yadav MC, Singh N, Gaba S, Jacob SR: Identification, analysis and

development of salt responsive candidate gene based SSR markers in wheat. *Bmc Plant Biol* 2018, 18.

22. Cmejlova J, Rejlova M, Paprstein F, Cmejla R: A new one-tube reaction kit for the SSR genotyping of apple (Malus × domestica Borkh.). *Plant Sci* 2021, 303:110768.
23. Cavanagh CR, Chao SM, Wang SC, Huang BE, Stephen S, Kiani S, Forrest K, Saintenac C, Brown-Guedira GL, Akhunova A *et al*: Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences of the United States ofAmerica* 2013, 110(20):8057-8062.
24. Wang S, Wong D, Forrest K, Allen A, Akhunov E: Characterization of polyploid wheat genomic diversity using the high-density 90,000 SNP array. Plant Biotech J. *Plant Biotechnology Journal* 2014, 12(6).
25. Boeven PHG, Longin CFH, Leiser WL, Kollers S, Ebmeyer E, Wurschum T: Genetic architecture of male floral traits required for hybrid wheat breeding. *Theor Appl Genet* 2016, 129(12):2343-2357.
26. Winfield MO, Allen AM, Burridge AJ, Barker GLA, Edwards KJ: High‐density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. *Plant Biotechnology Journal* 2016,
14(5).

27. Allen AM, Winfield MO, Burridge AJ, Downie RC, Edwards KJ:

Characterization of a Wheat Breeders' Array suitable for high‐ throughput SNP genotyping of global accessions of hexaploid bread wheat (Triticum aestivum). *Plant Biotechnology Journal* 2017, 15(3):390-401.

28. Sun C, Dong Z, Zhao L, RenY, Zhang N, Chen F: The Wheat 660K SNP array demonstrates great potential for marker‐assisted selection in polyploid wheat. *Plant Biotechnology Journal* 2020, 18(6).

29. Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD: Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 1988, 335(6192):721.

30. Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J: Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Tagtheoretical & Applied Geneticstheoretische Und Angewandte Genetik* 1986, 72(6):761-769.

31. Miller JC, Tanksley SD: Effect of different restriction enzymes, probe source, and probe length on detecting restriction fragment length polymorphism in tomato. *Tagtheoretical & Applied Geneticstheoretische UndAngewandte Genetik* 1990, 80(3):385-389.

32. Li JX, Yu SB, Xu CG, TanYF, Gao YJ, Li XH, Zhang Q: Analyzing quantitative trait loci for yield using a vegetatively replicated F-2

population from a cross between the parents of an elite rice hybrid. *Theor Appl Genet* 2000, 101(1-2):248-254.

33. Brown J, Schnell R, Motamayor J, Lopes U, Kuhn D: Resistance Gene Mapping for Witches' Broom Disease in Theobroma cacao L. in an F2 Population using SSR Markers and Candidate Genes. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet* 2005, 87(87):519-524.

34. Xiong LZ, Liu KD, Dai XK, Xu CG, Zhang Q: Identification of genetic factors controlling domestication-related traits of rice using an F2 population of across between Oryza sativa and O. rufipogon. *Theoretical & Applied Genetics* 1999.

35. Mapping quantitative trait loci (QTL) for grain size in rice using a RIL population from Basmati× indica cross showing high segregation distortion. *Euphytica* 2013, 194(3):401-416.

36. Wang L, Cheng J, Lai Y, Du W, Huang X, Wang Z, Zhang H: Identification of QTLs with additive, epistatic and QTL×development interaction effects for seed dormancy in rice. *Planta* 2014, 239(2):411-420.

37. Williams K, Sorrells ME: Three-Dimensional Seed Size and Shape QTL in Hexaploid Wheat (Triticum aestivum L.) Populations. *Crop Sci* 2014, 54(1):98-110.

38. Bai CH, Liang YL, Hawkesford MJ: Identification of QTLs

associated with seedling root traits and their correlation with plant height in wheat. *J Exp Bot* 2013, 64(6):1745-1753.

39. Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T: Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. *Theor Appl Genet* 1997, 95(7):1025-1032.

40. Cavanagh C, Morell M, Mackay I, Powell W: From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Curr Opin Plant Biol* 2008, 11(2):215-221.

41. Yu JM, Holland JB, McMullen MD, Buckler ES: Genetic design and statistical power of nested association mapping in maize. *Genetics* 2008, 178(1):539-551.

42. Keightley PD, Bulfield G: Detection of Quantitative Trait Loci from Frequency Changes of Marker Alleles under Selection. *Genet Res* 1993, 62(3):195-203.

43. Michelmore RW, Kesseli I: Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences of the United States of America* 1991.

44. Mohan M, Nair S, Bentur JS, Rao UP, Bennett J: Rflp and Rapd Mapping of the Rice Gm2 Gene That Confers Resistance To

Biotype- 1 of Gall Midge (Orseolia Oryzae). *Theor Appl Genet* 1994, 87(7):782-788.

45. SollerM, Beckmann JS: Marker-based mapping of quantitative trait loci using replicated progenies. *Theoretical & Applied Genetics* 1990, 80(2):205-208.

46. Edwards M, Stuber CW, Wendel JF: Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. *Genetics* 1987, 116(1):113-125.

47. Tanksley SD, Hewitt J: Use of molecular markers in breeding for soluble solids content in tomato — a re-examination. *Theor Appl Genet* 1988, 75(5):811-823.

48. Weller JI, SollerM, Brody T: Linkage analysis of quantitative traits in an interspecific cross of tomato (lycopersicon esculentum x lycopersicon pimpinellifolium) by means of genetic markers. *Genetics* 1988, 118(2):329-339.

49. Lander E: Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 1989, 121.

50. Zeng ZB: Precision mapping of quantitative trait loci. *Genetics* 1994, 136(4):1457-1468.

51. 王建康: 数量性状基因的完备区间作图方法. *作物学报* 2009, 35(2):239-245.

52. Li H, Peter B, Elhan E, Buckler ES, Wang J, Daniel K: Joint QTL Linkage Mapping for Multiple-Cross Mating Design Sharing One Common Parent. *Plos One* 2011, 6(3):e17573.

53. 朱军: 数量性状遗传分析的新方法及其在育种中的应用. *浙江大学学报:农业与生命科学版* 2000.

54. Churchill GA, Doerge RW: Empirical Threshold Values for Quantitative Trait Mapping. *Genetics* 1994, 138(3):963-971.

55. Guo ZF, Chen DJ, Alqudah AM, Roder MS, Ganal MW, Schnurbusch T: Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. *New Phytol* 2017, 214(1):257-270.

56. Golan G, Ayalon I, Perry A, Zimran G, Ade-Ajayi T, Mosquna A, Distelfeld A, Peleg Z: GNI-A1 mediates trade-off between grain number and grain weight in tetraploid wheat. *Theor Appl Genet* 2019, 132(8):2353-2365.

57. Gao XQ, Wang N, Wang XL, Zhang XS: Architecture of Wheat Inflorescence: Insights from Rice. *Trends Plant Sci* 2019, 24(9):802-809.

58. Singh VK, Ellur RK, Singh AK, Nagarajan M, Singh BD, Singh NK: Effect of qGN4.1 QTL for Grain Number per Panicle in Genetic Backgrounds of Twelve Different Mega Varieties of Rice. *Rice* 2018, 11.

59. Li T, Deng GB, TangYY, Su Y, Wang JH, Cheng J, Yang Z, QiuXB, Pu X, Zhang HL*etal*: Identification and Validation of a Novel Locus Controlling Spikelet Number in Bread Wheat (TriticumaestivumL.). *Front Plant Sci* 2021, 12.

60. MaJ, Ding PY, Liu JJ, LiT, Zou YY, Habib A, MuY, Tang HP, Jiang QT, Liu YX *etal*: Identification and validation of a major and stably expressed QTL for spikelet number per spike in bread wheat. *Theor Appl Genet* 2019, 132(11):3155-3167.

61. Sakuma S, Golan G, Guo ZF, Ogawa T, Tagiri A, Sugimoto K, Bernhardt N, Brassac J, Mascher M, Hensel G *et al*: Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States ofAmerica* 2019, 116(11):5182-5187.

62. Voss-Fels KP, Keeble-GagnereG, Hickey LT, Tibbits J, NagornyyS, Hayden MJ, Pasam RK, Kant S, Friedt W, Snowdon R *et al*: High-resolution mapping of rachis nodes per rachis, a critical determinant of grain yield components in wheat. *Theor Appl Genet* 2019, 132(9):2707-2719.

63. Liu G, Jia LJ, Lu LH, Qin DD, Zhang JP, Guan PF, Ni ZF, Yao YY, Sun QX, Peng HR: Mapping QTLs of yield-related traits using RIL population derived from common wheat and Tibetan semi-wild wheat. *Theor Appl Genet* 2014, 127(11):2415-2432.

64. Guan PF, DiN, Mu Q, Shen XY, Wang YF, Wang XB, Yu KH, Song WJ, Chen YM,Xin MM *etal*: Use ofnear-isogeniclines to precisely map and validate a major QTL for grain weight on chromosome 4AL in bread wheat (Triticum aestivum L.). *Theor Appl Genet* 2019, 132(8):2367-2379.

65. Yang L, Zhao DH, Meng ZL, Xu KJ, Yan J, Xia XH, Cao SH, Tian YB, He ZH, Zhang Y: QTL mapping for grain yield-related traits in bread wheat via SNP-based selective genotyping. *Theor Appl Genet* 2020, 133(3):857-872.

66. Guan P, Shen X, Mu Q, Wang Y, Peng H: Dissection and validation of a QTL cluster linked to Rht-B1 locus controlling grain weight in common wheat (Triticum aestivum L.) using near-isogenic lines. *Theor Appl Genet* 2020(9):1-15.

67. Guan PF, Lu LH, Jia LJ, Kabir MR, Zhang JB, Lan TY, Zhao Y, Xin MM, Hu ZR, Yao YY *et al*: Global QTL Analysis Identifies Genomic Regions on Chromosomes 4A and 4B Harboring Stable Loci for Yield-Related Traits Across Different Environments in Wheat (Triticum aestivum L.). *Frontiers in Plant Science* 2018, 9.

68. Mikolajczak K, Ogrodowicz P, Gudys K, Krystkowiak K, Sawikowska A, Frohmberg W, Gorny A, KedzioraA, Jankowiak J, Jozefczyk D *et al*: Quantitative Trait Loci for Yield and Yield-Related Traits in Spring Barley Populations Derived from Crosses

between European and Syrian Cultivars. *Plos One* 2016, 11(5).

69. Bhusal N, SarialAK, Sharma P, Sareen S: Mapping QTLs for grain yield components in wheat under heat stress. *Plos One* 2017, 12(12).

70. Garcia M, Eckermann P, Haefele S, Satija S, Sznajder B, Timmins A, Baumann U, Wolters P, Mather DE, Fleury D: Genome-wide association mapping of grain yield in a diverse collection of spring wheat (Triticum aestivum L.) evaluated in southern Australia. *Plos One* 2019, 14(2).

71. Isham K, Wang R, Zhao WD, Wheeler J, Klassen N, Akhunov E, Chen JL: QTL mapping for grain yield and three yield components in a population derived from two high-yielding spring wheat cultivars. *Theor Appl Genet* 2021, 134(7):2079-2095.

72. Dura S, DuwayriM, Nachit M, Al SheyabF: Detection of molecular markers associated with yield and yield components in durum wheat (Triticum turgidum L. var. durum) under saline conditions. *Crop Pasture Sci* 2013, 64(10):957-964.

73. Dudley JW: Theory for Identification of Marker Locus-Qtl Associations in Population by Line Crosses. *Theor Appl Genet* 1992, 85(1):101-104.

74. Lee M: Comparative genetic and QTL mapping in sorghum and maize. *Symposia of the Society for Experimental Biology* 1996, 50:31.

75. Tanksley SD, Young ND, Paterson AH, Bonierbale MW: RFLP Mapping in Plant Breeding: New Tools for an Old Science. *Biotechnology* 1989, 7(3):257-264.

76. Zhao XR, Tan GQ, XingYX, Wei L, Chao Q, ZuoWL, Lubberstedt T, Xu ML: Marker-assisted introgression of qHSR1 to improve maize resistance to head smut. *Mol Breeding* 2012, 30(2):1077-1088.

77. Gupta HS, Agrawal PK, Mahajan V, Bisht GS, Kumar A, Verma P, Srivastava A, Saha S, BabuR, Pant MC *et al*: Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular marker assisted breeding. *Curr Sci India* 2009, 96(2):230-237.

78. Liu L, Du YF, Shen XM, Li MF, Sun W, Huang J, Liu ZJ, Tao YS, Zheng YL, Yan JB *et al*: KRN4 Controls Quantitative Variation in Maize Kernel Row Number. *Plos Genet* 2015, 11(11).

79. Nelson JC, Sorrells ME, Vandeynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA: Molecular Mapping of Wheat - Major Genes and Rearrangements in Homoeologous Group-4, Group-5, and Group-7. *Genetics* 1995, 141(2):721-731.

80. Li K, Debernardi JM, Li C, Lin H, Dubcovsky J: Interactions Between SQUAMOSA and SVP MADS-box Proteins Regulate Meristem Transitions During Wheat Spike Development. 2020.

81. Muellner AE, Buerstmayr M, Eshonkulov B, Hole D, Michel S,

Hagenguth JF, Pachler B, Pernold R, Buerstmayr H: Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. *Theor Appl Genet* 2021, 134(2):489-503.

82. Zhang CY, Anarjan MB, Win KT, Begum S, Lee SH: QTL-seq analysis of powdery mildew resistance in a Korean cucumber inbred line. *Theor Appl Genet* 2021, 134(2):435-451.

83. Tian BH, Zhang LX, Liu YL, Wu PP, Wang W, Zhang Y, Li HJ: Identification of QTL for resistance to leaf blast in foxtail millet by genome re-sequencing analysis. *Theor Appl Genet* 2021, 134(2):743-754.

84. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 2004, 116(2):281-297.

85. Mallory AC, Vaucheret H: Functions of microRNAs and related small RNAs in plants. *Nat Genet* 2006, 38:S31-S36.

86. Zhang BH, Pan XP, Cobb GP, Anderson TA: Plant microRNA: A small regulatory molecule with big impact. *Dev Biol* 2006, 289(1):3-16.

87. Shukla LI, Chinnusamy V, Sunkar R: The role of microRNAs and other endogenous small RNAs in plant stress responses. *Bba-Gene RegulMech* 2008, 1779(11):743-748.

88. Lee RC, Feinbaum RL, Ambros V: The C-Elegans Heterochronic

Gene Lin-4 Encodes Small Rnas with Antisense Complementarity To Lin-14. *Cell* 1993, 75(5):843-854.

89. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G: The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 2000, 403(6772):901-906.

90. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P *et al*: Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 2000, 408(6808):86-89.

91. Lagos-Quintana, M.: Identification of Novel Genes Coding for Small Expressed RNAs. *Science* 2001, 294(5543):853-858.

92. Lau NC, Lim LP, Weinstein EG, Bartel DP: An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science* 2001, 294(5543):858-862.

93. Lee RC, Ambros V: An extensive class of small RNAs in Caenorhabditis elegans. *Science* 2001, 294(5543):862-864.

94. Llave C, Xie ZX, Kasschau KD, Carrington JC: Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. *Science* 2002, 297(5589):2053-2056.

95. Reinhart, Brenda J, Weinstein, Earl G, Rhoades, Matthew W, Bartel, Bonnie, Bartel, David P: MicroRNAs in plants.

96. Jin XJ, Jia LL, Wang YX, Li BQ, Sun DF, Chen XY: Identification of Fusarium graminearum-responsive miRNAs and their targets in wheat by sRNA sequencing and degradome analysis. *Funct Integr Genomic* 2020, 20(1):51-61.

97. Liu YX, Chang W, Han YP, Zou Q, Guo MZ, Li WB: In silico Detection ofNovel MicroRNAs Genes in Soybean Genome. *Agr Sci China* 2011, 10(9):1336-1345.

98. Wang XF, EllingAA, Li XY, LiN, Peng ZY, He GM, Sun H, Qi YJ, Liu XS, Deng XW: Genome-Wide and Organ-Specific Landscapes of Epigenetic Modifications and Their Relationships to mRNA and Small RNA Transcriptomes in Maize. *Plant Cell* 2009, 21(4):1053-1069.

99. Jones-Rhoades MW, Bartel DP, Bartel B: MicroRNAs and their regulatory roles in plants. *Annual Review of Plant Biology* 2006, 57:19-53.

100. Qi Y, Denli AM, Hannon GJ: Biochemical specialization within Arabidopsis RNA silencing pathways. *Mol Cell* 2005, 19(3):421-428.

101. Tang GL, Reinhart BJ, Bartel DP, Zamore PD: A biochemical framework for RNA silencing in plants. *Gene Dev* 2003, 17(1):49-63.

102. Sandberg R, Neilson JR, Sarma A, Sharp PA, Burge CB:

Proliferating cells express mRNAs with shortened 3 ' untranslated regions and fewer microRNA target sites. *Science* 2008, 320(5883):1643-1647.

103. Wu L, Belasco JG: Let me count the ways: Mechanisms of gene regulation by miRNAs and siRNAs. *Mol Cell* 2008, 29(1):1-7.

104. Jover-Gil S, Candela H, Ponce MR: Plant microRNAs and development. *IntJDev Biol* 2005, 49(5-6):733-744.

105. Bird A: DNA methylation patterns and epigenetic memory. *Gene Dev* 2002, 16(1):6-21.

106. Tate PH, Bird AP: Effects of DNA Methylation on DNA-Binding Proteins and Gene-Expression. *Curr Opin Genet Dev* 1993, 3(2):226-231.

107. Li C, Zhang BH: MicroRNAs in Control of Plant Development. *J Cell Physiol* 2016, 231(2):303-313.

108. Uberti-Manassero NG, Lucero LE, Viola IL, Vegetti AC, Gonzalez DH: The class I protein AtTCP15 modulates plant development through a pathway that overlaps with the one affected by CIN-like TCP proteins. *JExp Bot* 2012, 63(2):809-823.

109. HuijserP, Schmid M: The control ofdevelopmental phase transitions in plants. *Development* 2011, 138(19):4117-4129.

110. Xue LJ, Zhang JJ, Xue HW: Characterization and expression profiles of miRNAs in rice seeds. *Nucleic Acids Res* 2009,

37(3):916-930.

111. Li DT, Wang LW, Liu X, Cui DZ, Chen TT, Zhang H, Jiang C, Xu CY, LiP, Li S*etal*: Deep Sequencing ofMaize Small RNAs Reveals a Diverse Set of MicroRNA in Dry and Imbibed Seeds. *Plos One* 2013, 8(1).

112. CurabaJ, Spriggs A, Taylor J, Li Z, Helliwell C: miRNA regulation in the early development ofbarley seed. *Bmc Plant Biol* 2012, 12.

113. Zhao YT, Wang M, Fu SX, Yang WC, Qi CK, Wang XJ: Small RNA Profiling in Two Brassica napus Cultivars Identifies MicroRNAs with Oil Production- and Development-Correlated Expression and New Small RNA Classes. *Plant Physiology* 2012, 158(2):813-823.

114. Meng FR, Liu H, Wang KT, Liu LL, Wang SH, Zhao YH, Yin J, Li YC: Development-associated microRNAs in grains of wheat (Triticum aestivum L.). *Bmc Plant Biol* 2013, 13.

115. Hou GG, Du CY, Gao HH, Liu SJ, Sun W, Lu HF, Kang J, Xie YX, Ma DY, Wang CY: Identification of microRNAs in developing wheat grain that are potentially involved in regulating grain characteristics and the response to nitrogen levels. *Bmc Plant Biol* 2020, 20(1).

116. Li T, Ma L, Geng YK, Hao CY, Chen XH, Zhang XY: Small RNA and Degradome Sequencing Reveal Complex Roles ofmiRNAs and Their Targets in Developing Wheat Grains. *Plos One* 2015, 10(10).

117. Yu Y, Sun FY, Chen N, Sun GL, Wang CY, Wu DX: MiR396 regulatory network and its expression during grain development in wheat. *Protoplasma* 2021, 258(1):103-113.

118. Sun YD, Luo WR, Chang HC, Li ZX, Zhou JG, Li XZ, Zheng JL, Hao MX: Identification of miRNAs and Their Target Genes Involved in Cucumber Fruit Expansion Using Small RNA and Degradome Sequencing. *Biomolecules* 2019, 9(9).

119. Guo HS, Xie Q, Fei JF, Chua NH: MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for Arabidopsis lateral root development. *Plant Cell* 2005, 17(5):1376-1386.

120. Kim J, Jung JH, Reyes JL, Kim YS, Kim SY, Chung KS, Kim JA, Lee M, Lee Y, Kim VN *et al*: microRNA-directed cleavage of ATHB15 mRNA regulates vascular development in Arabidopsis inflorescence stems. *Plant J* 2005, 42(1):84-94.

121. Yang XY, Wang LC, Yuan DJ, Lindsey K, Zhang XL: Small RNA and degradome sequencing reveal complex miRNA regulation during cotton somatic embryogenesis. *J Exp Bot* 2013, 64(6):1521-1536.

122. Song CH, Zhang D, Zheng LW, Zhang J, Zhang BJ, Luo WW, Li YM, Li GF, Ma JJ, Han MY: miRNA and Degradome Sequencing Reveal miRNA and Their Target Genes That May Mediate Shoot

Growth in Spur Type Mutant "Yanfu 6". *Front Plant Sci* 2017, 8.

123. Wang L, Mai YX, Zhang YC, Luo QA, Yang HQ: MicroRNA171c-Targeted SCL6-II, SCL6-III, and SCL6-IV Genes Regulate Shoot Branching in Arabidopsis. *Mol Plant* 2010, 3(5):794-806.

124. Wang L, Gu XL, Xu DY, Wang W, Wang H, Zeng MH, Chang ZY, Huang H, Cui XF: miR396-targeted AtGRF transcription factors are required for coordination of cell division and differentiation during leaf development in Arabidopsis. *J Exp Bot* 2011, 62(2):761-773.

125. Debernardi JM, Rodriguez RE, Mecchia MA, Palatnik JF: Functional Specialization of the Plant miR396 Regulatory Network through Distinct MicroRNA–Target Interactions. *Plos Genet* 2012, 8(1).

126. Glazinska P, Kulasek M, Glinkowski W, Wojciechowski W, Kosinski J: Integrated Analysis of Small RNA, Transcriptome and Degradome Sequencing Provides New Insights into Floral Development and Abscission in Yellow Lupine (Lupinus luteus L.). *Int J Mol Sci* 2019, 20(20).

127. Chen XM: A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. *Science* 2004, 303(5666):2022-2025.

128. Zhao L, Kim YJ, Dinh TT, Chen XM: miR172 regulates stem cell fate and defines the inner boundary of APETALA3 and

PISTILLATA expression domain in Arabidopsis floral meristems. *Plant J* 2007, 51(5):840-849.

129. Gupta OP, Permar V, Koundal V, Singh UD, Praveen S: MicroRNA regulated defense responses in TriticumaestivumL. during Puccinia graminis f.sptritici infection. *Mol Biol Rep* 2012, 39(2):817-824.

130. Wang PF, Yang Y, Shi HM, Wang YM, Ren FS: Small RNA and degradome deep sequencing reveal respective roles of cold-related microRNAs across Chinese wild grapevine and cultivated grapevine. *Bmc Genomics* 2019, 20(1).

131. Xu XW, Zhong CL, Tan M, Song Y, Qi XH, Xu Q, Chen XH: Identification of MicroRNAs and Their Targets That Respond to Powdery Mildew Infection in Cucumber by Small RNA and Degradome Sequencing. *Front Genet* 2020, 11.

132. Li WQ, Jia YL, Liu FQ, Wang FQ, Fan FJ, Wang J, Zhu JY, Xu Y, Zhong WG, Yang J: Integration Analysis of Small RNA and Degradome Sequencing Reveals MicroRNAs Responsive to Dickeya zeae in Resistant Rice. *IntJMol Sci* 2019, 20(1).

133. Liu H, Able AJ, Able JA: Multi-Omics Analysis of Small RNA, Transcriptome, and Degradome in T. turgidum—Regulatory Networks of Grain Development and Abiotic Stress Response. *IntJ Mol Sci* 2020, 21(20):7772.

134. Zhou XF, Wang GD, Sutoh K, Zhu JK, Zhang WX: Identification of

cold-inducible microRNAs in plants by transcriptome analysis. *Bba-Gene RegulMech* 2008, 1779(11):780-788.

135. Feng JX, Wang QM, Qi HU, Rao LQ: Differential Expression of Heat Stress Related microRNA in Arabidopsis thaliana. *Hunan Agricultural Sciences* 2012.

136. Yamasaki H, Abdel-Ghany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M: Regulation of copper homeostasis by micro-RNA in Arabidopsis. *J Biol Chem* 2007, 282(22):16369-16378.

137. Abdel-Ghany SE, Pilon M: MicroRNA-mediated systemic down-regulation of copper protein expression in response to low copper availability in arabidopsis. *J Biol Chem* 2008, 283(23):15932-15945.

138. Schuster SC: Next-generation sequencing transforms today's biology. *Nat Methods* 2008, 5(1):16-18.

139. Sultan M, Schulz MH, Richard H, Magen A, Klingenhoff A, Scherf M, Seifert M, Borodina T, Soldatov A, Parkhomchuk D *et al*: A global view of gene activity and alternative splicing by deep sequencing of the human transcriptome. *Science* 2008, 321(5891):956-960.

140. Shen Y, Jiang Z, Lu SF, Lin HJ, Gao SB, Peng HW, Yuan GS, Liu L, Zhang ZM, Zhao MJ *et al*: Combined small RNA and degradome sequencing reveals microRNA regulation during immature maize embryo dedifferentiation. *Biochem Bioph Res Co* 2013, 441(2):425-

430.

141. Peng T, Sun HZ, Du YX, Zhang J, Li JZ, Liu YX, Zhao YF, Zhao QZ: Characterization and Expression Patterns of microRNAs Involved in Rice Grain Filling. *Plos One* 2013, 8(1).

142. Wu FY, Tang CY, Guo YM, Yang MK, Yang RW, Lu GH, Yang YH: Comparison of miRNAs and Their Targets in Seed Development between Two Maize Inbred Lines by High-Throughput Sequencing and Degradome Analysis. *Plos One* 2016, 11(7).

143. Luo XY, Gao ZH, Shi T, Cheng ZM, Zhang Z, Ni ZJ: Identification of miRNAs and Their Target Genes in Peach (Prunus persica L.) Using High-Throughput Sequencing and Degradome Analysis. *Plos One* 2013, 8(11).

144. Jiang J, Lv M, Liang Y, Ma Z, Cao J: Identification of novel and conserved miRNAs involved in pollen development in Brassica campestris ssp. chinensi s by high-throughput sequencing and degradome analysis. *BMC Genomics,15,1(2014-02-21)* 2014, 15(1):146-146.

145. German MA, Luo SJ, Schroth G, Meyers BC, Green PJ: Construction of Parallel Analysis of RNA Ends (PARE) libraries for the study of cleaved miRNA targets and the RNA degradome. *Nat Protoc* 2009, 4(3):356-362.

146. Huang BS, Gan L, Chen DJ, Zhang YC, Zhang YJ, Liu XL, Chen S,

Wei ZS, Tong LQ, Song ZJ *et al*: Integration of small RNA, degradome and proteome sequencing in Oryza sativa reveals a delayed senescence network in tetraploid rice seed. *Plos One* 2020, 15(11).

147. Wu Y, Yang LY, Yu ML, Wang JB: Identification and expression analysis of microRNAs during ovule development in rice (Oryza sativa) by deep sequencing. *Plant Cell Rep* 2017, 36(11):1815-1827.

148. Cakir C, Gillespie ME, Scofield SR: Rapid Determination of Gene Function by Virus-induced Gene Silencing in Wheat and Barley. *Crop Sci* 2010, 50(2):S77-S84.

149. Wang JS, Liu WH, Wang H, Li LH, Wu J, Yang XM, Li XQ, Gao AN: QTL mapping of yield-related traits in the wheat germplasm 3228. *Euphytica* 2011, 177(2):277-292.

150. Li H, Zhang L, Wang J: Analysis and Answers to Frequently Asked Questions in Quantitative Trait Locus Mapping. *ACTA AGRONOMICA SINICA* 2010, 36(6):918-931.

151. Gresshoff PM: Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry* 1991, 196(1):80-83.

152. Li SS, Jia JZ, Wei XY, Zhang XC, Li LZ, Chen HM, Fan YD, Sun HY, Zhao XH, Lei TD *et al*: A intervarietal genetic map and QTL analysis for yield traits in wheat. *Mol Breeding* 2007, 20(2):167-178.

153. Cui F, Ding AM, Li J, Zhao CH, Wang L, Wang XQ, Qi XL, Li XF,

Li GY, Gao JR *etal*: QTL detection of seven spike-related traits and their genetic correlations in wheat using two related RIL populations. *Euphytica* 2012, 186(1):177-192.

154. Mccouch S, Cho Y, Yano M, Paul E, Blinstrub M, Morishima H, Mccouch S, Cho Y, Paul E, Morishima H: Report on QTL nomenclature. *Rice Genet Newsl* 1997, 14.
155. Cao G, Zhu J, He C, Gao Y, Yan J, Wu P: Impact of epistasis and QTL×environment interaction on the developmental behavior of plant height in rice (Oryza sativa L.). *Theoretical & Applied Genetics* 2001, 103(1):153-160.
156. Zhen LI, Yuan-Yuan PU, Chang-Bin G, Guang-Sheng Z, Jin-Xing TU, Ting-Dong FU: Evaluation of Waterlogging Tolerance in Rapeseed (Brassica napus L.) DH Lines at Seedling Stage. *Scientia Agricultura Sinica* 2010, 43(2):286-292.
157. Li QC, Li YX, Yang ZZ, Cheng L, Li Y: QTL Mapping for Plant Height and Ear Height by Using Multiple Related RIL Populations in Maize. *Acta Agronomica Sinica* 2013, 39(9):1521.
158. Ganiger M, Hittalmani S: association analysis ofF2 population of MAS26 x IR50 rice (Oryza sativa L.) cross for drought and yield traits under aerobic condition. 2019.
159. Kumar N, Kulwal PL, Balyan HS, Gupta PK: QTL mapping for yield and yield contributing traits in two mapping populations of

bread wheat. *Mol Breeding* 2007, 19(2):163-177.

160. Torada A, Koike M, MochidaK, OgiharaY: SSR-based linkage map with new markers using an intraspecific population of common wheat. *Theor Appl Genet* 2006, 112(6):1042-1051.

161. Korzun V, BornerA, Worland AJ, Law CN, Roder MS: Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat (Triticum aestivum L). *Euphytica* 1997, 95(2):149-155.

162. CheYH, Li-Hui LI: Potential Evaluation of Wheat SSR Markers for Genetic Analysis of Agropyron Geartn. *Journal of Agricultural Biotechnology* 2006.

163. Somers DJ, Isaac P, Edwards K: A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). *Theor Appl Genet* 2004, 109(6):1105-1114.

164. Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD: A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. *Theor Appl Genet* 2005, 111(4):782-794.

165. Xue SL, Zhang ZZ, Lin F, Kong ZX, Cao Y, Li CJ, Yi HY, Mei MF, Zhu HL, Wu J *et al*: A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence

tags. *Theor Appl Genet* 2008, 117(2):181-189.

166. Zhai HJ, Feng ZY, Du XF, Song YE, Liu XY, Qi ZQ, Song L, Li J, Li LH, Peng HR *et al*: A novel allele of TaGW2-A1 is located in a finely mapped QTL that increases grain weight but decreases grain number in wheat (Triticum aestivum L.). *Theor Appl Genet* 2018, 131(3):539-553.
167. Gao FM, Wen WE, Liu JD, Rasheed A, Yin GH, Xia XC, Wu XX, He ZH: Genome-Wide Linkage Mapping of QTL for Yield Components, Plant Height and Yield-Related Physiological Traits in the Chinese Wheat Cross Zhou 8425B/Chinese Spring. *Frontiers in Plant Science* 2015, 6.
168. Li FJ, Wen WE, He ZH, Liu JD, Jin H, Cao SH, Geng HW, Yan J, Zhang PZ, Wan YX *et al*: Genome-wide linkage mapping of yield-related traits in three Chinese bread wheat populations using high-density SNP markers. *Theor Appl Genet* 2018, 131(9):1903-1924.
169. YuM, Mao SL, Hou DB, Chen GY, Pu ZE, Li W, Lan XJ, Jiang QT, Liu YX, Deng M *et al*: Analysis of contributors to grain yield in wheat at the individual quantitative trait locus level. *Plant Breeding* 2018, 137(1):35-49.
170. Addo-Quaye C, Eshoo TW, Bartel DP, Axtell MJ: Endogenous siRNA and miRNA targets identified by sequencing of the Arabidopsis degradome. *CurrBiol* 2008, 18(10):758-762.

171. Sunkar, R.: Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. *The Plant cell* 2004, 16(8).

172. Nawrocki EP, Burge SW, Bateman A, Daub J, Eberhardt RY, Eddy SR, Floden EW, Gardner PP, Jones TA, Tate J *et al*: Rfam 12.0: updates to the RNA families database. *Nucleic Acids Research* 2015, 43(D1):D130-D137.

173. Kapitonov VV, Jurka J: A universal classification of eukaryotic transposable elements implemented in Repbase. *Nat Rev Genet* 2008, 9(5).

174. Rachel Brenchley MS, Matthias Pfeifer, Gary L. A. Barker, Rosalinda D'Amore, Alexandra M. Allen, Neil McKenzie, Melissa Kramer, Arnaud Kerhornou, Dan Bolser, Suzanne Kay, Darren Waite, Martin Trick, Ian Bancroft, Yong Gu, Naxin Huo, Ming-Cheng Luo, Sunish Sehgal, Bikram Gill, Sharyar Kianian, Olin Anderson, Paul Kersey, Jan Dvorak, W. Richard McCombie, Anthony Hall, Klaus F. X. Mayer, Keith J. Edwards, Michael W. Bevan & Neil Hall: Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature* 2012, 491(7426):705-710.

175. Chu ZL, Chen JY, Xu HX, Dong ZD, Chen F, Cui DQ: Identification and Comparative Analysis of microRNA in Wheat (Triticum aestivum L.) Callus Derived from Mature and Immature Embryos

during In vitro Culture. *Frontiers in Plant Science* 2016, 7.

176. Hofacker IL, Fontana W, Stadler PF, Bonhoeffer LS, Schuster P: Fast folding and comparison of RNA secondary structures. *MonatshefteFür Chemie* 1994, 125(2):167-188.

177. German MA, PillayM, Jeong DH, HetawalA, Luo SJ, Janardhanan P, Kannan V, Rymarquis LA, Nobuta K, German R *et al*: Global identification of microRNA-target RNA pairs by parallel analysis of RNA ends. *Nat Biotechnol* 2008, 26(8):941-946.

178. Livak KJ, Schmittgen T: Analysis of relative gene expression data using real-time quantitative PCR and the 2-DDCt method. *Methods* 2001, 25(4):402–408.

179. Wei B, Cai T, Zhang RZ, Li AL, HuoNX, Li S, GuYQ, Vogel J, Jia JZ, Qi YJ *et al*: Novel microRNAs uncovered by deep sequencing of small RNA transcriptomes in bread wheat (Triticumaestivum L.) and Brachypodium distachyon (L.) Beauv. *Funct Integr Genomic* 2009, 9(4):499-511.

180. Su C, Yang X, Gao S, Tang Y, Zhao C, Li L: *Genomics* 2014, 103(4):298-307.

181. Friedlander MR, Mackowiak SD, Li N, Chen W, Rajewsky N: miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Research* 2012, 40(1):37-52.

182. Meyers BC,Axtell MJ, Bartel B, BartelDP, BaulcombeD, Bowman JL, Cao X, Carrington JC, Chen XM, Green PJ *et al*: Criteria for Annotation of Plant MicroRNAs. *The Plant cell* 2008, 20(12):3186-3190.

183. Jones-Rhoades MW, Bartel DP: Computational identification of plant MicroRNAs and their targets, including a stress-induced miRNA. *Molecular Cell* 2004, 14(6):787-799.

184. Voinnet O: Origin, Biogenesis, and Activity of Plant MicroRNAs. *Cell* 2009, 136(4):669-687.

185. Fahlgren N, Howell MD, Kasschau KD, Chapman EJ, Sullivan CM, Cumbie JS, Givan SA, Law TF, Grant SR, Dangl JL *et al*: High-Throughput Sequencing of Arabidopsis microRNAs: Evidence for Frequent Birth and Death of MIRNA Genes. *Plos One* 2007, 2(2).

186. Wang SK, Wu K, Yuan QB, Liu XY, Liu ZB, Lin XY, Zeng RZ, Zhu HT, Dong GJ, Qian Q *etal*: Control of grain size, shape and quality by OsSPL16 in rice. *Nat Genet* 2012, 44(8):950-+.

187. Zhu QH, Spriggs A, Matthew L, Fan LJ, Kennedy G, Gubler F, Helliwell C: A diverse set of microRNAs and microRNA-like small RNAs in developing rice grains. *Genome Res* 2008, 18(9):1456-1465.

188. Han R, Jian C, Lv JY, Yan Y, Chi Q, Li ZJ, Wang Q, Zhang J, Liu XL, Zhao HX: Identification and characterization of microRNAs in

the flag leaf and developing seed of wheat (Triticum aestivum L.). *Bmc Genomics* 2014, 15.

189. Wang JW, Czech B, Weigel D: miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. *Cell* 2009, 138(4):738-749.

190. Si LZ, Chen JY, Huang XH, Gong H, Luo JH, Hou QQ, Zhou TY, Lu TT, Zhu JJ, ShangguanYY *et al*: OsSPL13 controls grain size in cultivated rice. *Nat Genet* 2016, 48(4):447-+.

191. Cao RF, Guo LJ, MaM, Zhang WJ, Liu XL, Zhao HX: Identification and Functional Characterization of Squamosa Promoter Binding Protein-Like Gene TaSPL16 in Wheat (Triticum aestivum L.). *Frontiers in Plant Science* 2019, 10.

192. Mallory AC, Dugas DV, Bartel DP, Bartel B: MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *CurrBiol* 2004, 14(12):1035-1046.

193. Olsen AN, Ernst HA, Lo Leggio L, Skriver K: NAC transcription factors: structurally distinct, functionally diverse. *Trends in Plant Science* 2005, 10(2):79-87.

194. Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ: The AUXIN RESPONSE FACTOR 2 gene of Arabidopsis links auxin signalling, cell division, and the size of seeds and other organs.

Development 2006, 133(2):251-261.

195. Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J: A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 2006, 314(5803):1298-1301.

196. Koyama T, MitsudaN, Seki M, Shinozaki K, Ohme-Takagi M: TCP Transcription Factors Regulate the Activities of ASYMMETRIC LEAVES1 and miR164, as Well as the Auxin Response, during Differentiation of Leaves in Arabidopsis. *The Plant cell* 2010, 22(11):3574-3588.

197. Ru P, Xu L, Ma H, Huang H: Plant fertility defects induced by the enhanced expression of microRNA167. *Cell Res* 2006, 16(5):457-465.

198. Goetz M, Vivian-Smith A, Johnson SD, Koltunow AM: AUXIN RESPONSE FACTOR8 is a negative regulator of fruit initiation in Arabidopsis. *The Plant cell* 2006, 18(8):1873-1886.

199. Phookaew P, Netrphan S, Sojikul P, Narangajavana J: Involvement of miR164-and miR167-mediated target gene expressions in responses to water deficit in cassava. *Biol Plantarum* 2014, 58(3):469-478.

200. Grunewald W, De Smet I, De Rybel B, Robert HS, van de Cotte B, Willemsen V, Gheysen G, Weijers D, Friml J, Beeckman T: Tightly controlled WRKY23 expression mediates Arabidopsis embryo

development. *Embo Rep* 2013, 14(12):1136-1142.

201. Grunewald W, De Smet I, Lewis DR, Lofke C, Jansen L, Goeminne G, Bossche RV, Karimi M, De Rybel B, Vanholme B *et al*: Transcription factor WRKY23 assists auxin distribution patterns during Arabidopsis root development through local control on flavonol biosynthesis. *Proceedings of the National Academy of Sciences ofthe United States ofAmerica* 2012, 109(5):1554-1559.

202. Thakare D, Tang W, Hill K, Perry SE: The MADS-Domain Transcriptional Regulator AGAMOUS-LIKE15 Promotes Somatic Embryo Development in Arabidopsis and Soybean1[OA].

203. Wang H, Caruso LV, Downie AB, Perry SE: The embryo MADS domain protein AGAMOUS-Like 15 directly regulates expression of a gene encoding an enzyme involved in gibberellin metabolism. *The Plant cell* 2004, 16(5):1206-1219.

204. Greaves J, Chamberlain LH: DHHC palmitoyl transferases: substrate interactions and (patho)physiology. *Trends in Biochemical Sciences* 2011, 36(5):245-253.

APPENDEXES

Table S1. MiRNA expression list

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc__1dl_v1_2280510_73848	ta_iwgsc__1dl_v1_2280510_73848	novel	--	20	ggcggauguagccaagugga	ggggcggauguagccaaguggaucaaggcaguggauuaugaauccaucaugcgugguucaauucccgucguucgcccaucacauuauugcaaauuccaaaaaugcaa	14254	3.9	1.2	0	27957	27955
ta_iwgsc_2al_v1_369279_90974	ta_iwgsc_2al_v1_369279_90974	novel	--	22	ugagaagguagaucauaauagc	auuggaccgcuaaacaagucuuuagaugagaagguagaucauaauagcugagcgaaaugaaagugauauuguugcuuaauuguuaugaucugcuucucaucugaagacuag	3487.3	3.9	2.1	0	6837	6084
ta_iwgsc__1ds_v1__1896382_84215	ta_iwgsc__1ds_v1__1896382_84215	novel		22	ugagaagguagaucauaauagc	ccgcuaaacaagucuuuagaugagaagguagaucauaauagcugagcgaaaugaaagugauauuguugcucaauuguuaugaucugcuucucaucugaagacuag	3487.4	3.9	2.2	0	6837	6084
ta_iwgsc_6dl_v1_3251654_629440	ta_iwgsc_6dl_v1_3251654_629440	novel	--	19	aaaaaagauugagccgaau	aaaagagaaaaaaaucaguuaugaaauguaguaauucuucuuuauucuucuaauugauugcaauuaaacucggcucaaucuuuuuuuuucuaaaaaagauugagccgaaua	2332.9	3.9	0.9	0	4575	4553
ta_iwgsc_2bl_v1_7931332__135085	ta_iwgsc_2bl_v1_7931332__135085	novel	--	21	uccacaggcuuucuugaacug	cucuccacaggcuuucuugaacugugaacucgcggggauuguggcgcugcugccuucucuucggccgccuccgccuguugaugguucaagaaagcccauggaaaccaugc	2.5	-1.3	2.2	0	2991	2991
ta_iwgsc_5as_v1__1551865_470905	ta_iwgsc_5as_v1__1551865_470905	novel	--	22	ugaagcugccagcaugaucuga	ccacaagcuggugaagcugccagcaugaucugaugaccuaacucauggaucagaauccaugucaaucaggucaugcuggaguuucaucugcuggucggagcacaacga	2.6	-1.3	2.3	0	2248	2180
ta_iwgsc_5ds_v1_2769792_560237	ta_iwgsc_5ds_v1_2769792_560237	novel	--	22	ugaagcugccagcaugaucuga	ccacaagcuggugaagcugccagcaugaucugaugaccuaagucauggaucagaauccaugucaaucaggucaugcuggaguuucaucugcuggucggagcacaacga	2.6	-1.3	2.3	0	2248	2180
ta_iwgsc__1as_v1_3293291_24189	ta_iwgsc__1as_v1_3293291_24189	novel	--	22	ugaagcugccagcaugaucuga	ccacgagcuggugaagcugccagcaugaucugaugaccuaacucauggaucagaguccaugucaaucaggucaugcuggaguuucaucugcuggucggagcaccacnn	2.7	-1.3	2.4	0	2248	2180
ta_iwgsc_5bs_v1_2252788_516635	ta_iwgsc_5bs_v1_2252788_516635	novel	--	22	ugaagcugccagcaugaucuga	ccacgagcuggugaagcugccagcaugaucugaugaccuaacucauggaucagaguccaugucaaucaggucaugcuggaguuucaucugcuggucggagcaccacga	2.7	-1.3	2.4	0	2248	2180
ta_iwgsc_6as_v1_4350592_581627	tae-miR9670-3p	tae-miR9670-3p	--	21	agguggaauacuugaagaaga	gagauccgccaagcuggaguaccugaacgucuucuucaaguacuccacuuuuuuuuucuugcggagaaaagguggaauacuugaagaagauuuucaggucguccauu	1271.9	3.9	2.4	0	2491	1080
ta_iwgsc_4al_v2_7133883_350482	ta_iwgsc_4al_v2_7133883_350482	novel	--	24	aacgauagauccauggcgaagugg	ccaacgacaacaacaacgacaacgauagauccauggcgaagugguagagcuaucacuacgcuaugggccaaccguuuuuguugcagaucaauaggucacugguucgaa	509.4	3.9	1.1	0	998	991
ta_iwgsc_5dl_v1_4584209_545883	ta_iwgsc_5dl_v1_4584209_545883	novel	--	21	ucggaccaggcuucauucccc	gucaugguugucgaggggaaugacgccggguccgaaagagagacgcgcgcauggcgugcguguggcgcguuucggaccaggcuucauuccccaugacuccaucaugguu	2.9	-1.3	2.6	0	1088	1087
ta_iwgsc_5bl_v1__10899037_498563	ta_iwgsc_5bl_v1__10899037_498563	novel	--	21	ucggaccaggcuucauucccc	gucaugguugucgaggggaaugacgccggguccgaaagagagacgcgcgcauggugugcguguggcgcguuucggaccaggcuucauuccccaugacuccaucaugguu	2.9	-1.3	2.6	0	1088	1087
ta_iwgsc_4dl_v3__14441658_420598	ta_iwgsc_4dl_v3__14441658_420598	novel	--	21	ucggaccaggcuucauucccc	ggggaauguugucugguuggagaccuggcucgcacgacggaucgaucggcggcgggcgugcgcgcgcgguuucggaccaggcuucauucccccccaacucaacccuucuucc	551	3.9	2.4	0	1077	1075
ta_iwgsc_6ds_v1_2106101_646368	ta_iwgsc_6ds_v1_2106101_646368	novel	--	18	agagguugucugacagac	aacaacugaaacagagguugucugacagacuccacaaaucuuuuuuuuuccuuuuuggcuguagagcuaggaugugguuguacaacaucuggcaauuacauucaguuguuguuc	0.8	-1.3	0.5	0	1405	1405
ta_iwgsc_2bl_v1_8046566__149453	ta_iwgsc_2bl_v1_8046566__149453	novel	--	24	uuaggaacaugugugcucucuugu	ucaucauuuaugaugauacaagagagcacacgucuuccuaaacaagagaucauaaacuaacaucacuuguuuaggaacaugugugcucucuuguaucaucauaaaugaugau	3.1	-1.3	2.8	0	691	691
ta_iwgsc_3al_v1_4447263_238317	ta_iwgsc_3al_v1_4447263_238317	novel	--	20	acgaccuuacuugaacagga	cucgguuguuuagcggaagcaacacgguuuaaaaggacaggcccugcgcacauuggcacgaccuuacuugaacaggaucuguucuauaguagucguaccgcugcauccuuuacca	1422.9	3.9	0.7	0	2798	2795
ta_iwgsc_3al_v1_4447263_238318	ta_iwgsc_3al_v1_4447263_238318	novel	--	20	acgaccuuacuugaacagga	cauuggcacgaccuuacuugaacaggaucuguucuauaguagucguaccgcugcauccuuuaccaauaaggaggcaagcacuuaacccugauugaugacaugacaccugugggcc	1422.6	3.9	0.4	0	2798	2795
ta_iwgsc_3b_v1__10373685_254581	ta_iwgsc_3b_v1__10373685_254581	novel		20	acgaccuuacuugaacagga	ucgguuguuuagcgggagcaacaccgguuuaaaaggagaggcccugcgcacaucagcacgaccuuacuugaacaggaucuguucuauaguagucguaccgcugcauccuuuauca	1423	3.9	0.8	0	2798	2795

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_3b_v1_10373685_254582	ta_iwgsc_3b_v1_10373685_254582	novel	--	20	acgaccuuacuugaacagga	caucagcacgaccuuacuugaacaggaucuguucuauagu agucguaccgcugcauccuuuaucaauaaggaggcaagca cuuaagccugguugaugacaugacaccugugggcc	1426.8	3.9	0.8	0	2798	2795
ta_iwgsc_5bl_v1_10925918_508613	ta_iwgsc_5bl_v1_10925918_508613	novel	--	20	acgaccuuacuugaacagga	uuacagggagcagaaccagcuuauaagccgaggcgcaucc ugagguagcacgaccuuacuugaacaggaucuguucugua ggcucguaccgcugcauccuuuaccaau	1434.9	3.9	0.9	0	2821	2795
ta_iwgsc_6ds_v1_2084038_643711	tae-miR9674b-5p	tae-miR9674b-5p	--	21	auagcaucauccauccuaccc	guugaacuuguccauagcaucauccauccuacccuacccu ucaacggcggccgcuggugccccacgguggguaggauggc uggugcuaugguagcuucaacccgauggu	325.6	3.9	2.4	0	635	549
ta_iwgsc_4bl_v1_1655130_374210	tae-miR9674b-5p	tae-miR9674b-5p	--	21	auagcaucauccauccuaccc	uugaacuuguccauagcaucauccauccuacccuugaacg cugguugcugauuccccacgguggguaagauggcugaugc uauggauaacuucgaaccagauuauaguugu	2.3	-1.3	2	0	548	548
ta_iwgsc_6bs_v1_3017544_617934	tae-miR156	tae-miR156	--	21	ugacagaagagagugagcaca	uguggagaggcccucgagagauugacagaagagagugagc acacggcgugaugccggcauaacauguaugccgucuucgc cgcgugcucacuccucuuucugucagccuc	258.4	3.9	2.4	0	503	501
ta_iwgsc_6ds_v1_732466_650398	tae-miR156	tae-miR156	--	21	ugacagaagagagugagcaca	uguggggaggcccucgagagauugacagaagagagugagc acauggcgugaugccggcauaacauguaugccgucuucgc cgcgugcucacuccucuuucugucagccuc	258.4	3.9	2.5	0	503	501
ta_iwgsc_6as_v1_4354848_582116	tae-miR156	tae-miR156	--	21	ugacagaagagagugagcaca	uguggggaggcccucgagagauugacagaagagagugagc acacggcgugaugccggcauaacauguaugccgucuucgc cgcgugcucacuccucuuucugucagccuc	258.5	3.9	2.5	0	503	501
ta_iwgsc_6dl_v1_3325061_636528	tae-miR156	tae-miR156	--	21	ugacagaagagagugagcaca	acggugacagaagagagugagcacacggccggucguuacg gcaccgcccgggugugccgucgcggccgcgugcucacugc ucuuccugucauccacucucccgcuucccuucc	202.4	3.9	2.5	0	393	389
ta_iwgsc_7as_v1_4208248_676353	ta_iwgsc_7as_v1_4208248_676353	novel	--	21	uuccacagcuuucuugaacug	cuuccacagcuuucuugaacugcaucugcaauugguggau gcuagcuucuaccggcaagaucugcaguucaauaaagcug ugggaaauugcagagagagaccaauugggca	2.3	-1.3	2	0	461	402
ta_iwgsc_6ds_v1_2125042_649323	tae-miR9655-3p	tae-miR9655-3p		21	caagggaaggaaguagccaac	auccaacucaggcguuggcuacuuccuuucccuugccggu gcgccggcaugugcagccccuugcacuggcaagggaagg aaguagccaacaucuugggcggaucuagagc	163.8	3.9	2.7	0	317	283
ta_iwgsc_6as_v1_4386089_585750	tae-miR9655-3p	tae-miR9655-3p	--	21	caagggaaggaaguagccaac	caggcgucggcuacuuccuuucccuugccggugcgccggu caugccggugggugcaccccucuugcauggcaagggaagg aaguagccaacgucuugggcggaucuagcau	260	3.9	2.5	0	506	339
ta_iwgsc_7bl_v1_6686739_696777	ta_iwgsc_7bl_v1_6686739_696777	novel	--	19	auaguuacucugauagggc	ucugcuugucuuagcccuacaugaugaacuauguuuuagc uauggauguauguccagguggagcuauauuugacauaguu acucugauagggcaaagacuguuaauuucc	353.6	3.9	1.3	0	692	689
ta_iwgsc_3b_v1_10413964_256745	tae-miR9677a	tae-miR9677a	--	22	uggccguugguagaguaggaga	caaaggcggccucgcuucucuuccacucuaccaacagcca cgcugccacgucuagcccgcgaccauggcguggccguugg uagaguaggagacgagcauggccaccugu	106.5	3.9	2.5	0	205	202
ta_iwgsc_3b_v1_10767060_293368	tae-miR9677a	tae-miR9677a	--	22	uggccguugguagaguaggaga	caaaggcggccucgcuucucuuccacucuaccaacagcca cgcugccacgucuagcccgcgaccauggcguggccguugg uagaguaggagacgagcauggccaccuguccc	106.5	3.9	2.5	0	205	202
ta_iwgsc_5ds_v1_2769792_560233	tae-miR167a	tae-miR167a	--	21	ugaagcugccagcaugaucua	cccaugugaaugagugaagcugccagcaugaucuaccuuu gauucgcuucugcgugugaauccuuguuagaucaugacug acagccucauuucuucccagcaaugggcau	139.2	3.9	2	0	270	208
ta_iwgsc_5bs_v1_2252788_516633	tae-miR167a	tae-miR167a	--	21	ugaagcugccagcaugaucua	cccaugugaaugagugaagcugccagcaugaucuaucuuu gauucgcuucugcgugugaauccuuguuagaucaugacug acagccucauuucuucccagcaaugggcau	136.7	3.9	2.1	0	265	203
ta_iwgsc_1as_v1_2760709_18776	tae-miR167a	tae-miR167a	--	21	ugaagcugccagcaugaucua	ugugaaugagugaagcugccagcaugaucuaucuuugauu cgcuucugcgugugaauccuuguuagaucaugacugacag ccucauuucuucccagcaaugggcaua	136.5	3.9	1.9	0	265	203
ta_iwgsc_5dl_v1_4581919_545423	tae-miR167a	tae-miR167a	--	21	ugaagcugccagcaugaucua	cccaagagaaagcgugaagcugccagcaugaucuaacucu ugaaccagcuagcuuccgcgggugguuagaucgugcugu gacaguuucacccuuucccuguugggcacaug	106.8	3.9	2.3	0	206	204
ta_iwgsc_5bl_v1_10821195_482313	tae-miR167a	tae-miR167a	--	21	ugaagcugccagcaugaucua	gugcccaagagaaagcgugaagcugccagcaugaucuaac ucuugaagcuggccgcguggugcugguuagaucgugcugu gacaguuucacucuuucccuguugggcacau	2.8	-1.3	2.5	0	204	204

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_5al_v1_2750956_448905	tae-miR167a	tae-miR167a	--	21	ugaagcugccagcaugaucua	uaguuaguucgagugcccaagagaaagcgugaagcugcca gcaugaucuaacucuugaagcugugguuagaucgugcugu gacaguuucacucuuucccuguugggcacau	2.7	-1.3	2.4	0	204	204
ta_iwgsc_3as_v1_3444703_253082	ta_iwgsc_3as_v1_3444703_253082	novel	--	21	acaaguauuuccggacggagg	cugcuagcuacucccuccguccgaaaauaguugucaucaa uaguuuuagauacaucgcuuuuuauccauuuuggugacaa guauuuccggacggagggaguauauaccaau	2.5	-1.3	2.2	0	362	362
ta_iwgsc_2dl_v1_9822247_187290	ta_iwgsc_2dl_v1_9822247_187290	novel	--	21	acaaguauuuccggacggagg	uauaauuauguuguaucuaccuccuaaugccuaauauggu acucccuccauccggaaauacuuguccaaggaauacaagu auuuccggacggagggaguaugaaauaguugucau	2.4	-1.3	2.1	0	309	309
ta_iwgsc_1ds_v1_1884529_81868	ta_iwgsc_1ds_v1_1884529_81868	novel	--	21	acaaguauuuccggacggagg	cauggcuuuugugaguaguacucccuucgucuggaaauac uugucggguauccauuuccccgacaaguauuuccggacgg agggaguacauguuaaugaugguugaagcuuagu	2.8	-1.3	2.5	0	324	324
ta_iwgsc_3as_v1_3280614_244290	ta_iwgsc_3as_v1_3280614_244290	novel	--	21	acaaguauuuccggacggagg	ggggaucagcuagccuaguacucccuucgucuggaaauac uugucucaaccauuucgaggacaaguauuuccggacggag ggaguauauaguuauguaauguucauuuuguaca	166	3.9	2.3	0	322	321
ta_iwgsc_3b_v1_10686187_281121	ta_iwgsc_3b_v1_10686187_281121	novel	--	21	ugaauuuguccauagcaucag	gagccaacccucugguugaauuuguccauagcaucagcca gccaucuugcgcauuuuugcacgaugggaaugauggcugg ugcuauggauaaauucaacccgauggguc	92.3	3.9	2.5	0	177	160
ta_iwgsc_3b_v1_10771022_294102	ta_iwgsc_3b_v1_10771022_294102	novel	--	21	ucuguuaacuaauauaagagu	uugcuugcuaauauacuccaucuguuaacuaauauaagag uguuuacaaacacucuuauauuaguuaaccgagggaguac uauuuuguguggugccuaucuuuucauucg	2.1	-1.3	1.8	0	87	87
ta_iwgsc_5dl_v1_953728_553095	ta_iwgsc_5dl_v1_953728_553095	novel	--	21	uccguaaacuaauauaagagu	agccagcauggugauccuuaaaucguacucccuccguaaa cuaauauaagagugaucuaaaugcucuuauauuaguuuac agagggaauaccaucuugcucuugugauucaaa	2.2	-1.3	1.9	0	116	111
ta_iwgsc_1ds_v1_1899930_84816	ta_iwgsc_1ds_v1_1899930_84816	novel	--	22	cuuguuugucauuaagcuucug	agauacuucuccgccauuuggccaaaugguggucaagugca gaauguaucaagacaagauuuguuugccacuuguuuguca uuaagcuucugaguaucugugccauguggcu	0.8	-1.3	0.5	0	81	81
ta_iwgsc_1bl_v1_3895330_43722	ta_iwgsc_1bl_v1_3895330_43722	novel	--	24	agguuguagagcccacugccgagg	cuggguggucggggcagcgagguuguagagcccgccgccg aggucgccuccuuggguggccugggcggcaagguuguaga gcccacugccgagguuguuguaugccugggaggc	2.8	-1.3	2.5	0	83	80
ta_iwgsc_2bl_v1_2544267_131709	tae-miR9652-3p	tae-miR9652-3p, tae-miR9652-5p	--	20	aagcuuaaugagaacaugug	ucaccuguuugucauuaaguuucuuagcgccuuugccauc cuagugacuagugaaaggucaaaagguacuuagaagcuua augagaacaugugacaaacguauuucauaac	1.9	-1.3	1.6	0	94	87
ta_iwgsc_2al_v1_6372865_101073	tae-miR9652-3p	tae-miR9652-3p, tae-miR9652-5p	--	20	aagcuuaaugagaacaugug	ucaccuguuugucauuaaguuucuuagcgccuuugccauc cuagugacuagugaaaggucaaaagguacuuagaagcuua augagaacaugugacaaacguauuucauaac	1.9	-1.3	1.6	0	94	87
ta_iwgsc_2bl_v1_7960512_138722	tae-miR9652-3p	tae-miR9652-3p, tae-miR9652-5p	--	20	aagcuuaaugagaacaugug	guguuugucgccuguuugucauuaaguuucuuagugccuu ugccauccuagugaaaggucaaaagguacuuagaagcuua augagaacaugugacuaacguacuuugaaac	2.3	-1.3	2	0	96	87
ta_iwgsc_2dl_v1_9828429_188164	tae-miR9652-3p	tae-miR9652-3p, tae-miR9652-5p	--	20	aagcuuaaugagaacaugug	guguuugucaccuguuugucauuaaguuucuuagugccuu ugccaucuuagugaagggucaaaagguacuuagaagcuua augagaacaugugacaaacguacuucgaggc	2.5	-1.3	2.2	0	94	87
ta_iwgsc_2al_v1_6416280_107395	ta_iwgsc_2al_v1_6416280_107395	novel	--	20	uucgucggacgagcgugccu	auggcggcaugguggccccguucgucggacgagcgugccu aggcaucggccgcgcgcuccuccgucgaacggcaccugcc gugucgcacgcaugcaguuaggcggcgggg	75.1	3.9	2.6	0	143	142
ta_iwgsc_2al_v1_6419642_107902	ta_iwgsc_2al_v1_6419642_107902	novel	--	20	uucgucggacgagcgugccu	auggcggcaugguggccccguucgucggacgagcgugccu aggccaucggccgcgcgcuccuccgucgaacggcgccugc cgugucgcgcgcaugcaguuaggcggcgg	75.2	3.9	2.8	0	143	142
ta_iwgsc_4bs_v1_4896990_397564	ta_iwgsc_4bs_v1_4896990_397564	novel	--	21	cgcggcuccgucgacuggugc	gcagcagcggcggaagagaacgacgccggugggcgccuuu cgucggauucgcgugcaaaucaagguugcgcgcggcuccg ucgacuggugcucgucgguguccgcgaggcg	47.4	3.9	2.5	0	89	72
ta_iwgsc_1ds_v1_1900880_84979	ta_iwgsc_1ds_v1_1900880_84979	novel	--	21	cgcggcuccgucgacuggugc	gcagcagcggcggaagagaacgacgccggugggcgccuuu cgucggauucgcgugcagaucaagguugcgcgcggcuccg ucgacuggugcucgucgguguccuucgcga	48.4	3.9	2.5	0	91	72
ta_iwgsc_1al_v2_3909165_6487	ta_iwgsc_1al_v2_3909165_6487	novel	--	21	ugauugagccgcgccaauauc	aaaugaaaucgcagcugcgguauuggcucggcucacucag gucaucguguugcagaagcauaugcuucucgguauaaucu gauugagccgcgccaauaucucagugccauu	61.9	3.9	2.2	0	118	73
ta_iwgsc_1bl_v1_3820936_34131	ta_iwgsc_1bl_v1_3820936_34131	novel	--	21	ugauugagccgcgccaauauc	aaaugaaaucgcagcugcgguauuggcucggcucacucag gucaucguguugcagaagcauaugcuucucgguauaaucu gauugagccgcgccaauaucucagugccauu	61.9	3.9	2.2	0	118	73

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_4bl_v1_7008051_382875	ta_iwgsc_4bl_v1_7008051_382875	novel	--	21	ucuguaaagaaauauaagagc	uagcuauacauuuguucuacagacuaguacuccaucuguaaagaaauauaagagcguuacagucuuuauaucucuuuacggagggaguaauagacaaaaaaaaagggacuaug	1.1	-1.3	0.8	0	100	100
ta_iwgsc_7al_v1_4561451_668755	ta_iwgsc_7al_v1_4561451_668755	novel	--	20	cauaucauaugacugagcaa	cauugcggugaugaauuuauguauuuucgcaugucagacuucagagaugccccuggcgucagcgagaauucauaucauaugacugagcaaugaaauaccaucuuuaauau	0.7	-1.3	0.4	0	126	123
ta_iwgsc_6ds_v1_2075519_642817	ta_iwgsc_6ds_v1_2075519_642817	novel	--	21	ucgcuuggugcagaucgggac	ccgucgccgccgccucgggcucgcuuggugcagaucgggacccucagcccgccccgacgggccggaucccgccuugcaccaagugaaucggagccggcgcagcgaccacgc	69	3.9	2.7	0	131	97
ta_iwgsc_2bs_v1_5170420_162412	ta_iwgsc_2bs_v1_5170420_162412	novel	--	21	ucuguaaacugauauaagagc	nnnnnnnnnnnnnnnauucccucuguaaacugauauaagagcguuacgcucuuauauuaguuuauggagggaguauuauuuauguauuauuuucuuccuuauuugugcaaugug	2.1	-1.3	1.8	0	57	56
ta_iwgsc_5dl_v1_4557929_540612	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cgguagcagcuguugagaugagauuaccccauacccugcagagagcgucagcucgacgccguacacgcguauggguaaucucaacucaacagccgcucccugugagguuu	55.5	3.9	2.5	0	105	103
ta_iwgsc_5bl_v1_10926395_508865	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cuuuugcucucgguagcagcuguugagaugagauuaccccauacccugcagagagcgucagcuccgacgccguacacgcguauggguaaucucaacucaacagccgcuccc	2.6	-1.3	2.3	0	103	103
ta_iwgsc_5bl_v1_10926542_508919	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cuuuugcucucgguagcagccguugagaugagauuaccccauacccugcagagagcgucagcccgacgccgcacaugcguauaggguaaucuuaccucaacagccgcuccc	2.4	-1.3	2.1	0	103	103
ta_iwgsc_4al_v2_7122935_348714	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cuuuugcucucgguagcagccguugagaugagauuaccccauacccuggagcgucagcucgaugccguacacgcguaugggguaaucucaccucaacagccgcucccugug	2.6	-1.3	2.3	0	103	103
ta_iwgsc_5dl_v1_4544441_538060	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cuuuuucucucugcagcggccguugagaugagauuaccccauacccuguagcggcagcucgacuccgugcaugcguagggggcaaucucaccucaacagcugcucccagug	2.4	-1.3	2.1	0	104	104
ta_iwgsc_5dl_v1_4559949_541033	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cuuuugcucacuguagcggccguugagaugagauuaccccauaccauauagcggcagcucgacuccgcacacgcguagggggcaaucucaccucaacagcuucuccgugug	2.2	-1.3	1.9	0	103	103
ta_iwgsc_5bl_v1_10802619_478679	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cuuuuucucucuguagcggccguugagaugagauuaccccauacccuauagcggcagcucgacuccgcacacgcguagggggcaaucucaccucaacagcugcucucagug	2.4	-1.3	2.1	0	103	103
ta_iwgsc_5dl_v1_4577478_544606	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	cgguagcagcgguugagaugagauuaccccauacccugcagagagcgucagcucgacaccguacacgcguauggguaaucucaacucaacagccgcuccaugugagcuuu	55.3	3.9	2.2	0	105	103
ta_iwgsc_4al_v2_7073985_340626	tae-miR9772	tae-miR9772	--	21	ugagaugagauuaccccauac	uacuugugcucucuagcagcuguugagaugagauuaccccauaccccgcggagagcgugagcucgacgccguacaugcguaugggguaaucucaacucaacagccgcuccc	55.5	3.9	2.5	0	105	103
ta_iwgsc_1ds_v1_1899319_84656	ta_iwgsc_1ds_v1_1899319_84656	novel	--	24	uuaaggauaugagucgucacuaug	cuucggucuucauuacacucuuaaggauaugagucgucacuaugaaucuucaccucuuccgggcagagaagguccauagugacacuccauauuccucaugagagcaaucau	36.1	3.9	1.4	0	69	60
ta_iwgsc_1dl_v1_2289383_75664	ta_iwgsc_1dl_v1_2289383_75664	novel	--	24	cauggccaaggccucugagguucg	cauggcauguaucccacggccauggccaaggccucugagguucgcgugaccuuuuaggccucgcccauggcuaugguguucauggucuuauuugucuaucagaucuagu	2.2	-1.3	1.9	0	273	272
ta_iwgsc_5al_v1_2661813_441885	ta_iwgsc_5al_v1_2661813_441885	novel	--	24	auuuguaguguucggauugaguuu	aaaccacuuuugcaaacuuagucuuggcacuauaaauuuaaaaugugcaugugaaauaugcucauuauaaauuuguaguguucggauugaguuuauaauagucguucu	2.2	-1.3	1.9	0	85	85
ta_iwgsc_5dl_v1_4495350_527831	ta_iwgsc_5dl_v1_4495350_527831	novel	--	24	auuuguaguguucggauugaguuu	accacuuuugcaaacuuaguccuggcacuauaaauuuaaaaaugugcaugugaaaauaugcucauuauaaauuuguaguguucggauugaguuuauaauagucguucu	2.2	-1.3	1.9	0	85	85
ta_iwgsc_5bl_v1_10917894_502681	ta_iwgsc_5bl_v1_10917894_502681	novel	--	24	auuuguaguguucggauugaguuu	aaccacuuuugcaaacuuagucuuggcacuauaaauuuaaaaugugcaugugaaaauaugcucauuauaaauuuguaguguucggauugaguuuauaauagucguucu	2.3	-1.3	2	0	85	85
ta_iwgsc_4dl_v3_14400158_417314	ta_iwgsc_4dl_v3_14400158_417314	novel	--	21	uuucuucuuccucuuccuacc	aggaggaggaaggaggaagaagaggguuuugcuacguggcauggaauucauggcauaguugguucuuucuucuuccucuuccuaccucugccucuugcucuuccuucuucg	35.2	3.9	2	0	66	65
ta_iwgsc_2al_v1_6418433_107731	ta_iwgsc_2al_v1_6418433_107731	novel	--	21	uggacgaggaugugcagcugc	guacgguccugguuagaguuuggacgaggaugugcagcugcgguaggacguucagcuacgaccggcagcugcacauccacuuccaagcgcuagcuaggaucgucaacaacg	3	-1.3	2.7	0	43	43

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_2al_v1_6363060_99722	ta_iwgsc_2al_v1_6363060_99722	novel	--	21	uggacgaggaugugcagcugc	guacgguccugguuagaguuuggacgaggaugugcagcugcgguaggacguucagcuacgaccggcagcugcacauccacuuccaagcgcuagcuaggaucgucaaca	3	-1.3	2.7	0	43	43
ta_iwgsc_2dl_v1_9907038_200671	ta_iwgsc_2dl_v1_9907038_200671	novel	--	21	uggacgaggaugugcagcugc	guacgacccuaguuagaguuuggacgaggaugugcagcugcgguaggacucucagcuacgaccggcagcugcacauccacguccaagcgcuagcuaggaucgucaacaacg	3	-1.3	2.7	0	43	43
ta_iwgsc_2bl_v1_8012957_144903	ta_iwgsc_2bl_v1_8012957_144903	novel	--	21	uggacgaggaugugcagcugc	guacgauccuaguuagaguuuggacgaggaugugcagcugcgguaggacguucagcuacgaccggcagcugcacauccacuuccaagcgcuagcuaggaucgucaacaacg	3	-1.3	2.7	0	43	43
ta_iwgsc_6bs_v1_2953939_613069	ta_iwgsc_6bs_v1_2953939_613069	novel	--	24	cucuggucagccaaguagggcuuc	gcuuucucuggucagccaaguagggcuucgcccagucaacaaggcucuguugcccguucggacgguuggguccagcgcucgcuuaccagacagcaucu	1.8	-1.3	1.5	0	143	77
ta_iwgsc_4as_v2_5986012_369720	ta_iwgsc_4as_v2_5986012_369720	novel	--	20	ggcggacguagccaagugga	aauccuuuagcuggauaaggggcggacguagccaaguggaucaaggcaguggauugugaauccaccaugcgcgguucaauucccgucguucgcccaucgcauuauug	23.8	3.9	1.6	0	52	50
ta_iwgsc_4as_v2_5969649_367860	ta_iwgsc_4as_v2_5969649_367860	novel	--	20	ggcggacguagccaagugga	aauccuuuagcuggauaaggggcggacguagccaaguggaucaaggcaguggauugugaauccaccaugcgcgguucaauucccgucguucgcccaucgcauuauug	23.8	3.9	1.6	0	52	50
ta_iwgsc_1al_v2_3976442_15235	ta_iwgsc_1al_v2_3976442_15235	novel	--	21	cauggccaaggccucugaggu	auggcauguaucccacggccauggccaaggccucugaggucgcgugaccuuuuaggccucgcccauggcuaugguguucauggucuuauuuguccaucagaucuaucuaaa	2.3	-1.3	2	0	77	76
ta_iwgsc_1ds_v1_1895716_84068	ta_iwgsc_1ds_v1_1895716_84068	novel	--	24	caaguauuuccggacggagggagu	aggcugcugcuuugccucauaaaauagauuauacucccuccguccagaaauacugccaucaaaauguuugauggcaaguauuuccggacggagggaguacuaucuguaaag	2.9	-1.3	2.6	0	161	161
ta_iwgsc_1bl_v1_3855386_38625	ta_iwgsc_1bl_v1_3855386_38625	novel	--	24	caaguauuuccggacggagggagu	nnnnnnnnnnnnnnnnnnnnncuacuacucccuccguccguagauacaucucauuuuauccauuuugaugccaaguauuuccggacggagggaguaguacuuaagaaacag	2.2	-1.3	1.9	0	159	159
ta_iwgsc_7as_v1_4233997_678927	ta_iwgsc_7as_v1_4233997_678927	novel	--	24	ucuucggcucagugcugagcgugu	ggcagcagcgguguugggguucuucggcucagugcugagcguguucgucacacgguacaccacggcgccucuggacuacgccgccgagcnnnnnnnnnnnnnnnnnnnnnnnnn	1.4	-1.3	1.1	0	77	77
ta_iwgsc_1as_v1_1364010_17358	ta_iwgsc_1as_v1_1364010_17358	novel	--	21	acaaguauuuucggacggagg	uaaguuauacucccuccauccgaaaauacuugucuucaaaauagauaaaauggaauguaucuaauuugaagacaaguauuuucggacggagggaguacucacuagaaua	2.7	-1.3	2.4	0	112	111
ta_iwgsc_5bs_v1_2270715_518588	ta_iwgsc_5bs_v1_2270715_518588	novel	--	21	acaaguauuuucggacggagg	uaaguuauacucccuccauccgaaaauacuugucuucaaaauagauaaaauggaauguaucuaauuugaagacaaguauuuucggacggagggaguacucacuagaaua	2.7	-1.3	2.4	0	112	111
ta_iwgsc_3al_v1_4438896_237342	ta_iwgsc_3al_v1_4438896_237342	novel	--	18	ucacuguuuggaauggua	ugcuaccugagcaaacuugccuuuccugcagccauccuagccccacgauuuaggacgagugggccggagaucacuguuuggaaugguaggacugccuagguagcacaccaua	2.4	-1.3	2.1	0	114	114
ta_iwgsc_3dl_v1_6952438_318170	ta_iwgsc_3dl_v1_6952438_318170	novel	--	18	ucacuguuuggaauggua	ugcuaccugagcaagccugccuuuccugcagccauccuagccccacgauuuaggacaugugggcugagaucacuguuuggaaugguaggacugcccagguagcaaaccaua	2.8	-1.3	2.5	0	114	114
ta_iwgsc_5dl_v1_3183779_524821	ta_iwgsc_5dl_v1_3183779_524821	novel	--	24	agcagcagguccauaacgcagugg	guaugaccaacaacaaccacagcagcagguccauaacgcagugguagcucuaccacugcgcuauggacccacugcuuuugcuguuguuagagcaauugacugcagaucaauagg	31.7	3.9	2.1	0	59	40
ta_iwgsc_6bs_v1_1636307_609064	tae-miR9663-5p	tae-miR9663-5p	--	21	aagcguagucgaacgaaucug	aagagaagcuuggucaucuaaagcguagucgaacgaaucugccguugaugaggauauguucagauucguucuauuacacuuuacaugagcagacuacucacccauggu	1.9	-1.3	1.6	0	44	44
ta_iwgsc_2ds_v1_1133030_204840	ta_iwgsc_2ds_v1_1133030_204840	novel	--	21	uugaggaugggaugauuuaga	uagucuaaaucaucccauccucaacauucuuucacagccauggccaagcuacauauauugugaaaguauauugaggauggggaugauuuagacaaaaagcucugguaauac	2.5	-1.3	2.2	0	31	31
ta_iwgsc_3b_v1_10476564_262267	ta_iwgsc_3b_v1_10476564_262267	novel	--	24	agcagaggauauguucaugagaug	ugacgauuguggcaguuguguggugacaacuacaaggauucgcucuguugcggaugacuguuacaaacacagcagaggauauguucaugagaugaaaccacuaaauggccaaga	1.6	-1.3	1.3	0	35	35
ta_iwgsc_7bs_v1_3090810_711519	ta_iwgsc_7bs_v1_3090810_711519	novel	--	21	aucuaggacaaguaauucggg	ugaucacuagcaauuucguaauuccucugucccaaaagcuugucuuagauuuuucuagauauggauguaucuaggacaaguaauucgggauggcgggaguaguuuagua	1.8	-1.3	1.5	0	31	31
ta_iwgsc_3as_v1_3368216_248077	ta_iwgsc_3as_v1_3368216_248077	novel	--	21	cuuaugacuucucuuagacag	ugaaacacugugugaaagaacucauaggugugaaacacucuccgaugaugaugacguagcugcaaacacuuaugacuucucuuagacaguguuucacacucuaccagc	1.5	-1.3	1.2	0	53	53

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_4al_v2_6019133_336852	ta_iwgsc_4al_v2_6019133_336852	novel	--	21	cuuaugacuucucuuagacag	ugaaacauugugugagagaagucauaagugugaaacacucuacgaugaugaugacgaagcugcaaacacuuaugacuucucuuagacaguguuucacacuccaccagcagc	30	3.9	2	0	56	53
ta_iwgsc_1as_v1_2129014_18194	ta_iwgsc_1as_v1_2129014_18194	novel	--	21	uugugcuugaucuaaccaugu	agucgcugcggggcuuuccuuugugcuugaucuaaccaugugguggaacgauggaaacggaacaugguucugucaagcaccgcggaaagcaccgugcucuccugcagca	2.4	-1.3	2.1	0	30	30
ta_iwgsc_4bl_v1_7036829_387971	ta_iwgsc_4bl_v1_7036829_387971	novel	--	21	uagaauggcuggugcuaugga	uuugacuuguccauagcaucauccauccugccauucugcugagguagcugcugcccuuuugcacgaugguagaauggcuggugcuauggaugaauucgaccagauggc	22	3.9	2.1	0	40	25
ta_iwgsc_2dl_v1_9896699_198560	ta_iwgsc_2dl_v1_9896699_198560	novel	--	18	acuguacucacugauggc	aucgguguuuccagggcacuacuguacucacugauggcguugagagagaccagaacgucuucucgagugcgguggcgaggagggcuuucccuugccuggaacgaagca	2.1	-1.3	1.8	0	25	25
ta_iwgsc_1dl_v1_2291843_77257	ta_iwgsc_1dl_v1_2291843_77257	novel	--	20	agggggacuucuuacuuaaa	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnagacuaauuuaaguaagaagucucccagauagggggacuucuuacuuaaaugaaauuauuuuauucuuuu	74.9	3.9	0.5	0	147	128
ta_iwgsc_6al_v1_5814619_573477	ta_iwgsc_6al_v1_5814619_573477	novel	--	24	cagacguguagcgucuaucuguga	gucuggggugcaacgcuggccagacguguagcgucuaucugugaggcguugcacaguauaguguggcgguuucgugucagacgucacacaaaaggccagccgcgugaaauaguu	2.2	-1.3	1.9	0	26	25
ta_iwgsc_6bl_v1_4272750_597574	ta_iwgsc_6bl_v1_4272750_597574	novel	--	20	ugggccuguagcucagagga	ucgguaucagugaagucgguuaggaugccuccgcugcauacaucacugcacuucauccaguggagacagcugggccuguagcucagaggauuagagcccucuucacugaa	2.2	-1.3	1.9	0	40	40
ta_iwgsc_3b_v1_10612331_274290	ta_iwgsc_3b_v1_10612331_274290	novel	--	24	ucaacaaggcugcuagugaacugc	ucauuauggagauuugcaaacagccuuuaggauggaucaucccuaggucucugccuagugaaagcauucaacaaggcugcuagugaacugcccgcuaaugugacauauccu	0.8	-1.3	0.5	0	90	86
ta_iwgsc_4bl_v1_6981217_379591	ta_iwgsc_4bl_v1_6981217_379591	novel	--	21	ucaucuuguggaaauauucug	aggaauaccacugaguacuuccauaagaugaccaguuacccgaugaccagcauggugucggcguaaccggucaucuuguggaaauauucugugggcaccguguguuuc	2.7	-1.3	2.4	0	39	39
ta_iwgsc_5al_v1_2769943_452200	ta_iwgsc_5al_v1_2769943_452200	novel	--	21	ucaucuuguggaaauauucug	aggacugucaccgaguacuuccauaagauaaccaguuacccgacgaccaacauggugucagcguaaccggucaucuuguggaaauauucuguggacaccaugucuuuaugu	2.3	-1.3	2	0	39	39
ta_iwgsc_4bl_v1_6987671_380418	ta_iwgsc_4bl_v1_6987671_380418	novel	--	21	ucaucuuguggaaauauucug	gggacugucacugaguacuuccauaagauaaccaguuaccugacgaccaacauggugucagcguaaccggucaucuuguggaaauauucuguggacaccaugucuuucucu	2.5	-1.3	2.2	0	39	39
ta_iwgsc_5dl_v1_4516462_532507	ta_iwgsc_5dl_v1_4516462_532507	novel	--	24	agauguacuaucacuuugaauguu	cauuacacucuuguuaaggagauguacuaucacuuugaauguucaccugucucgagcagagaacauucaaagugauaggccaucuucuuaauggugga ugccgguguuuuuuu	2.5	-1.3	2.2	0	25	25
ta_iwgsc_2bs_v1_5203117_168082	ta_iwgsc_2bs_v1_5203117_168082	novel	--	24	agauggaguaucacuuugaauguu	ucauuacagucuugcuaaggagauggaguaucacuuugaauguucacaugucucgagcggagaacauucaaagugauaggccaucuucuuaaugguggaugucgguggu uuuuu	2.3	-1.3	2	0	23	23
ta_iwgsc_7ds_v1_3935183_753242	ta_iwgsc_7ds_v1_3935183_753242	novel	--	24	agauggacuaucaauuugaauguu	uucauuacacucuuguuaaggagauggacuaucaauuugaauguucacuugucuugauacgagaacauucaaagugauacgccaucuucuuaauggaggaugccgacguuuuuu	2.1	-1.3	1.8	0	23	23
ta_iwgsc_2bs_v1_5202683_168029	ta_iwgsc_2bs_v1_5202683_168029	novel	--	24	agauggacuaucaauuugaauguu	uucauuacacucuuguuaaagagauggacuaucaauuugaauguucaccucucucgagcagagaacauucaaagugauagaccauauucuuaaugguggaugcuggcguuuuuu	1.6	-1.3	1.3	0	23	23
ta_iwgsc_6as_v1_4385667_585667	tae-miR397-5p	tae-miR397-5p	--	21	ucaccggcgcugcacacaaug	gaagagacgcaaaggugccauugagugcagcguugaugaaccguccuacuccuccgaggccggagcgguucaccggcgcugcacacaaugacggcucugcuuucuccggcg	21.2	3.9	2.4	0	38	28
ta_iwgsc_7bl_v1_6642049_692138	ta_iwgsc_7bl_v1_6642049_692138	novel	--	21	aggacaaguauuuccggacgg	caagguggauauauauuacguaugagaaaaaugcaaugaaguacucccuccguccagaaauacuugucuucuagauacaaccauuucgaggacaaguauuuccggacggaagg	2	-1.3	1.7	0	42	41
ta_iwgsc_7bl_v1_6642049_692137	ta_iwgsc_7bl_v1_6642049_692137	novel	--	21	aggacaaguauuuccggacgg	cguccagaaauacuugucuucuagauacaaccauuucgaggacaaguauuuccggacggaaggaguaugagguaucucguucccggucuaugagccacgaccgucucgucaca	2.3	-1.3	2	0	42	41
ta_iwgsc_1dl_v1_2203114_61853	ta_iwgsc_1dl_v1_2203114_61853	novel	--	21	aucuauuuuggaacggaggga	ucagcccauguuuuggccuaauggcuuguuagcgacuuccuccguuccaaaauaaaugacuuaacuuuguacuaauugucaucuauuuuggaacggagggagnnnnnnnnnnn	2.2	-1.3	1.9	0	56	56
ta_iwgsc_2dl_v1_9884245_196663	tae-miR171b	tae-miR171b	--	21	uugagccgugccaauaucacg	uccugcgaggggagugaacgcgguauuggugcgguucaaucagagagcuggcgccccaggaggcaaggguuccucccuucgauugagccgugccaauaucacgcgguuuc	13	3.9	2.3	0	22	21

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_2al_v1_6339230_96306	tae-miR171b	tae-miR171b	—	21	uugagccgugccaauaucacg	cguaccugcgaggggagugaacgcgguauuggugcgguuc aaucagagagcuggugccccaggagacaagggguuccgcc cuucgauugagccgugccaauaucacgcgguuuc	13.1	3.9	2.3	0	22	21
ta_iwgsc_3as_v1_3441441_251919	tae-miR9673-5p	tae-miR9673-5p	—	20	uaagaagcaaauagcacaug	uccaugcggggaaucaccucgagguaagaagcaaauagca caugcaggagguaagaagcaaacgaaugugugugcucuuu ccuucuuacccucgagccgacucccggaacc	13.7	3.9	2	0	24	18
ta_iwgsc_2bl_v1_8059865_151225	ta_iwgsc_2bl_v1_8059865_151225	novel	—	20	ugaaguguuugggcgaacuc	gcaagacauguuugguauuaccaugguucccugcaagca cuucaugaggcauuguuccaaaugccacugugaaguguuu gggcgaacucuuggcgaugccaagcuuuac	2.5	-1.3	2.2	0	22	22
ta_iwgsc_2bl_v1_7981518_141271	ta_iwgsc_2bl_v1_7981518_141271	novel	—	20	ugaaguguuuggggaaacuc	gcaaaucacguuugguauuaccaggaguuccuuucaaaca cuuuauggaucaccuuuccgaggucucacgugaaguguuu ggggaaacucuugcugcaaccaagcacuua	2.2	-1.3	1.9	0	22	22
ta_iwgsc_2bs_v1_5224439_172196	ta_iwgsc_2bs_v1_5224439_172196	novel	—	23	cgacccaaacggacgaaaaacgg	ggauauggacgagcggacgggcgacccaaacggacgaaaa acggacaaaaucgauguccguuuggucaccccguuggag uugcccuaacuugucauauccagggggcgaagc	2.4	-1.3	2.1	0	63	63
ta_iwgsc_5dl_v1_4606464_551851	ta_iwgsc_5dl_v1_4606464_551851	novel	—	23	cgacccaaacggacgaaaaacgg	ugccagcauuggacacauggacgacccaaaaagaaaacug ggcagacaccgagcgggcggccgacccaaacggacgaaaa acggacaaaaucaccguccgcuugggucggcg	2.3	-1.3	2	0	44	44
ta_iwgsc_5dl_v1_4606464_551852	ta_iwgsc_5dl_v1_4606464_551852	novel	—	23	cgacccaaacggacgaaaaacgg	gagcgggcggccgacccaaacggacgaaaaacggacaaaa ucaccguccgcuugggucggcgcguugaaguugcucuuau uccacauguuuuaccauuaaagaaaucguuug	2	-1.3	1.7	0	44	44
ta_iwgsc_3b_v1_10581855_271803	ta_iwgsc_3b_v1_10581855_271803	novel	—	24	aaaauuggauugcaugaggaagug	gcgaauauugaugucauaugcauggaugguauuccacaug caaaagagcaagggguuaagacaccccugcaaaauuggau ugcaugaggaagugccgggguuaagacacccca	2	-1.3	1.7	0	52	52
ta_iwgsc_4al_v2_7045630_338345	ta_iwgsc_4al_v2_7045630_338345	novel	—	21	ugagacgagaucuccccauac	uugcucccuuuggcggccguugagacgagaucuccccaua ccacauagcggcagcucggcuccgugcacgcguauggga gaucuugccucaacagccgcuccgugugagc	2.9	-1.3	2.6	0	31	31
ta_iwgsc_5dl_v1_4575265_544124	ta_iwgsc_5dl_v1_4575265_544124	novel	—	21	ugagacgagaucuccccauac	uugcucccuguggcggccguugagacgagaucuccccaua ccacauagcggcagcucggcuccgugcacgcguauggga gaucucgccucaacagccgcuccgugug	2.8	-1.3	2.5	0	31	31
ta_iwgsc_3as_v1_3304528_245584	ta_iwgsc_3as_v1_3304528_245584	novel	—	21	gaucggcgaguuguucuuggc	ggggacuauggugcagccaaggaugacuugccgaucgaau aaugauacaucaucgucauauacauugaucgaucggcgag uuguucuuggcuacgucuuagcuccugcucc	22.7	3.9	2.3	0	41	28
ta_iwgsc_3b_v1_969170_298450	ta_iwgsc_3b_v1_969170_298450	novel	—	21	gaucggcgaguuguucuuggc	ggacuauggugcagccaaggaugacuugccgaucgaacaa ugauacaucaucaucauauacauugggaacgaucggcgag uuguucuuggcuacaucuuagcuccugcucc	14.5	3.9	2.3	0	25	19
ta_iwgsc_5dl_v1_4608197_552566	ta_iwgsc_5dl_v1_4608197_552566	novel	—	21	acaagccgacuuuagcucagu	gcauucuggauauauguucggaaugauucuccgcuagccc uuagauaaguaagcuguguucguccguuaaacacaagccg acuuuagcucaguugguagagcggaggacugua	1.9	-1.3	1.6	0	138	138
ta_iwgsc_7ds_v1_3852191_743827	ta_iwgsc_7ds_v1_3852191_743827	novel	—	21	ugugacggugucuucuuguga	gccauugcuuuccuuguccuugugacggugucuucuugug augguccuuaugguguugucuucuuccacgccauggcaag gcaaaagaagacaucaucauaaggaccggua	2.2	-1.3	1.9	0	19	15
ta_iwgsc_2bs_v1_5171660_162577	ta_iwgsc_2bs_v1_5171660_162577	novel	—	21	aguauuuccggacggagggag	cucccuccguccggaaauacaucucauuuuauucauauug acgaggaaauacaucucauuuuauccauuuugacgaagag uauuuccggacggagggaguaguuagaggac	2.3	-1.3	2	0	42	42
ta_iwgsc_2bl_v1_7987776_141982	ta_iwgsc_2bl_v1_7987776_141982	novel	—	21	aguauuuccggacggagggag	ccaggguggggccgcggcccacccuggacaccuuguagcuc cgccccugugucuagauacauccauuuccuugacgaguau uuccggacggagggaguaguaucuaaauuau	2.3	-1.3	2	0	52	52
ta_iwgsc_5bl_v1_10924917_507838	ta_iwgsc_5bl_v1_10924917_507838	novel	—	21	ugaaguagagcagagaccuca	auaacaagggcauuucgacguuggggaagaugaaguagag cagagaccucacgcucaagaagcggugaggugccugaccu acuucaucuucaccaacgaagagaacggc	2.6	-1.3	2.3	0	25	23
ta_iwgsc_5bs_v1_2294743_521969	ta_iwgsc_5bs_v1_2294743_521969	novel	—	20	aguuacagacuguauccauc	cacuuugugcuggauaugacugucacuaugaaaguugucu gcaugcauaugauaagcagauaacuuucauaguuacagac uguauccaucauuaagugcauugcugau	2.4	-1.3	2.1	0	35	35
ta_iwgsc_4al_v2_7176460_360578	tae-miR9774	tae-miR9774	—	22	caagauauugguauuucuguc	agaaagauuaagaagaaaaaaagauucaaaauaaagacag aaauaccuaauaucuuguucuagcaagauauugguauuu cugucuucaaaaauucuuauaucuuagu	2.1	-1.3	1.8	0	92	92
ta_iwgsc_2bl_v1_8036763_148264	ta_iwgsc_2bl_v1_8036763_148264	novel	—	21	uuccaaaggaucgcauugau	uucaggaagcuaguggaggauuccaaaggaucgcauuga ucgaucucuccgugcaaggucgauggaucagugcaaucc ucuggaauucuccgcuugccuccgccuccgu	17.3	3.9	2.5	0	30	18

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_2dl_v1_9826058_187814	ta_iwgsc_2dl_v1_9826058_187814	novel	--	21	uuccaaagggaucgcauugau	uucaggaagcuaguggaggauuccaaagggaucgcauugaucgaucucuccgucaucggcgugcaaggucgauggaucagugcaaucccucuggaauucuccgcucgccuc	17.2	3.9	2.4	0	30	18
ta_iwgsc_2al_v1_6315928_93023	ta_iwgsc_2al_v1_6315928_93023	novel	--	21	uuccaaagggaucgcauugau	uucaggaagcuaguggaggauuccaaagggaucgcauugauccaucucuccguaaccggcgcucaagaucgauggaucagugcaaucccucuggaauucuccccuugccuc	18.2	3.9	2.4	0	32	20
ta_iwgsc_4al_v2_7170245_357219	ta_iwgsc_4al_v2_7170245_357219	novel	—	24	uauuuggaggauaugcaagaugag	cgugggucuuccuugcacucuauuuggaggauaugcaagaugaguaugauuuaucaucuguauucaucguguauauucuucaaguggagagcauggaugacugauguauuuuuu	11.1	3.9	2.4	0	18	16
ta_iwgsc_5al_v1_2802373_457545	ta_iwgsc_5al_v1_2802373_457545	novel	--	24	aguuggggaguguauauuagacgg	aaauuguggccaguuuugagaguuggggaguguauauuagacgguauugaaguugggggguguauauuagacuaggccuagaguugggggugucauaugcacuucucucauuuc	1.2	-1.3	0.9	0	14	14
ta_iwgsc_2as_v1_5241552_121429	ta_iwgsc_2as_v1_5241552_121429	novel	--	24	gcucugccgcagcugcccggacag	auugcccgggcagcucugccgcagcugcccggacaguguuacacuguuugggcagcugcgguagugcugcuagugcauuguaauucagaucacaucauuauuauguaucau	11.2	3.9	2.5	0	18	15
ta_iwgsc_1al_v2_3954947_11882	ta_iwgsc_1al_v2_3954947_11882	novel	--	24	gcucugccgcagcugcccggacag	uugcauugcccgggcagcucugccgcagcugcccggacaguguuacauuguucgggcagcugcgguagugcugcuagugcauuguaaauucagaucacaucauuauuauguauc	10.3	3.9	2.7	0	16	15
ta_iwgsc_7ds_v1_3863327_745034	tae-miR9667-5p	tae-miR9667-5p	—	21	aaauauggcaaacaaugaaug	caaguaauaaaugaaaaucuaaauauggcaaacaaugaaugagaauccaaagauaggggguuaucuuuggauuuucauuuauuguuuuccaucuuugggauuucauuuagu	8.4	3.9	2.2	0	13	12
ta_iwgsc_7bs_v1_3068904_709603	tae-miR9667-5p	tae-miR9667-5p	--	21	aaauauggcaaacaaugaaug	caaguaauaaaugaaaaucuaaauauggcaaacaaugaaugaaaauccaaagauagguuaucuuuggauuuuuuucucauuuauuguuauccaucuuugggauuucauuu	2.1	-1.3	1.8	0	12	12
ta_iwgsc_7bs_v1_3084852_711041	tae-miR9667-5p	tae-miR9667-5p	--	21	aaauauggcaaacaaugaaug	caaguaauaaaugaaaaucuaaauauggcaaacaaugaaugagaauccaaagauagguuaucuuuggauuuucauuuauuguuuuccaucuuugggauuucauuuacacu	8.3	3.9	2.2	0	13	12
ta_iwgsc_3ds_v1_2047945_323559	ta_iwgsc_3ds_v1_2047945_323559	novel	—	21	ucuuauauugugggacggagg	uuuacugcauuauaguacucacuccgucucacaauguaugacguuuuugcaagcaaaaacagcuugcaaaaacaucuuauauugugggacggagggaguaggccuguaaac	2.7	-1.3	2.4	0	45	44
ta_iwgsc_1bl_v1_3750335_29214	tae-miR5175-5p	tae-miR5175-5p	--	21	uuccaaauuacucgucguggu	uacuacucccuccguuccaaauuacucgucgugguuuugaacuagaaccacgacgaguaacuuggaacagagggaguauuuguuaugggaagugcuaaaacucagucgacuga	2.7	-1.3	2.4	0	31	29
ta_iwgsc_3dl_v1_6953294_318861	ta_iwgsc_3dl_v1_6953294_318861	novel	--	21	augacaaguauuucgggacgg	aaaauuaaacagaacuacuuccucugucccaauauacuuguccugguuuuaguucaauugaacuaaaaccaugacaaguauuucgggacggagggaguaauauaaguagc	2.7	-1.3	2.4	0	13	13
ta_iwgsc_6bs_v1_2925635_610820	ta_iwgsc_6bs_v1_2925635_610820	novel	--	21	augacaaguauuucgggacgg	uugucuucauccugaaguaugauguauuuaaugauguacucccuccguacaucuagauacauucauuucaaugacaaguauuucgggacggagggaguacuuuagagucuag	1.6	-1.3	1.3	0	14	14
ta_iwgsc_2bs_v1_5198150_167246	ta_iwgsc_2bs_v1_5198150_167246	novel	—	21	ucaggacguugcaacauuaac	cagaacaagcugauacugguucaggacguugcaacauuaacaccugaacagguguuaauguugcaacguccugaaccaguaucguucuacauccauccacccggggacuga	2.6	-1.3	2.7	0	11	11
ta_iwgsc_2as_v1_5291774_126861	ta_iwgsc_2as_v1_5291774_126861	novel	--	20	cucaggagagaugacaccga	guguggguuagggcccugugcucaggagagaugacaccgacgccgaucagaugggucggcuuaaccaggcgucuguucggaguuggugucaccucgccugaacauagggcu	2.9	-1.3	2.6	0	19	19
ta_iwgsc_7al_v1_4384684_652467	ta_iwgsc_7al_v1_4384684_652467	novel	--	24	ggcucaugccggcuugguaggacg	ugcaauauagaugcgcccguaucgugucauuuggaccguccgugccaucuugccaauagccuccugggggcucaugccggcuugguaggacgacgucgccacaaaagacgca	2.1	-1.3	1.8	0	16	16
ta_iwgsc_3b_v1_10523953_266642	ta_iwgsc_3b_v1_10523953_266642	novel	--	24	cgacgcugcugacuugauacaggc	cgcuagccacuacgccacagcgacgcugcugacuugauacaggcgaaaguuuuauuauacuagcacacacacgcugggccggcagccgggccaagcaaagccugcaauau	1.4	-1.3	1.1	0	12	12
ta_iwgsc_7dl_v1_3342202_727231	ta_iwgsc_7dl_v1_3342202_727231	novel	—	24	aauuaauuucuacgcggacagugu	aagacucuuuggaggucacuaauuaauuucuacgcggacaguguuguuacaccuacgucaauugggaauguggaauucaugcauuguccaagagaaauagauaaugauguggcu	0.8	-1.3	0.5	0	14	14
ta_iwgsc_5bl_v1_1708426_509500	ta_iwgsc_5bl_v1_1708426_509500	novel	--	24	cgacggacgccgagacgagcacgu	gacagcacugcaacccugucuuaacguaacgcgagucgaggcggacgucaucgcgguagcaacgacggacgccgagacgagcacgugacaccac	29.9	3.9	1.1	0	65	56
ta_iwgsc_1al_v2_3950781_11300	tae-miR9664-3p	tae-miR9664-3p	--	21	uugcaguccucgaugucguag	gguuaacgaaaccacggcauagaggcacugcaaacauccgagaugagcaaaauuaagcuugucccaaguuugcaguccucgaugucguaguuuuguuuuuuccaucuauu	2.4	-1.3	2.1	0	15	15

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_1bl_v1_3878449_41408	tae-miR9664-3p	tae-miR9664-3p	—	21	uugcaguccucgaugucguag	ugguuaacgaaaccacggcauagaggcacugcaaacaucc gagaugagcaaacuaagcuuguccguaguuugcaguccuc gaugucguaguuuuguuuuuccaucuauug	2.4	-1.3	2.1	0	15	15
ta_iwgsc_1dl_v1_1024386_57991	tae-miR9664-3p	tae-miR9664-3p	—	21	uugcaguccucgaugucguag	ugguuaacgaaaccacggcauagaggcacugcaaacaucc gagaugagcaaacuaagcuugucccaaguuugcaguccuc gaugucguaguuuuguuuuuccaucuau	2.4	-1.3	2.1	0	15	15
ta_iwgsc_2bs_v1_5203705_168199	ta_iwgsc_2bs_v1_5203705_168199	novel	—	24	uacuauugcuuuggacugaccaau	gauauaugaaaugauugauaguuggaagcauaggugagua gguucuuagugaaguacuaacgaaaaguauuacuauugcu uuggacugaccaauguuauauauaucguuagccu	1.2	-1.3	0.9	0	20	17
ta_iwgsc_5al_v1_1913232_440073	tae-miR9666b-3p	tae-miR9666b-3p	—	22	cgguugggcuguaugauggcga	aauacaaaucauucagauucgccaucauacguccaacugu gcauuugauaugcauacauauauauaugcaucacgagcca cgguugggcuguaugauggcgauaucgauug	15.4	3.9	2.2	0	27	25
ta_iwgsc_5al_v1_2798343_456810	tae-miR9666b-3p	tae-miR9666b-3p	—	22	cgguugggcuguaugauggcga	aauacaaaucauucggauucgccaucauacauccaaccgu gcauuugauaugcauauauaugcaucacgagcaacgguug ggcuguaugauggcgauaccgauuaguuugu	17.2	3.9	2.4	0	30	28
ta_iwgsc_4al_v2_5995168_336837	tae-miR9666b-3p	tae-miR9666b-3p	—	22	cgguugggcuguaugauggcga	aauacaaaucauucggauucgccaucauacauccaaccgu gcauuugauaugcauauauaugcaucacgagccacgguug ggcuguaugauggcgauaccg	17	3.9	2.2	0	30	28
ta_iwgsc_6as_v1_4385493_585636	ta_iwgsc_6as_v1_4385493_585636	novel	—	24	guagacuaguguaguguaaccaug	aagggauccgucguggccgggcguagacuaguguagugua accaugucgcgagaguggagcuccgcgugacaguacaugc acauaguauugccuuuuuaguuaaannnnn	0.7	-1.3	0.4	0	24	24
ta_iwgsc_5bl_v1_10836047_485852	tae-miR6197-5p	tae-miR6197-5p	—	21	ucuguaaacaaauguaggacg	cuacuuccucuguaaacaaauguaggacguuuuuguaguu gaaauuaaacugcaaaaacgucuuauauuuguguucagag guaguacucccuccguccgaaaagcuuguc	2.4	-1.3	2.1	0	15	15
ta_iwgsc_7as_v1_4251563_683335	ta_iwgsc_7as_v1_4251563_683335	novel	—	21	uaagacaagaauuuugggacg	uguacuuucguacucccuccgucucaaaauucuuguuuua aauuagacuccguaucuagacuaauuuaagacaagaauuu ugggacguggcgaauacaagauaaaaaa	2.1	-1.3	1.8	0	31	31
ta_iwgsc_7bs_v1_2069090_708549	ta_iwgsc_7bs_v1_2069090_708549	novel	—	21	uccguccggaaauacuugucg	cuccguccggaaauacuugucgcucaacggauguaucuag acgugcuagauacauccguuugagcgacaaguguuuccag acagagggaguaugauauaaccuaagauguaaug	2.8	-1.3	2.5	0	18	16
ta_iwgsc_7as_v1_4248517_681681	ta_iwgsc_7as_v1_4248517_681681	novel	—	21	uccguccggaaauacuugucg	cuccguccggaaauacuugucgcucaacggauguaucuag acguguuagauacauccguuugagcgacaaguauuuccag acgggggaguaugauauaaccuaagauguaaugc	3	-1.3	2.7	0	23	16
ta_iwgsc_6bl_v1_4402482_607877	ta_iwgsc_6bl_v1_4402482_607877	novel	—	24	aaggccugcauacgacuagaaacc	agcaggagaauucauacaccaaggccugcauacgacuaga aaccuagccguucauggucc aggaugcaggucguggugcc aaauauaucugcugcuaguaggccccacagggca	2.4	-1.3	2.1	0	12	12
ta_iwgsc_3b_v1_10498210_264110	ta_iwgsc_3b_v1_10498210_264110	novel	—	20	ucacuguuuggaaagguagg	ugcuaccugaguaaacuugccuuuccugcagccauccuag ccccacgauuuaggacgaguggggcugagaucacuguuug gaaagguaggacugcccagguagcacacca	2.8	-1.3	2.5	0	26	25
ta_iwgsc_7as_v1_4198132_675331	ta_iwgsc_7as_v1_4198132_675331	novel	—	24	agcugcgacugcgagcauccuaga	guguggaugauucuuuggcuagcugcgacugcgagcaucc uagaauagggcugcacaccauuguuguggacgauguacuc guaguuacaguagcaaguaguuuaaaaacauaca	1.6	-1.3	1.3	0	19	19
ta_iwgsc_6bs_v1_2933828_611337	ta_iwgsc_6bs_v1_2933828_611337	novel	—	21	uccguccggaaauacuugucc	acgguucuucuuuuaaaaauuaaaauauacucccuccguc cggaaauacuuguccuaggcauuucuaggacaaguauuuc cggacugagggaguauauguucuugucaaccuau	11.7	3.9	2.5	0	19	13
ta_iwgsc_7ds_v1_3926074_752239	ta_iwgsc_7ds_v1_3926074_752239	novel	—	21	ucuuacauuaugggacggagg	auggcauaaccuuuggacauauaaucuugcauguuacucc uccgcccauaauguaagauguuuuuuuaacacuacaucuu acauuaugggacggagggaguagauuauauuuug	2.3	-1.3	2	0	26	26
ta_iwgsc_1dl_v1_1338648_59081	ta_iwgsc_1dl_v1_1338648_59081	novel	—	21	uccgucccauaauauaagagc	cguuguuuuccauuaacugaacgaggagugugacuguaaa accaaaacaguaagaaaaguaguacuccuuccgucccaua auauaagagcgcucuuauauuaugagacggaggg	1.8	-1.3	1.5	0	14	14
ta_iwgsc_1dl_v1_1338648_59080	ta_iwgsc_1dl_v1_1338648_59080	novel	—	21	uccgucccauaauauaagagc	guaguacuccuuccgucccauaauauaagagcgcucuuau auuaugagacggagggaguaguacucagugaugguguagg cgccugguaacacccgcacgagaaugaccccgcu	2.6	-1.3	2.3	0	18	14
ta_iwgsc_3b_v1_10573976_271127	ta_iwgsc_3b_v1_10573976_271127	novel	—	21	uccgucccauaauauaagagc	gcuuuuacagcgucgggcagugggguaaucgaacucccucc gucccauaauauaagagcgucuuugacacugcacuaggau gggacggagggaguaauaaauaaaaaug	9	3.9	1.8	0	15	14
ta_iwgsc_2al_v1_6434749_110305	ta_iwgsc_2al_v1_6434749_110305	novel	—	21	uccgucccauaauauaagagc	aauuccacucccuccgucccauaauauaagagcguggugu aguguaaaaaacgcucuuauauuucgggaccgaucgaggg aguaccaugcuaccuacgauaaaauaccauagc	2	-1.3	1.7	0	14	14

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_3dl_v1_6929245_313687	tae-miR1130b-3p	tae-miR1130b-3p	—	21	ucuuauauuaugggacggagg	augcuauuagauuuaaugccaguuacacccagaacuacucccuccgucccauaauauaagagcguuuuucacacuugucagaaacacucuuauauuaugggacggagggagu	19.2	3.9	2.4	0	34	14
ta_iwgsc_3b_v1_10765406_292572	ta_iwgsc_3b_v1_10765406_292572	novel	—	18	ccugugcgaaagaugagc	guauaagcaaacgggcuggcccaucgcgucgcuucggcagcucgccugugcgaaagaugagcacuuugacgcaacgugcgucaaauaggauuucacccaaacccucuugcugc	5.2	3.9	1.6	0	8	7
ta_iwgsc_5bl_v1_10919911_504294	ta_iwgsc_5bl_v1_10919911_504294	novel	—	22	ccucgagaugaacggcgacggc	ggcgacggcgguuccauccaccucgagaugaacggcgacggcagucccauccaccucgagguguacggcaauggcgucccauccaccucgagguguacggcgacggccgucc	5.4	3.9	2.3	0	7	6
ta_iwgsc_5bl_v1_10799490_478046	ta_iwgsc_5bl_v1_10799490_478046	novel	—	21	uugagacgagaucuccccaua	uugcucccuguggcggccguugagacgagaucuccccauaggcccauaccacauagcggcagcucggcuccgugcacgcguauggggagaucucgccucaacagccgc	2.8	-1.3	2.5	0	15	15
ta_iwgsc_7bl_v1_6677650_695716	ta_iwgsc_7bl_v1_6677650_695716	novel	—	24	gcaucugauguggaaccauggggc	uuuuucaccgauuuucuccacggcagcaugcaucugauguggaaccauggggcagcuguccaagcuggaccauuggccaugcccuuacuaguuccaauagugugcauga	1.7	-1.3	1.4	0	13	13
ta_iwgsc_6dl_v1_1937876_623440	ta_iwgsc_6dl_v1_1937876_623440	novel	—	22	cuuccuuggaugucugaugcaa	gcugcauugcaaaugaugacaaaaucaaggaauguuucucccagccaggcgagcuagugcuguuagaaccuuccuuggaugucugaugcaaugcuggacauauauuugcuu	1.4	-1.3	1.1	0	16	16
ta_iwgsc_6bs_v1_2956936_613321	ta_iwgsc_6bs_v1_2956936_613321	novel	—	19	aacagcgucgcuguggcgu	aguggguccggcccguacgcgauaguggcuugugcgucucucguguaauaaguauuccgcaugguucaucaacagcgucgcuguggcguuggggcuagugggcgcacc	2.4	-1.3	2.1	0	13	13
ta_iwgsc_4ds_v1_2274320_428458	ta_iwgsc_4ds_v1_2274320_428458	novel	—	18	uugggccuugggccgggu	cgcuguugggccgggggucuguugggccuugggccgggugcgcuguugggucggggucugcugggcccuaggcuggcuccuggccugccuagcccucgcccgcuugggc	2.7	-1.3	2.4	0	12	12
ta_iwgsc_7al_v1_4463437_656061	ta_iwgsc_7al_v1_4463437_656061	novel	—	24	gaauccguugcuuauguucauugc	acaucuuaagguaguuauuaccaugccguccaugggggugaugaagcgugaagaggcugacgaugccgaugaauccguugcuuauguucauugccuugucauauggcauauu	6.1	3.9	0.7	0	19	11
ta_iwgsc_2bl_v1_1818501_131355	ta_iwgsc_2bl_v1_1818501_131355	novel	—	21	auugaacuaaggaggggugga	ggcaaccuucaacccucuuuaguucaaucaaguggauuaaauuuccaaaauuacagauuuaguucauuugauugaacuaaggagggguggagggcacccucccuaaag	2.6	-1.3	2.3	0	12	12
ta_iwgsc_4al_v2_7112033_346866	ta_iwgsc_4al_v2_7112033_346866	novel	—	23	ccaaaugacagucugauuaaaac	uuauuaucacuuauauccagccaaaugacagucugauuaaaacaugcacgguuauuggcaggaaggaacgugcguguguuggucaggcguuaugagggaccggucgaacgugc	1.9	-1.3	1.6	0	12	12
ta_iwgsc_6bl_v1_4397078_606663	ta_iwgsc_6bl_v1_4397078_606663	novel	—	24	cgguggaaggauaacgggcugcug	ccgcuucgaacgguggaaggauaacgggcugcugcccaaccuggccgcuugggccucgcagcccacuccaacgaaccaccauuuuuuuccgauuuuucuuuucuuuuu	51.9	3.9	0.9	0	101	99
ta_iwgsc_1dl_v1_374662_77766	ta_iwgsc_1dl_v1_374662_77766	novel	—	21	ucuguaaacgaauauaagacg	ugauauuaugauauacucccucuguaaacgaauauaagacguuuuauagacuugacacaagucucuaaaacaucuuauauuuguuuacagagguuguauuuuuuaaucugu	0	-1.3	2.1	0	7	6
ta_iwgsc_5al_v1_841568_463602	ta_iwgsc_5al_v1_841568_463602	novel	—	24	uuauuugaacuagcuuguagagcu	guaaaauuauuuguacuagguuacagugcuaguucaacucuaggguaaaauuauuugaacuagcuuguagagcuaguuuaacucuaguguaauguuauuuuaacuagagcu	1.1	-1.3	0.8	0	16	15
ta_iwgsc_2dl_v1_994825_204770	ta_iwgsc_2dl_v1_994825_204770	novel	—	21	ugcauuugcaccugcaccuac	uauggcugcauuugcaccugcaccuacgaugaagaacaaaggaaacgccuuuuguuaucaucaaguugcuaggugcaguggcacaugcagccguaucacaugaauuuuccg	5.7	3.9	2.1	0	8	7
ta_iwgsc_2bl_v1_7959372_138555	ta_iwgsc_2bl_v1_7959372_138555	novel	—	21	ugcauuugcaccugcaccuac	uauggcugcauuugcaccugcaccuacgacgaagaacaaaggaaacgccuuuugucauccucaaguugcuaggugcaguggcacaugcagccguaucacaugaauucucug	5.7	3.9	2.1	0	8	7
ta_iwgsc_4al_v2_7170222_357181	ta_iwgsc_4al_v2_7170222_357181	novel	—	22	ucuguaccgaaauacuuguagu	uacucccucuguaccgaaauacuuguaguuggaggaaacuaguacaaguacccccaacuacaaguauuucgguacggagguaguagugcuuuugcaugagcauuuugcccagu	2.8	-1.3	2.5	0	13	11
ta_iwgsc_5bl_v1_10864849_491510	ta_iwgsc_5bl_v1_10864849_491510	novel	—	24	guguuguaacccgacuguaaccac	ccagacaagcaauugcaaguguuguaacccgacuguaaccacgcccaaaaaagugaucgccaauugggguaagguuuuugaggcaaguugcauguccaacacuacacaugca	1.3	-1.3	1	0	26	17
ta_iwgsc_5bl_v1_10907489_500366	ta_iwgsc_5bl_v1_10907489_500366	novel	—	21	cgauccagauuaauugacgcu	guguauucugcuacucccuccgauccagauuaauugacgcuccuuuaguagaacauugugaccgucaauuaauuuggaucggagggaguuuugcagcugaauggaguacca	4.4	3.9	2.3	0	5	4
ta_iwgsc_3ds_v1_1051651_321607	ta_iwgsc_3ds_v1_1051651_321607	novel	—	24	ccacccguggcucgcgauuucagc	ucgguuguuccacaucaccacccguggcucgcgauuucagcaaguuuguguuugguacaacccauaguuguggcguagcuaggauuuugagucaggguggucccaauugaua	0.7	-1.3	1.3	0	10	10

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_6dl_v1_3232322_627670	ta_iwgsc_6dl_v1_3232322_627670	novel	--	24	gccugcuggaacuggaagucuagc	nnnnnnnnnnnnnnnnngccgccugcuggaacuggaagucuagcgcgucauucgacgugcagaucauguacgagggugacgagaagacaugcuccgguguaaggaagcuucugc	1.5	-1.3	1.2	0	23	19
ta_iwgsc_5dl_v1_4553317_539609	ta_iwgsc_5dl_v1_4553317_539609	novel	--	24	gacgugggccauagucuuaggcug	accucaauaagagucaccaaccgcagcuaaagaggcccaccuccggagcaccuacugcaacuuaaucugcgacgugggccauagucuuaggcuguaagcauccuguuuaucccc	0.4	-1.3	1	0	10	10
ta_iwgsc_2al_v1_6350300_98147	ta_iwgsc_2al_v1_6350300_98147	novel	--	21	uauuaguugucgcucaaacgg	gucccauauuaguugucgcucaaacggaugaaucuggcacugaaguacgccuagauaguagauacauccauuucagcgagaacuaaugugagacggagggaguauaagac	9.3	3.9	1.6	0	16	15
ta_iwgsc_6dl_v1_2672984_624748	ta_iwgsc_6dl_v1_2672984_624748	novel	--	21	uauuaauugucgcugaaacgg	gucccauauuaauugucgcugaaacggauguaucuugacguauuucagugcuagauacaucuguuugagcgacaauuaaugugggauggagggaguagca	2.3	-1.3	2.3	0	11	11
ta_iwgsc_7as_v1_4104611_671584	ta_iwgsc_7as_v1_4104611_671584	novel	--	21	cggugccuaaggcugucggcu	uuggugacggcggggccuggcggugccuaaggcugucggcuugaggcacucgucagugacgacgaugcuauugcaagcauaucuccugaagcagcugccagccggcgucag	1.1	-1.3	2.2	0	9	7
ta_iwgsc_6as_v1_4430433_591464	ta_iwgsc_6as_v1_4430433_591464	novel	--	21	aaacgaucuuauauuaugggga	caaagaugagguaaaauguuacucgcuccaucucauaauauaagaucauuucgcaaguuauuuuuaacuugcaaaacgaucuuauauuaugggacggaugggguaauaauuuga	1	-1.3	2.1	0	9	9
ta_iwgsc_5as_v1_1482713_465350	ta_iwgsc_5as_v1_1482713_465350	novel	--	24	cagccuuuggggcacuguagcacu	gaaaagaguagcuucucaggcagccuuuggggcacuguagcacuacauuaccgcuacaguacccaaaggaaaugcaucucacgccucaggcagcgcccgggcugccuaggaucu	2.4	-1.3	2.1	0	13	13
ta_iwgsc_7al_v1_4468840_656505	ta_iwgsc_7al_v1_4468840_656505	novel	--	21	aggacaaguauuuccgaacgg	uuuucuacuacucccuccguucagaaauacuuguucuagaaaugguuguaucuagacugauacaaucaucuguaggacaaguauuuccgaacggaggaaguaggccgga	0.4	-1.3	2.5	0	7	7
ta_iwgsc_6dl_v1_3328699_637442	ta_iwgsc_6dl_v1_3328699_637442	novel	--	21	auauuaguuuacggagggagu	agcauuuagaucacuacuuuagugaucuauaauauuaguuuacggagggaguauaagucuuucuagagacuccaauguggacuacauacggagcaaaauugccgcucgu	5.7	3.9	0.6	0	11	10
ta_iwgsc_3as_v1_3414319_250294	ta_iwgsc_3as_v1_3414319_250294	novel	--	22	ccacuguuggcaaggaagcugc	ugcccuguuuggcacaaaggccacuguuggcaaggaagcugcguucguacgcugcaauuugcagcgacgguggcaccucacccgugagguuguugcaggacagguucagcac	1	-1.3	2.1	0	9	9
ta_iwgsc_3b_v1_10429966_257948	ta_iwgsc_3b_v1_10429966_257948	novel	--	22	ccacuguuggcaaggaagcugc	ugcccuguuuggcacaaaggccacuguuggcaaggaagcugcguucguacgcugcaauuugcagcgacaguggcaccucgcccgugagguuguugcaggacagguucagcac	1	-1.3	2.1	0	9	9
ta_iwgsc_3b_v1_10429966_257953	ta_iwgsc_3b_v1_10429966_257953	novel	--	22	ccacuguuggcaaggaagcugc	ugcccuguuuggcacaaaggccacuguuggcaaggaagcugcguucguacgcugcaauuugcagcgacgguggcaccucgccccugagguguugcaggacagguucugcaca	1	-1.3	2.1	0	9	9
ta_iwgsc_5ds_v1_187063_553953	ta_iwgsc_5ds_v1_187063_553953	novel	--	20	uuguacuaggcuauagagcu	caguguaaaauuguuuguacuaggcuauagagcuaguucaauuguaguguaaaauuauuugaacuagcauauagagcuaguucaacaauaguguaaaauuauuuguacaa	0.8	-1.3	0.5	0	21	10
ta_iwgsc_4dl_v3_14441801_420605	ta_iwgsc_4dl_v3_14441801_420605	novel	--	21	uccguaaacugauauaagagc	augggagauguaccacucccuccguaaacugauauaagagcauuuauauaguauaaacacucuuauauuaguuuacagagggaauacuuuauuaguagcugacccaucuua	1.2	-1.3	1.8	0	10	5
ta_iwgsc_5ds_v1_2772789_560631	ta_iwgsc_5ds_v1_2772789_560631	novel	--	21	uccguuccaaaauagaugacc	cccuccguuccaaaauagaugacccaaccuuauacuaacuuuguguacuaaaguuaguauaagguugggucaucgauuuuggauugaagggaauaguacagaauaaccuauugg	10.1	3.9	2.5	0	16	15
ta_iwgsc_6bs_v1_3003075_616845	ta_iwgsc_6bs_v1_3003075_616845	novel	--	24	aaucuagcuagucggauauauagu	agugcucgcagaagccagauaaucuagcuagucggauauauaguccgacuuuuagucggauauauaguccgacuuuuagucggauguaauaucugacacaagccagauuuuuuu	0	-1.3	2.1	0	7	6
ta_iwgsc_6as_v1_4385667_585666	ta_iwgsc_6as_v1_4385667_585666	novel	--	24	auugugugcagcgccggugaaccg	ccggagaaagcagagccgucauugugugcagcgccggugaaccgcuccggccucggaggaguaggacgguucaucaacgcugcacucaauggcaccuuugcgucucuucccuccu	0	-1.3	2.5	0	6	5
ta_iwgsc_4as_v2_6003347_371590	ta_iwgsc_4as_v2_6003347_371590	novel	--	24	acugggcggacaccuggcggcacg	gagacgacggcgucggcgcggcggcggcagugcagcggcgagaacgccggcucguacgugagccggcgccacugggcggacaccuggcggcacgcggagaggucgucgagggag	1.9	-1.3	2.5	0	10	10
ta_iwgsc_3b_v1_10640029_276849	ta_iwgsc_3b_v1_10640029_276849	novel	--	21	uuugcaugaccgaggagccgc	ucuaccggaagguuugugcagcugcucguucaugguucccacuauccuaucuccauagaaaacgaggagguaggccugugguuugcaugaccgaggagccgcuucgaucc	3.1	3.9	2.1	0	3	2
ta_iwgsc_7al_v1_3729801_651667	ta_iwgsc_7al_v1_3729801_651667	novel	--	20	ucaucuauuuuggaacggag	uuauauacuacucccuccauuccaaaauagaugacuccacuuuauacuaacuuuagacuaaaguuaguacaaaguggagucaucuauuuuggaacggaggaaguagugcac	3	-1.3	2.7	0	26	25

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_6dl_v1_3311975_634716	ta_iwgsc_6dl_v1_3311975_634716	novel	--	21	agaaucuugaugaugcugcau	cacagucgguucuugcaggugcagcaccaccaagauucacaucggauccgucguaaaaucaaauuuaugcgacgcgccaggugaugagaaucuugaugaugcugcaucagc	5	3.9	1.9	0	7	6
ta_iwgsc_6al_v1_5830987_576438	ta_iwgsc_6al_v1_5830987_576438	novel	--	21	agaaucuugaugaugcugcau	cacagucgguucuugcaggugcagcaccaccaagauucacaucggauccgucgucguaaauuaaauuuaugcgacgcgccaggugaugagaaucuugaugaugcugcauca	5	3.9	1.9	0	7	6
ta_iwgsc_6bl_v1_4279451_597977	ta_iwgsc_6bl_v1_4279451_597977	novel	--	21	agaaucuugaugaugcugcau	cacagucgguucuugcaggugcagcaccaccaagauucacaucgcauccgucguaaaauuaaauuuaugcgacgcgccagaugaugagaaucuugaugaugcugcaucagc	4.9	3.9	1.8	0	7	6
ta_iwgsc_7bl_v1_6632794_691049	ta_iwgsc_7bl_v1_6632794_691049	novel	--	25	cgacucgacucguuacuauaacaag	guuucauuaacgagcuagcucgacucgacucguuacuauaacaagcucaaauucaagauuggcucgacucguauaacucgagagcuggcucguuuagcuuguuaagcgcguuaaa	1.6	3.9	0.6	0	3	2
ta_iwgsc_5ds_v1_2212184_554224	ta_iwgsc_5ds_v1_2212184_554224	novel	--	24	ccucuguaaacuaauauaggacgu	cucccucuguaaacuaauauaggacguuuuagagacuugacacguccuauauuagucaagucucuaaaacguccuauauuaguuuacagagggaguacaucaugugauauuu	2.7	-1.3	2.8	0	11	5
ta_iwgsc_5ds_v1_2212184_554225	ta_iwgsc_5ds_v1_2212184_554225	novel	--	24	ccucuguaaacuaauauaggacgu	ucccucuguaaacuaauauaggacguuuuagagacuugacuaauauaggacgugucaagucucuaaaacguccuauauuaguuuacagagggaguauuuucuauuuugcau	3	-1.3	2.7	0	14	5
ta_iwgsc_4as_v2_5993628_370509	ta_iwgsc_4as_v2_5993628_370509	novel	--	24	ccucuguaaacuaauauaggacgu	guacucccucuguaaacuaauauaggacguuuuagagacaucucuaaaacguccuauauuaguuuacagagggaguaguuauuaugauuauaacuucguggcuuacucgcaagcu	2.6	-1.3	2.7	0	11	5
ta_iwgsc_2dl_v1_9845563_190777	ta_iwgsc_2dl_v1_9845563_190777	novel	--	24	ccucuguaaacuaauguaagacgu	uuuuguucuccauuugguauuccucuguaaacuaauguaagacguuuuugguauucuaaaaugcccuaaaacgucuuauauuaguuuacaaagggaguauauuauuuugu	0.5	-1.3	2.1	0	8	8
ta_iwgsc_1as_v1_3301476_25066	ta_iwgsc_1as_v1_3301476_25066	novel	--	22	cccgucuagcucaguugguagg	uagaaaaaaugcagcacauaacccgucuagcucaguugguagggcgcaagccucuuaaccuucuggucaugguuugaggagucugggguuugagccccacggcgggcga	1.7	-1.3	1.4	0	15	15
ta_iwgsc_2bs_v1_5167335_161892	ta_iwgsc_2bs_v1_5167335_161892	novel	--	22	ccgaacuggaccgugcagcugg	auuugaguagaucguaucauucuggucaaguagauccucacacagacugauggcgugcaguucgggggcccgaacuggaccgugcagcugggguggagagaugcucucacggca	0.7	-1.3	1.8	0	9	9
ta_iwgsc_6ds_v1_793750_650453	ta_iwgsc_6ds_v1_793750_650453	novel	--	24	agcucuggagaccgcuuagcaagc	ucugguguacccgcaauaagcuagucaucgagcgugugauuccauaccgugugacugaugcaaucuacaaaauauguggcuucuuacagcucuggagaccgcuuagcaagcgg	0.7	3.9	0.4	0	9	5
ta_iwgsc_4dl_v3_7747508_426648	ta_iwgsc_4dl_v3_7747508_426648	novel	--	24	aacgcgagucgaggcggacgucgu	acgugagacgaaugccacccugucuaucuuauacuaacgcgagucgaggcggacgucgucgcgguagcaacgauggacgccgaaacgagcacguuacacgaaugccaccc	1.2	3.9	0.9	0	9	6
ta_iwgsc_5dl_v1_513605_552788	ta_iwgsc_5dl_v1_513605_552788	novel	--	19	auauaagaacguuuuugac	uccgucuguccuacaauauaagaacguuuuugacacaacacuagaguaaaagacacucuuauacuaugggacagagggaguaaauacuaugcuugaaaccaaacuuagg	0.1	-1.3	0.7	0	10	10
ta_iwgsc_7dl_v1_3393859_738145	ta_iwgsc_7dl_v1_3393859_738145	novel	--	21	uaagacguuuucugacacuac	ucccucuguccuauaauauaagacguuuucugacacuacauacuagugucauaaaacgucuuaaauuaugaaacagagcgcauaucgaaaacccaauucagugcccgggcucau	0.1	-1.3	1.7	0	8	8
ta_iwgsc_2dl_v1_6838803_181654	ta_iwgsc_2dl_v1_6838803_181654	novel	--	21	uaagacguuuucugacacuac	cucccuucgucucauaauguaagacguuuucugacacuacacuaaugucaaaaaacgucuuauauuauggcauggagggaauacuauacuuugaacaaugagguuuuuucuuuc	0.3	-1.3	1.9	0	8	8
ta_iwgsc_5dl_v1_4534720_535921	ta_iwgsc_5dl_v1_4534720_535921	novel	--	24	cuuauauuaguuuacagagggagu	auccguuccaugguaucgcaauguaagaacucuuaucuagaaacuacuacucccucuguaaacgcucuuauauuaguugcucuuauauuaguuuacagagggaguaccacacaac	0.8	-1.3	0.9	0	11	11
ta_iwgsc_1bl_v1_3799762_31183	ta_iwgsc_1bl_v1_3799762_31183	novel	--	24	uugcacugucuguuuuuugggcgc	cgcuaaaaguuuuugcgcgcgcgcugaaaaacucuauagugcgcagcauauuuggggcgccggauugcgcgcgcuucacaauuugcacugucuguuuuuugggcgcgc	2.9	3.9	1.8	0	3	2
ta_iwgsc_4bs_v1_4925439_400988	tae-miR1847-5p	tae-miR1847-5p	--	21	accugcaguugggccaaugac	uucagugccguaauugcaccugcaguugggccaaugacaugugggccuaacagacggauggcccacaugccauugacccaacugcaggugcaguuacggcacccaagcagc	0	-1.3	3.1	0	5	2
ta_iwgsc_1bs_v1_3484048_57455	ta_iwgsc_1bs_v1_3484048_57455	novel	--	21	auauuaugggacagagggagu	acucacuccgucccauaauauaggacguuuuuuggcacuacacaaaaaacuuccuauauuaugggacagagggaguacuauauauuuguuuuuauuuuucguuuaguuuuc	0.9	-1.3	2	0	9	8
ta_iwgsc_4bs_v1_4878896_395209	ta_iwgsc_4bs_v1_4878896_395209	novel	--	21	guauuggaaucucuaaaaaga	ucaaucuuaguaauuacucccuguauuucuaaauauaagucauuuuacugauuuuacuccguauauaguuuguauuggaaucucuaaaaagacuuauauuuagaaauau	0	-1.3	0.5	0	10	10

Table S1 (continued)

Tag ID	Mature miRNA ID	Potential ID	Conserved miRNAs	Mature length	Mature sequence	Precursor sequence	Total score	Score for start	Mfe	Mismatch	Total read count	Mature read count
ta_iwgsc_1bs_v1_3450821_54079	ta_iwgsc_1bs_v1_3450821_54079	novel	—	22	acaaguauuuaggaacagaggg	uagcauguauucccucuguaccugaauauuuugucguugggcaaacccagucguucaacaucucccagcgacaaguauuuaggaacagagggaguaguauauucuaa	1.5	-1.3	2.1	0	10	10
ta_iwgsc_2ds_v1_5343631_212135	ta_iwgsc_2ds_v1_5343631_212135	novel	—	23	uggagaucaaacugugcaucacu	uccaugacacgcgcaacuguauugaaaccgugcacgaagacugacagugcaugcggaguuuggccucgugcauggagaucaaacugugcaucacucauggacagaucaaaccaug	0.6	-1.3	1.2	0	10	10
ta_iwgsc_5dl_v1_4521816_533383	ta_iwgsc_5dl_v1_4521816_533383	novel	—	19	ugacucaacuuuguacuaa	guacucacuucgucccaaaauaugugacucaacuuuguacuaaaguuaauacaaaguugagucacuuauuuuaggacagagagaguagcuucuuggacuauacaugcaag	2.3	-1.3	2	0	14	14
ta_iwgsc_3al_v1_4233262_224929	ta_iwgsc_3al_v1_4233262_224929	novel	—	21	ucauuugguugaacuaaagag	uuuaggguacccucugacccuacuuagcucaaccaauugaacugaaguuucaaaaacgaagugaauucugaaaauacaguucauuugguugaacuaaagagggucggagg	3.2	3.9	2.2	0	3	2
ta_iwgsc_2as_v1_5307484_130291	ta_iwgsc_2as_v1_5307484_130291	novel	—	20	aucccuguacgcugcacuuu	aacaugcacgcaugggcgaauugccuugaaguagcuguaugcauggcugcaugggugccaugcaucuaucccuguacgcugcacuuuuugcccuuuuucugcguguua	2.8	3.9	1.8	0	3	2
ta_iwgsc_6dl_v1_907982_638792	ta_iwgsc_6dl_v1_907982_638792	novel	—	18	acgacggacgccgagacg	accacugccacccugucuaucuuaggguaaagcgaguccaggcggacgucgucgcgguaaggacgacggacgccgagacgagcucgugacaccacugccacccuaucuaucuu	0.8	-1.3	1.9	0	9	7
ta_iwgsc_4al_v2_7062981_338678	ta_iwgsc_4al_v2_7062981_338678	novel	—	24	aagacacuuauuuugggacggagg	acucccuccgucccaaaauaagugucucacuauaguacaaaguuguacuaagcuuaagacacuuauuuugggacggagggaguaaaauacagcggaucauuuuucuucug	2.8	-1.3	2.5	0	19	17
ta_iwgsc_1al_v2_3895713_5164	ta_iwgsc_1al_v2_3895713_5164	novel	—	25	acgaguauuucacggaaagccgaca	cuucccuauucauugcggguugguuauuugucaguacagguuuagcuuaugacguguuuggaaguccuaggccaaacgaguauuucacggaaagccgacaaggaauuc	1.4	3.9	0.6	0	10	8
ta_iwgsc_1bl_v1_1649188_28412	ta_iwgsc_1bl_v1_1649188_28412	novel	—	21	aguuuaguuggcaagcguggc	uuguguucgccgaggauggcaaguuuaguuggcaagcguggcaaaucugccucaguucauuuuuugccaggaaaauugccgugcuugcaaacuaaauuugcuauccucgc	3.8	3.9	2.3	0	4	3
ta_iwgsc_6bl_v1_4258723_596468	ta_iwgsc_6bl_v1_4258723_596468	novel	—	19	cuugucggagaaauggaug	aagaaaaguacucuggaaaugcuugucggagaaauggauguaucuguauguauguacauccauuucucugacaaguauucucagauggagugaguagcaaagaacaacuugggg	4.1	3.9	2.1	0	5	4
ta_iwgsc_3b_v1_10460378_260742	ta_iwgsc_3b_v1_10460378_260742	novel	—	24	cuccgucccaaaauauaagaacgu	auuuuugaaauauuuguguccuacucccuccgucccaaaauauaagaacguuuuugaaaacguaaaaacguucuuauauuuuggggcggagggaguauuuuuuagcgcgac	5	3.9	2.5	0	6	5
ta_iwgsc_6as_v1_4431727_591704	ta_iwgsc_6as_v1_4431727_591704	novel	—	23	ccaaccgccgguggucgagagcu	cagggacguggaccugugcgggccaaccgccgguggucgagagcuccgcgacgccaugccgcgggcucgggcgcggcauggcaucgcggagcucuccgccagcgguugacccgc	4.8	3.9	3.3	0	4	3

Table S2: Known miRNAs from two small RNA libraries of 7DAP and 14DAP grains

miRNA	mature_sequence	7 DAP	14 DAP	miRNA	mature_sequence	7 DAP	14 DAP
tae-miR9777	AGCAAACATATCTGAGCACA	11595.45	7770.40	tae-miR1137a	TAGTACAAAGTTGAGTCATC	214.73	0.00
tae-miR9670-3p	AGGTGGAATACTTGAAGAAGA	5797.72	27569.78	tae-miR9658-3p	ATCGTTCTGGGTGAATAGGCC	0.00	3390.72
tae-miR7757-5p	ATAAAACCTTCAGCTATCCATC	5797.72	16307.75	tae-miR1127b-3p	ACAAGTATTTCTGGACGGAGG	0.00	1170.61
tae-miR9672b	TACCACGACTGTCATTAAGCA	5153.53	12190.45	tae-miR9663-5p	AAGCGTAGTCGAACGAATCTG	0.00	888.05
tae-miR531	CGCTCGCCGGAGCAGCGTGCA	4938.80	7064.00	tae-miR5062-5p	TGAACCTTAGGGAACAGCCGCAT	0.00	726.58
tae-miR5048-5p	TTTGCAGGTTTTAGGTCTAAGT	4294.61	7084.18	tae-miR408	CTGCACTGCCTCTTCCCTGGC	0.00	645.85
tae-miR9662a-3p	TTGAACATCCCAGAGCCACCG	3865.15	14309.65	tae-miR9652-3p	AAGCTTAATGAGAACATGTG	0.00	363.29
tae-miR9662b-3p	TGAACATCCCAGAGCCACCGG	3865.15	14309.65	tae-miR9676-5p	TGGATGTCATCGTGGCCGTACA	0.00	343.11
tae-miR9773	TTTGTTTTTATGTTATTTTGTGAA	3865.15	2543.04	tae-miR9666a-3p	CGGTAGGGCTGTATGATGGCGA	0.00	322.93
tae-miR9779	CTTATGCAACGTCTGAGGAT	3650.42	3451.27	tae-miR9664-3p	TTGCAGTCCTCGATGTCGTAG	0.00	302.74
tae-miR9677a	TGGCCGTTGGTAGAGTAGGAGA	3435.69	3754.01	tae-miR9666b-3p	CGGTTGGGCTGTATGATGGCGA	0.00	262.38
tae-miR159a	TTTGGATTGAAGGGAGCTCTG	3435.69	3713.65	tae-miR395a	GTGAAGTGTTTGGGGGAACTC	0.00	242.19
tae-miR159b	TTTGGATTGAAGGGAGCTCTG	3435.69	3713.65	tae-miR395b	TGAAGTGTTTGGGGGAACTC	0.00	242.19
tae-miR167c-5p	TGAAGCTGCCAGCATGATCTGC	3220.96	10434.54	tae-miR398	TGTGTTCTCAGGTCGCCCCCG	0.00	242.19
tae-miR9669-5p	TACTGTGGGCACTTATTTGAC	2362.04	8961.19	tae-miR9667-5p	AAATATGGCAAACAATGAATG	0.00	242.19
tae-miR9653b	TGGCCAAGGTCTCTTGAGGCT	2147.31	2421.94	tae-miR397-5p	TCACCGGCGCTGCACACAATG	0.00	201.83
tae-miR9774	CAAGATATTGGGTATTTCTGTC	2147.31	1251.34	tae-miR1137b-5p	TCCGTTCCAGAATAGATGACC	0.00	161.46
tae-miR9674b-5p	ATAGCATCATCCATCCTACCC	1932.57	10192.34	tae-miR1118	CACTACATTATGGAATGGAGGGA	0.00	161.46
tae-miR164	TGGAGAAGCAGGGCACGTGCA	1932.57	4460.41	tae-miR9665-3p	GCTAGCAGTGTAAACTCAAATCA	0.00	161.46
tae-miR1130b-3p	TCTTATATTATGGGACGGAGG	1717.84	908.23	tae-miR1120a	ACATTCTTATATTATGAGACGGAG	0.00	141.28
tae-miR9653a-3p	TTTGAGACTTTGGCCATGGCC	1288.38	1856.82	tae-miR9652-5p	CCTGTTTGTCATTAAGTTTCTT	0.00	141.28
tae-miR9772	TGAGATGAGATTACCCCATAC	1073.65	1977.92	tae-miR9657b-3p	CGTGCTTCCTCGTCGAACGGT	0.00	141.28
tae-miR1135	CTGCGACAAGTAATTCCGAACGGA	1073.65	1614.63	tae-miR9657c-3p	CGTGCTTCCTCGTCGAACGGT	0.00	141.28
tae-miR1121	AGTAGTGATCTAAACGCTCTTA	858.92	1170.61	tae-miR9675-3p	TTTATGATCACTCTCGTTTTG	0.00	141.28
tae-miR156	TGACAGAAGAGAGTGAGCACA	644.19	10091.43	tae-miR1122a	TAGATACATCCGTATCTAGA	0.00	121.10
tae-miR319	TTGGACTGAAGGGAGCTCCCT	644.19	2179.75	tae-miR9660-5p	TTGCGAGCAACGGATGAATC	0.00	121.10
tae-miR396-5p	AACTGTGAACTCGCGGGGATG	644.19	585.30	tae-miR5384-3p	TGAGCGCGCCGCCGTCGAATG	0.00	100.91
tae-miR1120b-3p	TTCTTATATTGTGGGACAGAG	644.19	544.94	tae-miR1125	AACCAACGAGACCAACTGCGGCGG	0.00	60.55
tae-miR171b	TTGAGCCGTGCCAATATCACG	644.19	343.11	tae-miR1847-5p	ACCTGCAGTTGGGCCAATGAC	0.00	60.55
tae-miR9655-3p	CAAGGGAAGGAAGTAGCCAAC	429.46	7104.37	tae-miR9679-5p	CAGAACCAGAATGAGTAGCTC	0.00	60.55
tae-miR9654b-3p	TTCCGAAAGGCTTGAAGCGAAT	429.46	5731.93	tae-miR5050	TTGAACGACCTCACCATGTCG	0.00	40.37
tae-miR9668-5p	CCAATGACAAGTATTTTCGGA	429.46	2442.13	tae-miR1117	TAGTACCGGTTCGTGGCACGAACC	0.00	40.37
tae-miR9657a-3p	TGTGCTTCCTCGTCGAACGGT	429.46	1251.34	tae-miR1122b-3p	AGACTTATATGTAGGAACGGA	0.00	40.37
tae-miR9776	TTGGACGAGGATGTGCAACTG	429.46	645.85	tae-miR1128	TACTACTCCCTCCGTCCGAAA	0.00	40.37
tae-miR6197-5p	TCTGTAAACAAATGTAGGACG	429.46	242.19	tae-miR1130a	CCTCCGTCTCGTAATGTAAGACG	0.00	40.37
tae-miR9672a-3p	CCACGACTGTCATTAAGCATC	214.73	1049.51	tae-miR9666b-5p	GCCATCATACGTCCAACCGTG	0.00	40.37
tae-miR167a	TGAAGCTGCCAGCATGATCTA	214.73	1029.33	tae-miR9666c-5p	GCCATCATACGTCCAACCGTG	0.00	40.37
tae-miR160	TGCCTGGCTCCCTGTATGCCA	214.73	464.21	tae-miR1127a	TCCTTCCGTTCGGAATTAC	0.00	20.18
tae-miR1120c-5p	TAATATAAGAACGTTTTTGAC	214.73	464.21	tae-miR9657b-5p	TTCGTCGGAGAAGCATGTTGC	0.00	20.18
tae-miR1136	TTGTCGCAGGTATGGATGTATCTA	214.73	423.84	tae-miR9661-5p	TGAAGTAGAGCAGGGACCTCA	0.00	20.18
tae-miR171a	TGATTGAGCCGTGCCAATATC	214.73	363.29	tae-miR9673-5p	TAAGAAGCAAATAGCACATG	0.00	20.18
tae-miR9656-3p	CTTCGAGACTCTGAACAGCGG	214.73	322.93	tae-miR9678-3p	TCTGGCGAGGGACATACACTGT	0.00	20.18
tae-miR1122c-3p	TCTAATATTATGGGACGGAGG	214.73	80.73	tae-miR9775	TGTGCGCAATAAGATTTTGCTA	0.00	20.18
tae-miR5049-3p	AATATGGATCGGAGGGAGTAC	214.73	60.55	tae-miR9783	ATAAGCACCGGTGCTTAAGAA	0.00	20.18
tae-miR1119	214.7305132	0.00					

Table S3: Detailed information of novel miRNAs from two small RNA libraries of 7DAP and 14DAP grains

Novel miRNA name	ID	Pre_seq	Strand	Start	End	Mfe_score	Total score	Star_score	miRNA sequence	Star_sequence	Genome ID	Hairpin_structure	Hairpin _energy	7 DAP	14 DAP
novel-miR0001-1	ta_iwgsc_2al_v1_6404501_5978	cuagccgcagcuuguggaaguuauggaaggcgaucaaauucgaguucgagccgguagauacuauugauuaacuagauaaaacuaaagau aaagaagguauaaauaaaaaagaaacgaaauaaaaagagaaaacaaucaguuaugaaaugcaguaauucuucuuuauucuucuaauuga uugcaauuaaacucggcuuaaucuuuuuuagaaaaaaagauugagccgaaucuuuacuugccgaagcacuucu	-	2680	2929	0.1	0.4	-13	aaaaaagauugagccgaau	ucggcuuaaucuuuuuuag	ta_iwgsc_2al_v1_6404501	...((..(.((..(((((.......((((((.((((....))).(((((...)))))......(((......)))((((((((((((..............(((.........................)))..............))))))).))))....)))))).........((((((((((((((....)))))))))))))..)))))..)).)..))......	-53.1	1717.84	1598.82
novel-miR0002-1	ta_iwgsc_4as_v2_5930276_20488	gugcgagcccauugccguccggcccuuucucacaucuccauuucucgguggaggggucaagaagucuaucccggaggaggaaucc agagaagcgaggcguuguauauaucaccgcugcacauucgagaggagagccagaggaaggaggaaggaggaaggaagaaggagggguuuu gcuacgugccauggaauucauggcauaguugguucuuucuucuucuccucuuccuacccucgccucuugcucuuccuucu	-	2180	2429	3.9	69.1	3.9	aaggaggaggaaggaggaaga	uuucuucuuccucuuccuacc	ta_iwgsc_4as_v2_5930276	..((((.....))))..(((((...(((((.((.((((((((....))))))).)).)..)))))......))))(((((.......((((.(.((((.((.......)).)))).).).))).(((((((((((.((.((((((((((((((((((.........(((..(((.((((.....)))).)))))...)))))))))))))))))).)).)))))))).)))))))).	-98.6	214.73	444.02
novel-miR0003-2	ta_iwgsc_1ds_v1_1911306_4982	gccaaugguggaagugggcggagaaguggaaccagaaguggaggucaacauauuacaggugaugccugcgugaauauguugaccuc cacuucugguuccacuuccucccgccugccccaccguccagguccagccccacucccccguacacucccucuacguccucugcag gugcucccccaucacucccccuucuccuccauggucuacugcagguucucuccucucugacccgcugccucauccauca	+	3959	4208	4.7	1.1	-13	aaguggaaccagaaguggagg	uccacuucugguuccacuucu	ta_iwgsc_1ds_v1_1911306	....((((((.(((((((((((((((((((((((((((((((((((((((.(((.....)).)))...))))))... ((..((((...........((((..............((((((((((.........)))..........((.....))....))))))..............))))).)))).)..)).........	-117.9	0.00	40.37
novel-miR0004-2	ta_iwgsc_7as_v1_4191171_37829	ccaaauacauacgacccucuagaauuuugagccuaacuuugacuaacaaaauaugaguauaugcuguaaaaaguauacuauuggau ucuuauucgaaagagguuuccaaauguauaguaagucuuauaacauacaacucauauuuauuguuaauuuuaaaaucgaaguuagcuc aaaacuugaggggcucucuuuuuucgacagagauaguaccuucugugagguauuauaaguuuguggguuugcuc	-	3545	3794	2	2.3	-13	aauuuugagccuaacuuugacu	ucgaaguuagcucaaaacuug	ta_iwgsc_7as_v1_4191171	.((((..((((...(((((.((.((((((.((((((((((.((((((((((.((((.(((((....((((((((((..((...((....)))..))))))))))....))))).)))).))))))))))).))))...........))))))))))))).)).)))))(((.((......)).)))(((((((((....))))))))).....)))).)))...	-83	0.00	222.01
novel-miR0005	ta_iwgsc_6bl_v1_2921472_32514	agaaauuuuuaaagguaaaaaauaugaauuuugaauuuugcaaauggggaccuucaugaaggccaggccuccaaaggcaauuuccgaga uugguuucuuuuucagauuccuuggauccguagacauacgauauguacugcaccacgaagaagaacauauacuucucuguccgg aaauaaaauacuugucggagaaauggccgacaaguauuuccggacggaggagcauaugcacuccgcuacuacgcu	-	334	583	1.8	2.1	-13	acaaguauuuccggacggagg	ucuguccggaaauaaaauacuugucg	ta_iwgsc_6bl_v1_2921472	....((((((.....))))))((.((((((.(..((((...(((((.(((..........))).)))))...))))..).)))))).))(((((.(((.(((((...)))).((((((((...))))).))))......)))))))((((.((((((((((((((.....((((((((((.......))))))))))))))))))))))))).))))).................	-81.7	3006.23	6438.33
novel-miR0006	ta_iwgsc_4dl_v3_14357443_22780	augaugccacacaauuugauccacacgucgagaaacaagaaugacaaaucugcucugguaguagcuaauucaaaucagaggugaa aauaguuuugugcauuauuaauuauacagcagaauuucucauuuuuguuucuucaugugugugucuauuuguuucuuguaacuu uguggcaucguaguugaaccuugugaacauaauuuucuaaugaccuuuuuucccuugaaauggguucuuaucacuu	+	1464	1713	0.8	2.3	3.9	acacgucgagaaacaagaaug	uuuuuguuucuucaugugugu	ta_iwgsc_4dl_v3_14357443	(((((((((((((...(((.((((((.(((((((((((((.(((((((((....(((((..((((.......(((((.((....)).)))))......)))).))))..)))))))))).))))))))))))))).))))))).)))....))))............))))))))).........((((.(((((.....))))((((.((((......)))).))))......))))..	-71	0.00	20.18
novel-miR0007-1	ta_iwgsc_5bs_v1_2260864_28356	ccaucccccugcauacggcucggagguggggaguuguuggcuggugcgcugaaaugaaaauaggagggaggggcggugggagcugg uugauggacaucggcggugggcggcaaaggggaccaaagucuuuauauuuuaggacuuuugucccuucgccgccgccgccacccuc ccauccaucccucaacuccgccgccucucucucuucaauuucagcccccagccaacaaccuccuaguucccaaccgugug	+	7431	7680	4.9	48.8	3.9	aggugggagguuguuggcugg	agccaacaaccuccuaguucc	ta_iwgsc_5bs_v1_2260864	..........((((((...((((.(((((((((((((((((.((((((((.......(((((((((((((((((((.(((..((((((...(.((((((((((..((((((.((((((((. ....)))))))).))))))).))))))))))).)......)))))..))).))))))))))))))))))).))))))))).))))))))))))))))).))))...)))))))	-166.4	0.00	343.11
novel-miR0008	ta_iwgsc_3al_v1_4448119_13495	acccgcguucgugcacauugcuauaugagacaguuaguaauaauuuugguuugaauuugcacugcuagggggcggcacugccgcacu gcccggucaguguaagcucauuacacugucccgggaagugcggccguacugcaagcaguguaaacucaaaucauacuaucauuacuauuu guuuuauuuuuauauuuuugccacacuaugcaaauuucaugaguacuaucuacaauauuuuuuuugacauccuucacaug	-	540	789	3.3	3.6	-13	auaugagacaguuaguaauaauu	ucauuacuauuuguuuuauuuuu	ta_iwgsc_3al_v1_4448119	.......((((((............(((((((((.((((((.((.((((((((.((((((((((....((((.((.((((((((.(((((.((((((((.....))))))).))))).))))))))).)).)))).)))))))))))).))))))).)).)))))))).)))))))))).........((((((.........))))))..))))))......................................	-93.5	0.00	60.55
novel-miR0009-4	ta_iwgsc_2as_v1_5291774_7109	ucuuguggguuagggguccugugaucaggagagaugacaccgacgccgaucggaugggucggcuuaaccaggccaugcauccgucu ggccggaguuggugucaccucgccugaacauaggcucacuauccugucaaagcaccagcuucagaggagagcacugcacaggug auaaaugcaacugcagcugagcaccgccucccucuuccucccucgcguuuccucuugcaggucaggucagcuggagaugcgg	-	11188	11437	4.7	5	-13	aucaggagagaugacaccga	ggugucaccucgccugaaca	ta_iwgsc_2as_v1_5291774	..(((.(((.((((((((((((.(((((.(((.((((((((((.(((.(((((((((((((((((....))))))..).))))))))))).))).)))))))))))).))).)))).)))))))).).))) .)))...))).((((((((..((((((((.((..(((.(((.((...............)).))).)))..)))))))).((((((.(((..........)))))..)))..))))))...))))..	-114.4	0.00	80.73
novel-miR0010-6	ta_iwgsc_7bl_v1_6747143_39914	aagcaguagguuuucaaguaaaaacacucaguagguuuucuuuuguggagauauuuagcaugucagguucaaguuuggcugaca auuagauuaaaauagguuguuauuugacaaagaaaauguauaucauugguuuuguacugaaaagcuuuuuccaauugguauuaucuc uuugucauauaacccacauuuuauugaucuaauuguuggucaaacuugaaucuugugaugcaugaugucuugcauaa	-	1902	2151	4.2	4.5	-13	auuguuggucaaacuugaaucuug	caaguuuggcugacaauu	ta_iwgsc_7bl_v1_6747143	....((((.(..((((....))))..))))..................(((((((((((..((((..((.(.((((.(((((((((..((((((((((((((...((..........))....)))))))))))))).)))))))))).)))).).)))..)))..))))))))))...	-103.8	0.00	827.50
novel-miR0011-2	ta_iwgsc_5dl_v1_4495350_28881	gucuuacugauauguagcugggucugucugcacgggcuuaucaggagaggcacccaacaggucccucuaauuggaagcauuuuug cucauguguagaguuuuaguuccaacaauuacacucauaaucagaucuuacaaacccacuuuugcaacuuaguccuggcacuaua aauuuaaaaaugugcauguaaaaaugucucauuauaaaauuuugauguguucggauugaguuuauaaaugcugucuaa	+	2699	2948	1.3	1.6	-13	auuuguaguguucggauugaguuu	acuuaguccuggcacuauaaauuu	ta_iwgsc_5dl_v1_4495350	.......((((((((.((((((((((((((.....))))))))...))).(((((..((((...)))))))))..((((((.....((((((((.......))))))......))))))..............))))).))))). .......(((.(((.(((.((((((((((((.((((((((((((((((..((((.((((((....)))))).)))).))))))))))))))))).))))))))))).))).))).)))....	-77.1	214.73	544.94
novel-miR0012-1	ta_iwgsc_7ds_v1_384317_42212	uaauuucuuguacuaauugagguccccagcaaggauacagguacaugcuggcugcaucccccuucaacagguguccguccuccaccg agguucaccuguauuuccuucuggauccuuuuaugcuucuucugcaacuuuuguuagcaugcaucaucauuuugguguggcuuug cugauuuguggcagcaaccaguggagaugaugcaugcuaacaaaagauguaggaggagcaaaugggauccaaaggu	-	1583	1832	4.8	5.1	-13	caugcuaacaaaagauguaggagg	uucugcaacuuuuguuagcaugca	ta_iwgsc_7ds_v1_384317	...................((((((..((((((..((((...)))).))))))..........))))))((((((.(((.........)).).)..))))))......((((.((((((((.((.((((((((((((((.((((.......)))).))).))))))))))).)).))))))).)))).	-120	0.00	141.28
novel-miR0013-1	ta_iwgsc_7al_v1_4481774_36686	ucuguccucagacuaguaguuuccaacuccgacaacauuacuaagggcauguguauuaauguauggcuauuaaucaagagaaagcaa ugccagagagcuuuagcaacggaaccugauuaucauuuuccugcguguugagcucuugcugcauugcuuuccugagauaguagu cuuguauuaaugcacaugcccuuaggcaaugcuguuggaguuggaagcaccggucugagcaccggucagcugaag	+	729	978	4.9	35.5	3.9	ccaacuccgacaacauuacuaagg	uuagcaaugcuguuggaguugg	ta_iwgsc_7al_v1_4481774	...((.((((((((.((.(((((((((((((((((.((((.((((((((((((((((.((((..((((((((.(((.(((((((((((((((((((((((((((.(((.......................))).)))))))))))).)))))))))))))))))))).))))))))..))))).)))).))))))))))))).)))).))))))))))))))))))))..))))))).))((((...))))...	-136.3	0.00	282.56

Table S3 (continued)

Novel miRNA name	ID	Pre_seq	Strand	Start	End	Mfe_score	Total score	Star_score	miRNA sequence	Star_sequence	Genome ID	Hairpin_structure	Hairpin_energy	7 DAP	14 DAP
novel-miR0014-1	ta_iwgsc_7bl_v1_6749817_39957	cuugaggaggaaggcgaggaucugcuugggccggugcuccgucuccuacaccuggcgccacaucuccgccacgcucuccugga ggcugucugcucccucucagccggaccauccccaucaccagcaucgugcugcugcucuccccaucaucccucccuuguucuu accguccuugccgccgcgcuugccaacgcucuggcgccggacgaugcggggcaggaggugcaucuggcuguggaggaagaag	+	818	1067	3.8	0.3	-1.3	ccggacgaugcggggcaggaggug	ccuccuccuuguucuuaccguccuugc	ta_iwgsc_7bl_v1_6749817	.((((((((..((((.((((.((...(.((((((............)))).))).))).)))).))))....))))))).(((.....))).((((.(((((((..........((.(((((...))))).))..........((.((((((((((......((((.(((((..(((.........)))..))))).)))))..)))))).)))))))..)))))))).)))))......	-97.7	429.46	60.55
novel-miR0015-1	ta_iwgsc_3al_v1_4448716_13502	uggcugaaaaccaucggcccccccguguggcgacugcuaucagaaucacuuuagggaggauugcagccgugacucacauuaugacu acaacuacuuaugcaaaaguucauggacgucaugagaaccgcauuugggaggcguacaucguccaugaacuguucccaugaguaga uguauucaucauacgagucaucgguggaauccucguagauguggucucauagcagucgcccaugcgggggccgaggc	+	951	1200	4.9	5.2	-1.3	ccguguggcgacugcuaucagaau	ucucauagcagucgcccaugcggg	ta_iwgsc_3al_v1_4448716	...........((.((((((((((((.((((((((((.(((((((.((.(.((((((.((.((((((((..((.((((.((((.((((((((....((((((((((..(((....((.... ..))....)))....))))))))))......))))))))).)))).)))).))..))))).))))).))))))).).)).))))))).)))))))))))))).)))))))))).	-134.6	0.00	100.91
novel-miR0016-1	ta_iwgsc_4al_v2_6019133_18858	uacuuucccgcaaacuuuuucccuucauuuuauuuucccgccauuucuuugccgccauuuucuuucccuccauucucuuccgcc acauaaggagugcuacaucgacggaggaugacgaugaugccuucauguguaguguagaaacauugugugagagaagucauaaggugu gaaacacucuacgaugaugaugacgaagcugcaaacacuuaugacuucucuuagacaguguuucacacuccacagcagc	-	777	1026	0.9	29	3.9	cuuaugacuucucuuagacag	gugugagagaagucauaagug	ta_iwgsc_4al_v2_6019133	....(((((.((((((((.((....((((.(((..........))))))))).......)).)))))))).))(((.....)))(((.(((((((((((((.((((((((((((((((((....((.((.((...........)).)).))...))))))))))))))))).))))))))))))).)))..)))).	-72.8	214.73	504.57
novel-miR0017-1	ta_iwgsc_5bs_v1_2254293_28306	ucagcuagagaacaagaguucagauggugcucaguuuaauuucacugcucaaggaccauggacauauuuuauauuauguccaaauu uuuugugucccaccuugagcagugaaacuaaacugcgcaacauuugaagcuuguucucuaccugaaauuccugacgauguuuguca agagcuccuuauuguuggucuuaucuaccagugucaucgaguguguagcacuugcuuucgguacaccaaaaaugacaaa	+	1473	1722	4	3.4	-1.3	gauggugcucaguuuaauuucacu	ugaaacuaaacugcgcaacauuug	ta_iwgsc_5bs_v1_2254293	((((.((((((((((..(((((((.(((.((((((.((((((((((((((....((((((((........................)))))))))))))))))))))).))))))).))).))))))))).)))))))))).)))..(((.((((((.....))))).)))....((((.(((((..((((..(.((((((((......))))))).)......)))).))))).))))))....	-100.1	0.00	100.91
novel-miR0018-3	ta_iwgsc_3b_v1_10775495_16427	cggcggccgucggugcagagaguucaggacgaggucucgauggccggcgaauuuggugcggcuccgcucgauuugaugcuccggaugg acgaaguugacgcgguguuguucccuggucgcggucucagggcuuggggaggccgguggccgcgucuguggaggcggcaugcgcg acggugucgcuuggggggggcgagcagggaggucgcagcauagggggagaggaaggaaggc	-	2382	2631	4.2	0.7	-1.3	gcggccgucggugcagaa	gagcucgauggcggcga	ta_iwgsc_3b_v1_10775495	..((.((((((((.((.....(((.....)))..))))))))).))....((.((((((((((((.(((.(.....((.((..((((..((((.(.(((.(((.(((((((.((((((.(..(((((. ...))))).).)))))).))).))))))))).)))))..))))..)).))).)))))))...))).)))))).))......................	-94.4	2576.77	0.00
novel-miR0019	ta_iwgsc_6al_v1_5834455_31604	aagagcagaagcacgcaguaggagucgacgacguccgcaugcgcguagccggaucgccaugcuccugccgcuccggacgcggc gccagagcgugcuccgggugguaggaggucgacgaggccggccgcgucgacgagcgucugcaccacgggggccucccagcuccgagca gagcagcacggcgccgcagcccgucggccacuuggagcagcgcgguggagccugcggcugcaccgcucgagcuuggcgcacu	-	9711	9960	4.7	0.3	-1.3	gcguggagccugcggcug	gccgcagcccgucggcc	ta_iwgsc_6al_v1_5834455	....(((((............(((((...))).)))((((.((((((((.(((((.((((...((((((((((..(((((.(((.(((....))))))))))))))))))))).).)))..))))).))).))))).))))))))......(((....))).((.((((((.((((.(((....((((((((((.((((...........)).)))))).).)))))))))...)))))..)))))).))...	-115.6	858.92	20.18
novel-miR0020-1	ta_iwgsc_2as_v1_5241552_6842	uugaguuugcauugcccgggcagcucugccgcagcugcccggacaguguuacacuguuuggcagcugcgguagugcugcuagu gcauuguaauucagaucacaucauuauuauguaucauauuuuugauaguuuagcauuuuaugcaaguuuguuuuauuauaucauaa aguuuguaauauuuuuguccggucagcguuuucguuguuucaaauucaugcaucauauuaagaaggucaauuuuuuga	+	81	330	2.7	11.4	3.9	gcucugccgcagcugcccggacag	guuugggcagcugcgguagugcug	ta_iwgsc_2as_v1_5241552	.((((((.(((.(((.(.((((((.((((((((((((((((((((((...))))))))))))))))))))))).)))))).).))).))))))))((((...((.(((.((((((((((......)))))............(((((.(((((((..........((((.........)))).............(((((....)))))...)))))).)))))))))).))))).)))))..........	-88.6	0.00	141.28
novel-miR0021	ta_iwgsc_7dl_v1_3377298_41627	gcgucgcuguaagccauccgauccggcacggcuugcagccggaacaaaacugcugaccggccuaacccuaggucacuuucuccc ugaccccucucgcguuuagcgcuacugagagcgacgacgacguucaacgguggcccaaggcacaccggccaaggcggcgcagcg gcugcacucaccggagcagcgugccguggacuuagucccggcgcgcuccuccgucgagcguggcugcugacgcugcgcg	+	13132	13381	4.8	5.1	-1.3	guccggcgcgcuccuccgucgagc	ucaccggagcagcgugccguggacuu	ta_iwgsc_7dl_v1_3377298	((((((.((((((((.((((...))).).))))))))(((((..((........)).)))))..........(((((............)))))...((((((.(((((......))))).)))).)))))))))... .((((((((...))).)))))((.....((((((((((((((((.(((..(((((.((((((((..((((...)))))))))))).))))))..))).))))))))))).))))))..)).	-121.4	0.00	141.28
novel-miR0022-3	ta_iwgsc_4bl_v1_6981217_21187	ugaucgaggaguucuuggguugaugaccauauagacaaccucagccuaugcguggucugcccauuggccauguucaccacgca aauaggacaagugugcaccaacuauggugccaucucucccaagccuaugaagccaggaauaccacugaguacuuccauaagaugacca guuacccgaugaccagcaugguguucggcguaaccggucaucuuguggaaauaucuguggcaccgugguuucucuu	+	1797	2046	4.4	4.7	-1.3	ucaucuuguggaaauauucug	gaguacuuccauaagaugacc	ta_iwgsc_4bl_v1_6981217	(((.(..((((...((.((((((...............)))))).))((((((((((((.((((...))))...)...)))))))..))))....(((.((((((.....))))))))..)))).).))). (((((((.(.....((((.(((((.((((((((((((((.(((((((((.((((...))))))))).))))).)))))))))))))))).))))).))))....).)).)))))....	-105.9	429.46	201.83
novel-miR0023-2	ta_iwgsc_2dl_v1_9872788_11101	accgaguguuaagcuuaagcacucccucuguacacaaauguaggauguuuuugcaguuuaaauuaaacugcaaaaguccuacauu uguuuacagagggaguagauguuugaacaauuuaagcaccauuauaacauugcuagcauugggagugucucucucuaagcacucaugc agguguaaauucaggccuaaauucuguguggggagcuucuuugucuguauauuuugcaguacuaaucaaaguaucu	-	8729	8978	3	1.9	-1.3	ucccucuguacacaaauguag	auuuguuuacagagggaguaga	ta_iwgsc_2dl_v1_9872788	.......((((.((((......((((((((((.((((((((((((((((((((((((((((...)))))))))))))))))))))))))))))).))))))))))))....))))).))))......((((... ....))))).(((..(((((.(((((....(((((.......((((.(.......).))))).......)))))))))))))).)))(((((......)))))......................	-90.8	0.00	60.55
novel-miR0024-1	ta_iwgsc_6bs_v1_2999099_34032	aaguguauugguaaguacucccucuguaaacuaauauaagggcauuuagaucacuaaagaucuaaacgcucuuauauuaguuuacgg cgggaguagaaauuagugguaggaauagaguaaggaaaauggaauaacaauuuguugcuuaaauacuacacaauucuuugauaug cugguaagucaacaugcacauacuugaaagaacacuuacuucaaacaaagucuggacggaugauaucgucggggga	+	4217	4466	2.1	399.8	3.9	ucuguaaacuaauauaagggc	ucuuauauuaguuuacggcg	ta_iwgsc_6bs_v1_2999099	.....(((((((...(((((((.((((((((((((((((((.((((((((.......)))))))).)))))))))))))))))))).))))))...))))))).((((.....((((((.(((.((.......)).))).))))))).....)))).......(((((...(((..((((((((((........((.......)).........)))))))))).))))((((.((((.........)))).))).	-84	2576.77	3047.61
novel-miR0025-1	ta_iwgsc_5dl_v1_4575265_29704	gcuugcucccuguggcggccguugagacgagaucuccccauaccacauagcggcagcucggcuccgugcacgcguauggggagau cucgcccucaacagccgcuccgugugagcuuuaccuguacguaugacgaugcuuuugguccuuuccugcaucgcuuguugguaac gcgcucuccucuacuaguugaucuugcaugagaggugccuuguucagccagccgagaguucacugcugggauuaaugcu	+	4101	4350	4	4.3	-1.3	ugagacgagaucuccccauac	augggggagaucucgccucaac	ta_iwgsc_5dl_v1_4575265	(((((.((((.((((((((.((((((.((((((((((((((((......((((.(((...)))))))......))))))))))))))))).)))))).)))))))....((((((((..(((.((....(((((.(((.......((((((.((((........((((((..............))))))).......)))).)))))).))).)))))..)).)))...))))))))).)).))))).))).)).	-100.9	0.00	262.38
novel-miR0026-1	ta_iwgsc_2dl_v1_9907038_11391	acguacgacccuaguuagaguuuggacgaggaugugcagcugcgguaggacucucagcuacgaccggcagcugcacauccacguc caagcgcuagcuaggaucgucaacaacggccggcagcaggacauccauguacguggagguuaauucuugauaggcgacgcgaguuc aggaaggagaaccugacggggguguucgaggugggaggugcgcugcugggcgaggucagguaccaggcgcacuccagcg	-	2657	2906	4.7	5	-1.3	uggacgaggaugugcagcugc	agcugcacauccacguccaag	ta_iwgsc_2dl_v1_9907038	....((((.(((((((((.((((((((.((((((((((((((((.(..............).))).))))))))))))))).)))))))).))))))))).)))).((..((.((((((((..(((.(((((....)))))).))).(((((.((....((((((....((((((.........))))))....))))))....)).)))))...))))))).)).)).)..............(((......)))	-114.9	0.00	201.83

Table S3 (continued)

Novel miRNA name	ID	Pre_seq	Strand	Start	End	Mfe_score	Total score	Star_score	miRNA sequence	Star_sequence	Genome ID	Hairpin_structure	Hairpin _energy	7 DAP	14 DAP
novel-miR0040	ta_iwgsc_5dl_v1_3183779_28677	auguaugaccaacaacaaccacagcagcagguccauaacgcagugguagcucuaccacugcgcuauggacccacugcuuuugcugu uguuagagcaauugacugcagaucaauaggucaccgguucgaacccgguugggcccuauuguuuucuuuuuguaauuuuauuucu uugucaacuacggucuuggccuuccacacuugcgucacagcuauucacuuucaaguuuuaaccauaucuagcuuuauuc	-	2440	2689	0.9	32.5	3.9	agcagcagguccauaacgcagugg	acugcgcuauggacccacugcuuu	ta_iwgsc_5dl_v1_3183779	..(((((.(.(((((((.(...(((((..(((((((.(((((((((....))))))))).))))))))..)))))...).))))))).)(((...((((.((((..((((((((((((((...... .)))))..)).)))))))........................(((((........)))))........)))))))..)))....................)))))............	-71.9	0.00	888.05
novel-miR0041	ta_iwgsc_4bs_v1_4945417_22357	uucaagcuuuuugcaagaauucagcggaaaaaaacaucagaggagcucucggcaaaaaaaacaaaaucaauguucuaaguuugcgcac uguuugagcacuguuugagcauugauuuuguuuuuuugugagagcuccucggauguuguuuuuagcugaaacuuugcaaaaagu ucaaacauuugaugaagcuuguugucucaagauuucagauuuuucggaccuuguuuuaucacauuuuucgaaguucc	-	7378	7627	4.2	3.1	-1.3	agcggaaaaaaacaucagagg	ucggauguuguuuuuagcuga	ta_iwgsc_4bs_v1_4945417	....((((((((((((..(((((.(((((.(((((.((((((((((.((((((((((((((((((((.((...(((.((.....)).)))...)).))))))))))))))))))))))))))))))))).))))))).))))).))))))..))))))))))))........((((((((..(..((((.((((........)))).)))))..))))))))..................	-103.1	0.00	80.73
novel-miR0042	ta_iwgsc_6alv1_5785293_31177	acauguuccauucucggacaccaagcucauaauguuauuuaggguacgugauaaccaaaauaccacaugucauuucuauacaucuuc cucuuaucaguacuuagcucaaagcuuaugucucuucaauggaauuucagcaaugugcuacucuauuaaugaaugcucccuccguu cggaaauacuuguccuagaaauaguuguauuucuaggacaaguauuuccggacguagguaguauuuauuuuuuauga	+	1660	1909	2	2.3	-1.3	aggacaaguauuuccggacgu	guucggaaauacuuguccuag	ta_iwgsc_6alv1_5785293	....((((((((...(((((..(((((......((((.....(((((.((((((...............(......)..............))))))))))))....))))).)))))....))))))).. ((((...)))).........((((((((((.(((.(((((((((((((((((((((((((((....))))))))))))))))))))))))))))).))).))))))))))........	-83.2	214.73	746.77
novel-miR0043	ta_iwgsc_3as_v1_3328771_13886	ugcacaccaaccucucuucuuucuucuuagagaggggcacacacaauucgcagagagagagagaaauugagaaauagagagagagag agugagggagggagggagggggagagagagcaacaaugccuuggggaagcauccaaagggaucgcauugauccuuccauccauggu guugauggcugucaucaaucaaccggcucgacagaucaugcgauccuuuuggaagcuuccucuagccauuacuccac	-	1064	1313	2.8	15	3.9	aucaugcgauccuuuuggaag	uccaaagggaucgcauugaucc	ta_iwgsc_3as_v1_3328771	......((..((((((((((((.(((((...............((((((..............).)))))...............))))).))))))))))))..)).((((.(((.(((....)))))(((((((((.((((((((((((((((.((((..((...((..((((((((((.....))))))))..)))))...))..))))))))))))))))))).)))))))).........))))..	-88.8	0.00	444.02
novel-miR0044	ta_iwgsc_7alv1_297285_36285	cuaugauuuuuguugcagauggcaaagugagcucuuugccgucugugcuuuuuuugcagacggcaaaguuuauuuugccgucagu uuuucuuugucgucug	-	0	101	4.5	3.3	-1.3	caaagugagcucuuugccgucugu	agacggcaaaguuuauuuugcc	ta_iwgsc_7alv1_297285	..(((((........((.((((((((((((((((..((((((((((((..........)))))))))))))))))))))))))))))))).)).........)))))...	-43.6	214.73	262.38
novel-miR0045	ta_iwgsc_7alv1_4536617_37090	agccucuuuuagcauauuuuaucaagaacuucuauuuuguuguagaguugcaugugacguuacagcgaagugccgaagaucuagca uuuuuauauguuuucuaaaaauaugagagaaacaaguugaaaguuuuacaacauucgugcuggaagagaagcgugugugcauuccca ugauauaauuuucauuuuaugaauguagacuacgcaacgagaaaugaacggguuuguuuuucaaauauuacaacauc	+	4539	4788	0.3	1.9	3.9	caagaacuucuauuuuguuguaga	acagcgaagugccgaagaucuagc	ta_iwgsc_7alv1_4536617	...(((((((((((..........(((.((((((((((((((((.(((......))).)))))))))))))..)))).)))....(((((.((((((((...)))))))).)))))....((((....... ...))))....)))))))))))..((((((.((((((((..((((.........))))......))))))).)).))))...((((((((((....))))))))))...............	-61.9	0.00	60.55
novel-miR0046	ta_iwgsc_7bl_v1_571312_38763	uuauaagcccugaguguagagacaaugaagggagaggcgcagugcucaccagcauguuucuuaccuucaagccuugaggaaguggc guagacugcacggagcuaugaggguuuuggacaagaacuacacauagcuccauguagucuacacuauuuccucaaggccugaagcu aagcaacgugcaggugagcauugcgccu	+	0	204	4.9	5.2	-1.3	caaggccugaagcuaagcaacgug	uguuucuuaccuucaagccuugag	ta_iwgsc_7bl_v1_571312	.......((((...(((....)))....)))).((((((((((((((.((((((.((((.(((((.(((((((((((((((.(((((((((.((((((((((..((((((......))))))...)))))))).))))))))).))))))))))))))).))))).)))).)))))).))))))))))))))	-124.7	214.73	1392.62
novel-miR0047	ta_iwgsc_3as_v1_3388517_14007	cuguuguccaaccggcuauggacacaaccucugaguccaagaacucuguuuccuugaacauaguacaccugugguuaagggcgcag aguucuuggacucagagauuguggccauagccggcugcacaacaaccccuuuuuguguggucugcuuacgugguuuccgccucgu acuguauaacaaauguuucaucucauuggaaacaucuuuggaaggagacgggcacgcccuuagacugcuacugccaccg	-	2812	3061	4.6	1.5	-1.3	cacaaccucugaguccaagaacuc	gucuuggacucagagauuguggc	ta_iwgsc_3as_v1_3388517	.((((((.((.((((((((((.(((((.(((((((((((((((((((((.(((((((.((((((.....)))))).))))))).)))))))))))))))))))))))).))))).)))))))))).)).))))))...........((((((((((.....((((...((((.(((.(.(((.........((((((((..........))))))))...))).).))).)))))))).....))))))))).))..........	-113.7	0.00	121.10
novel-miR0048	ta_iwgsc_7bl_v1_6691063_39345	uaauuuuccuuccaaguaaacccucuacuuuucauucacaaaauuguaaggccgggggggagggggggaaagcccccuccccugac uacacuaagcuccgccauuagugaguuguuucaauuucaaccccauguguccugcaugcgggagcuuguaguacauguaaaucaua gaaaaguaaaaaaauguuguccuuucuauguugcagguuuccuuagccuagacugugguuggaagaccggcgauuacac	+	3201	3450	2	2.3	-1.3	cagguuuccuuagccuagacugug	caugugccugcaugcgggagcuugua	ta_iwgsc_7bl_v1_6691063	...............(((((((........)))))).................(((((.((((((((((((((((.....))))))))))).((((.((((((((..........)))))))))).........(((((((((.((... (((..((.((.((((((((((((((...........((((((((.((((......))).).)))))))))))))))))))))).)))...))))).))))))))..)))))..))))).	-82.6	3006.23	1432.98
novel-miR0049	ta_iwgsc_6as_v1_4350518_31819	caucugguggcaaugagaagucauaucauaugacugagcaaugaaaugccaucuguaauguuucgggacugccucaccauugccuu ucaacuguggaugauuuugccauuuguacuugugaagaauguucauacgugcugcaacaaaacagauguuauuugugauguugcgg ucauggauuaaacuacaucauagacaagcauaugucugaauuuucuuauguggaacuuuguguguuucuuauaagugu	-	10502	10751	0.4	0.7	-1.3	cauaucauaugacugagcaa	gccuuucaacuguggaugau	ta_iwgsc_6as_v1_4350518	((...((((((((......((((((.((((((((..(.((((((((.....((..((((..........))))...))......))))))))..))))..))))))))))))))))))......((((((......))))))(((((((((((((..((((((...))))))..)))))))).))))(((.........))).((((((......))))).......((((((.(((((........))))).)))))))).	-64	5582.99	4642.06
novel-miR0050	ta_iwgsc_1alv2_3976442_839	ggcauggcauguaucccacggccauggccaaggccucugaggucgcgugaccuuuuaggccucgcccauggcuaugguguucaug gucuuauuuguccaucagaucuaucuaaaucaccaaagucugucuggaucuuucauguaugucggccaugcccagguauuguucu uuuugaucuacucccuccgucccgucccaaauucuugucuagauacaucuauguucagaaaaauuuaagacaagaauuuu	-	11955	12204	1.6	1.9	-1.3	cauggccaaggccucugaggu	uuaggccucgcccauggc	ta_iwgsc_1alv2_3976442	((((((((((((.((.(((.((((((((.(.((((((..((((((....))))))..)))))).).)))))))).))).)).)))).........(((((.((((((.................))))))..)))))..................))))))).((.....(.((.....)).)....))..............((((((((((((((((...(((......)))...)))).)))))))))))).	-79.4	429.46	766.95
novel-miR0051	ta_iwgsc_1dl_v1_2289383_4330	ggcauggcauguaucccacggccauggccaaggccucugagguucgcgugaccuuuuaggccucgcccauggcuaugguguucau ggucuuauuugucuaucagaucuaguaucuaucuaaaucaccgaagucuggauccuucauguauguugcguugacggccaugccc agguaaauuuuuuuuagaucuauauaagucgcucaauuuuuugcuaguucccuuuuaaagagcaaacucuaacagauccu	+	2906	3155	0.8	1.1	-1.3	cauggccaaggccucugagguucg	uuaggccucgcccauggc	ta_iwgsc_1dl_v1_2289383	((((((((.(((...(((.((((((((.(.((((((..((((((.....))))))..)))))).).)))))))).)))).....((((.(.......).)))).(((((((((.......................)))))))....((((((...))))))..)))))))))))..................(((((......(((.((((..........................)))).)))......))))))..	-70.7	4294.61	4359.50
novel-miR0052	ta_iwgsc_3ds_v1_1051651_17985	uaucgguuguuccacaucaccaccacccguggcucgcgauuucagcaaguuuguguuugguacaacccauaguuguggcguagcu aggauuuugagucaggguggucccaauugauauaaaaaaaguguacacca	-	0	134	0.7	0.1	-1.3	ccacccguggcucgcgauuucagc	uaggauuuugagucagggugguc	ta_iwgsc_3ds_v1_1051651	(((((((((.............((((((((.(((((((((.((((((.(((............((.((((((((.....)))))))).))..))).))))).)))))))))))))).)))))))))))................... .	-37.4	0.00	201.83

Table S3 (continued)

Novel miRNA name	ID	Pre_seq	Strand	Start	End	Mfe_score	Total score	Star_score	miRNA sequence	Star_sequence	Genome ID	Hairpin_structure	Hairpin _energy	7 DAP	14 DAP
novel-miR0027-1	ta_iwgsc_7ds_v1_3958882_43031	cugcgcgcggcuaauuaaugggucgccggggagccggcgaaccgcggcggggggaugcgagauucccagcgggagagagccgccggacucgccaaggaaacgccuaccuccaugcggggaaucgccucgagguaagaagcaaagagcacaugcauucaacugaucuaaauccuaaucccccacuucucaucagcccagaaaugaaugcgugugcucuuuccuucuuacccucgagccgacccccggagccgg	+	7724	7973	4.7	15.9	3.9	ugugcucuuuccuucuuaccc	uaagaagcaaagagcacaug	ta_iwgsc_7ds_v1_3958882	.......((((......(((.((((((...))))))).)))....((((..(((((.(((..(((((......))))).))))))).(((.....))).)))))...(((((..(((.((((((((((((((.(((((((((((((((.(((..((........................))..)))...))))))))))))))).)))))).)))))).))).))))).))))).	-116.8	0.00	100.91
novel-miR0028-1	ta_iwgsc_2al_v1_6416280_6063	ccauggcggcauggugggccccguucgucggacgagcgugccuaggcaucggccgcgcgcuccucgucgaacggcaccugccgugucgcacgcaugcaguuaggcggcggggaaaggggaugcguucgccggugcugcuggcgcauaaggaggugacggcaacgcguuuggcacgguggccuugcacgucgucguccaugggugugggcggcccggcgacuugaucuuccgcgcgacuucuccauggaga	-	2170	2419	4.8	77.2	3.9	uucgucggacgagcgugccu	cgcgcgcuccuccgucgaac	ta_iwgsc_2al_v1_6416280	(((((((((((((((..((((((.((((.((((((((...(((....))))))))))).)))).)))))))..)).))))))))..(((..........)))(((.(((....((((....(((((((.(((((..(.((((......(.(((((((.(((.((..(((......)))..)).))).))))))....)))).)))))))))))....))))))).)))........)))))...	-128.4	1503.11	1291.70
novel-miR0029	ta_iwgsc_5dl_v1_4571082_29689	ucgaccgugcugucccccccgacgguaucccuaugggcuauggcgacgcgcgugccucucucucucucccuucuuggcuggcuggggugaucaugcaugcacaugcugccaugcaucucgcacccaucccucauaauacuagcacacuuguacugcuuacucccucuguaccaaaauauaaaaacaauuuuaacacaauguaaaaucguucuauauuuugggacaaagggaguacuaguuaacuacuacu	+	965	1214	1.3	1.2	-1.3	uucuuauauuuugggacaaag	cuguaccaaaauauaaaaaca	ta_iwgsc_5dl_v1_4571082	......((((((((.......))))))))......((((.....(((...)))..))))...................((((((((...((((....((((((((......)).))))))).....))))).............(((((..........))))((((((((.(((.(((((((((((.(((.((((((((((...))))))))).))).)))))))))))).))).)))))))))))))))).........	-76.5	0.00	40.37
novel-miR0030-1	ta_iwgsc_5ds_v1_2782660_30771	guguuacuuugaaucuuauagguauuucacaggaaacagucuaacgaucaugucuaccgaacugaaagaguuuuuccugaccauggcuuuucuagucaauaggaauacaccauguguuggaagacaaacuucuuugcagcaucuaguagauguugaacuguuuccucugaaauuccguaaauuucauggaguaguuucguauuccuuugaaaaaccuauaugagacuucguaguuuuuucaaaggcucaaaauaug	-	10013	10262	3.2	153.6	3.9	uugaacuguuuccucugaaauucc	uauuucacaggaaacaguucaaug	ta_iwgsc_5ds_v1_2782660	...((((((((((..((((((((.((((((.((((((((((((((((((((.((.(((((((((((((((.((((((((..((((((......)))))).....))))).)))))))))...)).))))).)))..))..)))))))))))))))))))))).)))))).)))))))..)))).))))))...((((((((((((((((.....(((((.....)))))..))))))))))......))))))	-92	0.00	1836.64
novel-miR0031-1	ta_iwgsc_5ds_v1_2761097_30588	uuuuguuacuuuuauuuugauguuuguauuaucuagaaccuugcaagaucaaacaaagcuaucaauaauuaugauguuaacuugcauuugauguuucuuagaaacaucaaaucaaguuaacaucauaauuaaugauaaguuuguuugaaucuucccaagguucuagauaaauacaaauaucaaauacaaguaauaaaacguaugcuuuguaugagagacaaaaugagauacacuuauuggauauacaucu	+	7849	8098	4.8	4.2	-1.3	uuguauuaucuagaaccuu	gguucuagauaauacaaau	ta_iwgsc_5ds_v1_2761097	((((((((((.(((((((((((((((((((((((((((((.((((((((((((..((((((((((((((((((((((((.((((((((((((....)))))))))))).))))))))))))))))))))))))..)))))))))))))).)))))))))))))))))))))))))))))))).)))))))))))....((((((.....)))).)).((((((.....))))))............	-124.8	0.00	1432.98
novel-miR0032-2	ta_iwgsc_3al_v1_4435088_13419	cauugccauugaggccgccacacucaugacucagcugaaugccuaucuugccuucuaguacgccagccaagucacucaccucuaucgccaucgugugauugaggggaggacuuaccauggcguacuaagaggugagauggacgguuuagcuggguccugagcguggggccuccaugguuggucgccauuaaugaauagcuuggagugcaucaauggguccaaucguugcguggauguauagauguugg	-	1877	2126	4.8	5.1	-1.3	uuuagcuggguccugagcgug	cucaugacucagcugaaugcc	ta_iwgsc_3al_v1_4435088	....(((((.((((((.((((.((((.((((((((((((((.(((((((((((((.((((((((((.(..((((.(((.((((.(((((......))))).)))).))).)))))..).))))))))))).))))))))))))).)))))))))))))).)))).))))))))).)))))......(((((.(((.((.........)).))).)))))(((.(((((((((.....))))))).)))......	-125.7	0.00	2684.32
novel-miR0033	ta_iwgsc_7as_v1_4123870_37669	cuaacaaaauuugugaguguuuaaaaggcauguguucuacaaucuguaaaaaguucaaaucaaaauucgaaacacacaucgagaaacaaaaauaaaaaauuuguaguaugaauauguccacaaaaaggccaaaagccaaaagguacuauuuacguucagauuuuguccuuuuuguuucccuugugcauuuugaaauuugaguugaacuuuuuacagguuguucaacauauaugucuuaugaacacucauaauua	-	2260	2509	3.5	3.8	-1.3	aaaaggcaugugucuacaaucugu	agguugucaacauauaugucuuauga	ta_iwgsc_7as_v1_4123870	..........(((((((((((((.((((((((((..((((((((((((((((((((.((((((((((((((..((((..(.((((((((((....((((((....(((((((((((((......((.....))......)))))))))))))......))))))).....))))))))))))..))))..)))))).))))))))).)))))))))))))))))))))....)))))))))))).))))))))))))))...	-95.1	0.00	565.12
novel-miR0034	ta_iwgsc_1al_v2_3947972_580	ugcgccagaaacauauuuguaucguccccacgacaggugaacaaggcagugacggcacgguucuagcaagguuagaagucccuuggcaugucuuccuuccaaaaaucugauguauuuguguuagucaacacuccaaaaaauuauggaguggcauaauacucccucgguuccuaauuauaagucuuuguagagauucaaaucucuacaagacuuauauuuaggaacgggggggaguaguaaaagaguagaguc	-	5253	5502	3.7	4	-1.3	aagacuuauauuuaggaacgg	guuccuaauuauaagucuuug	ta_iwgsc_1al_v2_3947972	..........((((((..(((((((((........((.(((.((((((.(.(((((....((.((((..((((((...))))))).)).)))))).).))))))..)))))).........)))))))..)))))))......((((((((.........)))))))....((((((((((((((((((.((((((((((((((((((((....)))))))))))))))))))).))))))))))))))))))...............	-96.8	0.00	988.96
novel-miR0035	ta_iwgsc_4al_v2_7174831_20292	ccuaagcguuuuaaagauuauuugguuuucguaguguccuaaugcaccgguagggagggagugguacaugcuuacagcuuagggauggaugcuuuuaauaagcaugaugggauauggagagcgaaguaguggugcaggggcaccauggugcccuuuauuuuggaaaaaaaaauaaaacauauuuagaaguucuaaaaaauucaaaaaaauuauguggaaacuuguauguguucaccauggauucguaaaau	-	2526	2775	0.3	0.6	-1.3	aauuauguggaaacuuguaugugu	auauuuagaaguucuaaaaaauuca	ta_iwgsc_4al_v2_7174831	(((((((((........((((((((..((((((.....(((((.....)))))......))))).))))))))).........)).))))))))......((((((..........)))))..............((((((...((((((..(((((((((....))))))))((((((((.....))))))))(((((((..((.((((((((...((((......)))...))).)))))).))))))))))))))...)))))......	-62.2	644.19	706.40
novel-miR0036	ta_iwgsc_4bs_v1_4729575_21772	gcacgucuuuggcgaccagcaaacggagggcuaaaaccuguagcuuaaagaaaaaagauagggguguugcucuguguuugcuuugaggguuuccccuaguugggggagaccgaga	+	124	373	3.6	3.5	-1.3	acggagggcuaaaaccuguagc	gauagguguugcucuguuguuu	ta_iwgsc_4bs_v1_4729575	...((((...))))...((((((((((((((.....((((((..(((.........))))))))))...))))).))))))))))....(((((((((((.....)))))))))))....	-43.1	1717.84	60.55
novel-miR0037	ta_iwgsc_6dl_v1_2950520_34565	gugagguccgcuccaaauuucaacucauuuggauaucugaacaacucucggcaaaacgacaaucgggucaaaacaguucgugaacaauaacuuuuuuacagaccccgauuuugucuuuuuacagagagcugucuaaguccaaacgaguugaaauuugcaccgaaccucacgcaccaaauuaucuaccacacaaaaaaugggaauuugaugaaguuuucuacuauuuguuuugauuuuguuucgcg	-	3910	4159	1.3	0.7	-1.3	acucauuuggauaucugaacaacu	cugcucaaauguccaaacgaguug	ta_iwgsc_6dl_v1_2950520	(((((((.(((...((((((((((((((.((((((((((.((((.(((.((((((.(.((((((.((((((((((((((((((.((((((.((((......))))))).)))))...)).))))))..)))))))))).).))))))).)))).))))).))))))))))).))))))))))))))...)).)))))))).......................(((((......((((((((.....))))))))......)))))....................	-76.7	0.00	181.65
novel-miR0038	ta_iwgsc_5al_v1_2757370_24456	acacaauuagucuauaaacucgagaaucauaguagagcaaaacggcaagucaaagaaaguuuucauaagacugagacuaaaacaugagaacuaucuucccaaggguuaaaccaaagcagcaaggaguaauucucauguuuuaaucuucaagucuuauggaaacuuucuuugccuugccgcuuugcucgaguaugauucucagguuuauacgcuacuuauguuuuaucguccugcugaucauuucauua	+	2085	2334	4.3	15.1	3.9	agaaucauaguagagcaaaacgg	cgcuuugcucgaguaugauucuca	ta_iwgsc_5al_v1_2757370	(((.((.((((.((((((((.((((((((((((.(.((((((((.((((((((.((((((((((.((((((((((((((((((((((.((((((((((((((.(((..(((......(((.......))).....)))))).))))))))))))))).))))).))))))))))))))))))))))))).))))))).)))))))).).))))))))))).))))))))).)))).)).)))...((((((......))))...........	-105.3	0.00	262.38
novel-miR0039	ta_iwgsc_5bl_v1_3540269_27988	auuuauuacacucuuguuaaggagauggacuaucacuuugaguguucaccugucucgagcagagaacauucaaagugauaggccaucuucuuaaugguggaugccgguguuuuuuguuguguuuuauuucaugcuuggcaacaac	+	63	312	4.2	4.5	-1.3	agauggacuaucacuuugaguguu	cauucaaagugauaggccaucuuc	ta_iwgsc_5bl_v1_3540269	.......(((((...((((((((((((((.(((((((((((((((((((..((((((.....)))))).)))))))))))))))))))).))))))))))))))(((....)))))))).....(((((((((((...........))))))))))	-59.8	0.00	544.94

Table S3 (continued)

Novel miRNA name	ID	Pre_seq	Strand	Start	End	Mfe_score	Total score	Star_score	miRNA sequence	Star_sequence	Genome ID	Hairpin_structure	Hairpin_energy	7 DAP	14 DAP
novel-miR0053	ta_iwgsc_2bs_v1_5198356_9507	aaugcuucucuugcuauucacuguugagaccucuuggugugguuguaaacuuguaaugaaacgaaucgcaaagguggccggcaaccauuagaggaagguuuaggguucgaaugagcuuaaauaaauaaaacccucuguucuuaaauaaaaacguuugauaguuuaauaucccuccgucccauaauauaagaacguguuaaaaacguucuuauauuuugggacaaaggaauauuuugaaacaaguaac	+	8571	8820	0.7	10.5	3.9	ccgucccauaauauaagaacg	uucuuauauuuugggacaaag	ta_iwgsc_2bs_v1_5198356	..(((((((..((.........)).))))(((((((((.(((((...((((....(((......)))...))))...))))))))).)))))..(((((((((((....)))))).........))))))..(((((...((((........)))).)))))...((((.((((..(((((.(((((((((((((........))))))))))))).)))))..)))).)))........))))..	-70.5	0.00	242.19
novel-miR0054	ta_iwgsc_5bl_v1_10919911_27652	acggcgacggcgguuccauccaccucgagaugaacggcgacggcagucccauccaccucgagguguacggcaauggcguccc aauccaccucgagguguacggcgacggccguccc auccaccucgagguguauggcaacggcgucccgguggagaaccuagccgcagcuggucgccggcgcaccuccgcugcacgcccaacgugcccccacggaucugggcgaccaccgaggugagcucugccuaccu	+	1993	2242	4.8	2.7	-1.3	ccucgagaugaacggcgacggc	cgucccauccaccucgaggug	ta_iwgsc_5bl_v1_10919911	.(((.((((.((...((((.((((((((.((...((.((((((.(((((...((((((((((((...(((.......)))......))))))))))))...)).))).)))))))...)).)))))))))).)))...)).)))))))(((((...((.(((...(.((((((((...(((..((((..(((((.....)))))......))))..))))))))).)..))).))))))))......	-123.1	0.00	121.10
novel-miR0055	ta_iwgsc_2bs_v1_5197055_9481	ccuuaggcccaguucuuuuggccgauucucccagaauuuugagaaucgagccucccccagcuucucccagaaucuugaacauauauuucuuuuacaauucuagccaguuaugauguaaacagcuaggauuugucaaaaaaaauaugggucaagauuccgggagaagugagugagagguugauuuuuaagauuccgagagaaucgaccaaaagaacuggccuaaucacagugggacacaggucccccgga	-	1107	1356	4.8	5.1	-1.3	cgauucucccagaauuuugag	uaagauuccgagagaaucgac	ta_iwgsc_2bs_v1_5197055	..((((((((((((((((.((((((((.(.((((((((((((((.((((((((.((((((((((.((((((((((.((((((.(((.(((((((((((..(((.........))).))))))))))))).))).)))))))).))))))))))).))))))))))).).)).))))))))))))))))).).))))))).))))))))))))))))).......((.((....)).)).....	-130.2	0.00	121.10
novel-miR0056	ta_iwgsc_2bl_v1_8024098_8304	ugcgcgugacgcuggagcuacgcucgccggaggagcgugcgggacucggaccguugcacguugcuccagcgaggcuagccaccgcgccgccauggccagcgagccucgaagccgccgcucggcgccgcgccucccucggagagcgagugaaugcgggauggagcggggaaaugaggcuaggguuugacccaguggcaguuuugcuccacuccgggcgauuggagccaucggcucgauccgaaggacgaa	+	4451	4700	4.8	0	-1.3	cucgccggaggagcgugc	acguugcuccagcgaggc	ta_iwgsc_2bl_v1_8024098	....(((..(((.(..((((..(((((.((((.((((((((((((...))))..))))))).)))).)))))..)))).).)))..((((((((.(((.((((((...((((((((..(((((((((((....)).))))).))))......))))).)))......))))))...))).).)))).))))....((((((......))))))......(((((((.......))))))))..	-121.5	644.19	262.38
novel-miR0057	ta_iwgsc_2as_v1_5241042_6839	ggcagauucaugcacccuagcagauaagauagauaguaauaaaugugauuugacuuugcauugcccggcagcucugccgcaguugcccggacagaacacugucccgggcagcugcgguaugcugcuaugcaauguuaaauucagaucacaucauuaauacauaucuuauuuuugauaauuuaugcauuuuaugcaaguuuguuuaauaaucacauaaguuuuguaauaauuuuuguccgucagcguuuua	-	10376	10625	4.5	3.9	-1.3	gauaagauagauaguaauaaaugug	caucauuauuacauaucuuauuuu	ta_iwgsc_2as_v1_5241042	((((((....(((......)))(((((((((..((((((.((((((((((.(((((.(((.(.((((((.(((((((((((((((((((....))))))))))))))))))))).)))))).).))).)))))).)))))))))).)))))))..)))))))))......(((((.((((...)))).)))))..........((((.........))))......))))))..............	-109.3	0.00	201.83
novel-miR0058	ta_iwgsc_1bs_v1_3442002_3022	uggcacguaguggacgacugcggggagugaaugccguuuggggaccucaaccaugccgacgcgucaucaugcaagugcaugaccugcagacgacacugcagcuuccacgcaaaggugaugaggagagugcgccgccgugcgguagcggcggcgaccucuccaacauucuucggugcaagcuguaggcaauaguaggccgcccacuuguaugacgaugcgucgcggggucagauccccaa	-	12704	12953	4.9	5.2	-1.3	gccgcccacuuguaugacgaugcg	cgucaucaugcaagugcaugaccu	ta_iwgsc_1bs_v1_3442002	..((((((((((.....))))))..........))))....(((((.((.((((.((((((((((((.(((((((((((..((.(((((.......((((((((((.((((((((.((.((((((((((((((((((((((((.......))))))))))))))))))))))))).)))))))))).))))))))))))......))))).))..)))))))))).)))))))))))).))))).)).)))))...	-162.1	0.00	222.01
novel-miR0059	ta_iwgsc_1bs_v1_3428948_2970	uggcggaaccaauugguguacgccgccuuugccugcguuuccaggggguggucguacuacagucugccaacguacgccggcaguguacuaggccuaggcgugguguccaagugcaugucaauuuuggucguccguacgacuuuggccaggcggaaccacuggccgccuggaacgccaccgacggggacgccgugagggcgggguucucggugccgacggagauuugacgcccgcugguggcagcgaguggagg	-	3927	4176	4.3	0	-1.3	gcgacggaaguuugacgc	gucaauuuuggucgucc	ta_iwgsc_1bs_v1_3428948	((((((((....((..((((((((((((((...........)))))))).))))..))))))))).(((.((((((((...(((((.((.....)).)))))(((.....)))((((((((((.((((((.(((...(((((((((((((((..(((.(((..((..(((.....))).)).)))))))))))))))...)))))))))..))).))))).)))).))))))..)))))))..))).........	-105.4	429.46	161.46
novel-miR0060	ta_iwgsc_7dl_v1_3341310_41122	aaaaaugcaaguaaaaaaguaaggucaaccuuuguaaaaaaaacguaaguugcacuuuucauuagauugaguugcacuuuccugaaucgaucgggaaaaucacaguccacuagacauacaucgaucaugaaagcugugguagggaagcuacgaauggcuucguuuugucaugucgacuagcagauucgugaaguugccacccucacggguauguagcucagaugguagagcgcucguuagcaugcga	-	2286	2535	0.6	0.9	-1.3	ggguauguagcucagauggu	caugucgacuagcagauucgu	ta_iwgsc_7dl_v1_3341310	....((((((((..........(((((((((((.((((((((...(((.....)))....))))))))).)).......))))))))((((((((......))))))).........(((((((......(((......)))....)))))))....((((((((.....))))))))(((((((((((.(.(((((.((.((((((((.((.....)).)))))))))).)))))).))).)))))))))))....)))).))))....	-68.3	11165.99	787.13
novel-miR0061	ta_iwgsc_3b_v1_10686187_15724	aagagggagccaguuauccgguuuaauuuguccauagcaaccgcgauucccgccauucagcugugauagcugcugccuauugcacguugcggaagauagcugguggugcuauggauaaaauucaacccgauggguuggucaugcccgaaugaaggaagaauccugucuugaagcucgacugagccaacaguuugguuggagcagggaucucggucaucugcuuguaauuuuucuguauuauuaucauacaaccugc	+	385	634	2.5	15.2	3.9	gugcuauggauaaaauucaacc	uuaauuuguccauagcauccg	ta_iwgsc_3b_v1_10686187	....(((.((((((((((.((((.((((((((((((((((.((.(((((((.....((((((((((((.........))))))))).)))))))..))).)).)))))))))))))))))).)))).))))))))).)).)...))).((((..((.((.(((((.(((.((((..(((((......))))).)))).)))))))).)))).))))...............((((((........)))))......	-86.6	0.00	80.73
novel-miR0062	ta_iwgsc_2al_v1_6345017_5515	agcacgggaggugacugcucacagggggaauccaaauccaaaucccgcaccacaucacuauugcgguggggaagauugcaacuauaccuggccaucugucgggccgggccuaccaaagcccgaugcaaaaaaccccaggcccgagcccgaccagcccagccgcugagccuaaaaaucaagaccgagcccagcccaguagcgcuaaaagcguguugggccuugggccgggccucuucguuaacucaugaaua	+	8292	8541	3.5	0.4	-1.3	guugggccuugggccggg	cgagcccagcccauag	ta_iwgsc_2al_v1_6345017	...((((((((((((.((((....(((((((((....)))))...((((.(((((.((..........)).)))))))))))(((((.((.............)).))))((((((((((((((.......((......)).........)))))))).))))))))....))))))).)))..................(((.(((((((((((((.(((...))))))).))))))..)))))))))))).))))..............	-95	429.46	40.37
novel-miR0063	ta_iwgsc_4bl_v1_7036829_21561	uaaauccaaccggaaggguuggcgacauaaccgguugaaggaagcuuugcucccgcgcgcauccaggccgguuggugcagagugcccugguggcgccccagccggucaucgucgaccgcgggagccaauccucugguuugacuugucccauagcaucauccauccugccauucugcugaggugcgucugcccuuuugcacgauggugugaaaggcugguguggcuauggaugaaaucgaccagaaggugcugagc	+	1148	1397	4.8	24.7	3.9	uagaauggcuggugcuaugga	auagcaucauccauccugcca	ta_iwgsc_4bl_v1_7036829	......((((((((((........)))..)))))))).(((..((((((((.((((((..((.......))..))))).)))))))..)))..((((..((((.((.((((((........))))))))))).))))..((((((((((.((.((((((((((((((.((((.(((((..(((((.((((..((.....))))))..))).))..))))).)))).)))))))))))))).)).)))))))).)).....	-120.6	214.73	464.21
novel-miR0064	ta_iwgsc_4al_v2_7170245_20131	agcgugggucuuccuugcacucuauuuggaggauaugcaagaugaguaugauuuaucacucuguauucaucuguguauauucuucaaguggagagcaggaugacugaguuuuuuuuuuggagcuaacucggauaacccgagagguguucucgguaaacgugaaguugccgagaguagcccugguaaagauaaaggccaccaucaaaauuuugucccaccucgccuauagacaagcaacacgaccuguuaa	-	1622	1871	3.2	11.9	3.9	uauuuggaggauaugcaagaugag	caucguguauauucuucaagugga	ta_iwgsc_4al_v2_7170245	.(((.((((((.(((.(((.((((((((((((((((((((((.(((((((((((((......))).)))))))))).))))))))))))))))))))))))).))).))).))).(((..(((((..(((.(((.((.((...(((((.((.((...((((((((((.(......))))))))))))...)).)))))))..))).))).)))..)))..)))))...)))...)).)))...((((((.(.........).)))))).	-92.2	0.00	322.93
novel-miR0065	ta_iwgsc_2al_v1_6399394_5948	uguucaagcuuuuugcaagaguucagcggaaaaaaacaucagaggagcucucggcaaaaaaacaaaaucaauguucuaaguuugcgcacuguuuagcauugauuuuguuuuuuuuugugagagcucccucggauuguuuuuagcugaaucuuugcaaaaguucaaacauuuaaugaagcuuguuguucaagauuucagauuuucggaccuguuuuaucacauuuucgaaguuuugcaaacuc	+	4165	4414	3.9	2.8	-1.3	ucagcggaaaaaaacaucaga	ggauguuguuuuuagcugaaa	ta_iwgsc_2al_v1_6399394	((((..((((((((((((..((((((.(((((.((((((.((((((((((((..((((((((((((((((((((((..(((.......)))....)))))))))))))))))))))))..))))))))))))).))))))).))))))).)))))).))))))..)))))))))))))..))))......((((((((((.......)))).)))))))....(((((((...(((......)))...)))))))............	-99.7	0.00	80.73
novel-miR0066	ta_iwgsc_5dl_v1_4507581_29026	uauuuggcgcggacaaggcuccucugacguacugcagggugcaguuggcucacuaagagccaacugcacccuguaguacgucagaggagcugucuggcgcagaacaaggguagacagaaucgaaaaugcccuugcgcugcagcggggcgggaccgaagccuucucuuuuccuuacuggagaauaguaguggggaggcagaggaauagaauucgguggagaaagugaaggggggggggaauaaaagcggaaacguuuu	+	2019	2268	4.7	0.6	-1.3	ucugacguacugcagggugca	cacccuguaguacgucagagg	ta_iwgsc_5dl_v1_4507581	......((((((((..((((((((((((((((((((((((((((((((((((((......)))))))))))))))))))))))))))))))))))))))..)))).))))....((........((.((((.((.......((((((((.(((((((((......))))((((((......)))))...........))).))))))))........)).))).))........))......................((((((....)))))).	-117.9	0.00	60.55

Table S3 (continued)

Novel miRNA name	ID	Pre_seq	Strand	Start	End	Mfe_score	Total score	Star_score	miRNA sequence	Star_sequence	Genome ID	Hairpin_structure	Hairpin _energy	7 DAP	14 DAP
novel-miR0067	ta_iwgsc_5al_v1_2791901_24713	caaaaagguucacauagcuucuugaaccuuggggaaaagccgcauaacaccauuaacuugaaaaccauguauuuccuaucccaaaac ugcuccuagcacaaggggguaaaagaugguuuuuaguuguguggucaugcggauuuuucaccaagauucaagugguuaucuggaucu uuugacuuugaaaucuauacgugcuagugcacauuggauguaagugugucccuggugcaugugcucacugaagugca	-	7122	7371	2.8	20.1	3.9	ugaaccuuggggaaaagccgcau	gcggauuuuucaccaaga	ta_iwgsc_5al_v1_2791901	[illegible]	-88.9	429.46	686.22
novel-miR0068	ta_iwgsc_5bl_v1_10924917_27843	ggcauuucgacguuggggaagaugaaguagagcagagaccucacgcucaagaagcggugaggugccugaccuacuucaucuucacc aacgaagagaacggcgaaugcaacauugacggcugcuugcgcugcagaaaacuaucaaggagcucaacgagaccacaauaaaaaagaa uacauauaaggauauguucguauaauuaugucauuuuaaacuuauuuggcauaugcaguguagauguguuuacaac	-	10652	10901	1.8	2.1	-1.3	ugaaguagagcagagaccuca	aggugccugaccuacuucauc	ta_iwgsc_5bl_v1_10924917	[illegible]	-81.6	429.46	423.84
novel-miR0069	ta_iwgsc_1al_v2_3916116_359	uuaaaaagucuacauaaccucuugaaucuugggaaaaagcugcauaacuccaauaaauuggaaaccucauuuucuaucccaaaauug cuugcaaacacaauuucuggguaaaauauaugguuugaaguuaugagguuaugcagauuuuucaccaauauucaagugguuaucua gauuuuuuuagaaacuauggaacauauaucauugaagguacucuucgaccaaucuuacuuuauuuaggacaauuua	-	7634	7883	1	0.9	-1.3	ugaaucuugggaaaaagcug	gauuuuucaccaauauucaag	ta_iwgsc_1al_v2_3916116	[illegible]	-73.2	0.00	201.83
novel-miR0070	ta_iwgsc_2dl_v1_9769407_10602	auaucaaacuauauguccugcaagguagaagaccucagucuuaaaaaucucaagaacucuucgaugaaguauugcgcguccgagagu acauuagacguugguuacuugggagcgcaaaaacuuggguucuaauacauugguggaaccuagcaguaaccaacgucuaacaucauc uuagacauaugcaagauuucauccaagagcuuguugagaucuucaagacugagaucuuucgugcaaaagauauagu	-	1248	1497	3.8	4.1	-1.3	ugagaucuucaagacugaga	ucagucuuaaaaaucucaag	ta_iwgsc_2dl_v1_9769407	[illegible]	-97.8	0.00	787.13
novel-miR0071	ta_iwgsc_5ds_v1_2782660_30778	uucuuguacugaaagcaagagugucacacucgaugacacuaguagauaagaccaacaauaaggagcuuuugacaaaacagcgucagaa auuucagguagagaacacggcuucagauguugugcaguuuaguuucacuacucaagguggacgcaaaaaauuugagaaaugguauaa aauauguccaugguccuugaguagugaaauuaaacugagcaccaucugaacucuuguucucuagcugaagcuucu	-	12751	13000	3.9	1227.5	3.9	uuaaacugagcaccaucugaacuc	cuucagauguugugcaguuuaguu	ta_iwgsc_5ds_v1_2782660	[illegible]	-99.3	214.73	48418.67
novel-miR0072	ta_iwgsc_3dl_v1_2881909_16753	ggagcugugggugaacaguauaaguguccauuucuucagaaucuucagacuuuaggagcucgccaagauugggcccugacagggac gauggcuggugcuacggauaaauucaacccgauuggugugcaugcccgacugaaagaagaagccuugcugagaguuaagugagccg ggaaucaguuugaaacagggagccaguuauccgguuuaauuuguccauagcauccgcgaucccgccauucagcuguga	+	143	392	1.3	3.1	3.9	uuaauuuguccauagcauccg	ggugcuacggauaaauucaac	ta_iwgsc_3dl_v1_2881909	[illegible]	-76.5	0.00	60.55
novel-miR0073	ta_iwgsc_2bl_v1_8046566_8407	caauaucuaugagauaugagauacaaauuugucucauaauuaaagagaucuucuaggaucuuuauaucuaaauuuguucuugccauu aguuuucauauaacuuuccaacgcaguuucuuuuguugaacgcaaaaaaucaucauuuaugaugauacaagagagcacacgucuucc uaaacaagagaucauaaacuaacaucacuuguuuaggaacaugugugcucucuuguaucaucauaaaugaugauuu	+	15313	15562	3.6	3.9	-1.3	uuaggaacaugugugcucucuugu	aagagagcacacgucuuccuaaac	ta_iwgsc_2bl_v1_8046566	[illegible]	-95.7	214.73	13926.17
novel-miR0074	ta_iwgsc_5ds_v1_2732637_30405	ccuacgugagcaacgaugacgauuaugaagagaguacaacugaaggaggcagacucauggacauuuccucugaagaagacagcuagu uuugaccaccauguuaucuuaaauuuucaacaucggucuagacgauaaacagaaauuuaagauaacaugguggucgaaacuaguugu cuucuucagaggaaaugucaaugacuccgccuccuuuaguuguacucucuucauaaucgucaucguugcacacgua	-	13139	13388	4.9	3.3	-1.3	uuaugaagagaguacaacugaagg	uuuaguuguacucucuuc	ta_iwgsc_5ds_v1_2732637	[illegible]	-187	0.00	121.10
novel-miR0075	ta_iwgsc_5as_v1_1552033_25658	gauccuagaugagcuaggaaaugauuaaaaaaauccuauaguuuccaugcauaauaugguguuacuuugaaucuuacagguguuucac aggaaacaguucaaugucuaacgaacugaaaguuuuuuuuuccugaccauggcauuucuagucaauaggaauacgcuauguguugg aagacaaacuucuuuccagcaucuaguagauguugaacuguuuccucugacauuccuguaaauuucaugaguaguuu	-	2960	3209	2.2	2.5	-1.3	uugaacuguuuccucugacauuc	guuucacaggaaacaguucaaug	ta_iwgsc_5as_v1_1552033	[illegible]	-84.3	429.46	3612.73
novel-miR0076	ta_iwgsc_5bl_v1_10799490_26116	ggcuugcucccuguggcggccguugagacgagaucuccccauaggcccauaccacauagcggcagcucggcuccgugcacgcgua uggggagaucucgccucaacagccgcuccgugugagcuuuaccuuuacguacgaugaugcuuuugguccuuuccugcaucgcuu guugguaacgcgcucuccucuacuaguugaucuugcaugagagguagccuucuucaguccagccgagaguucacugcuggg	+	13156	13405	3.8	4.1	-1.3	uugagacgagaucuccccaua	ugggggagaucucgccucaaca	ta_iwgsc_5bl_v1_10799490	[illegible]	-97.8	0.00	100.91
novel-miR0077	ta_iwgsc_2ds_v1_1133030_11630	guggacgaagagggagcgaugagagccaaacauagcguuggaauacgagcaggagacgacggcguaggagaagcggcgcgggucgc gggggcgggcgcgcucccccuuguacucuuacaaccagaaguccucgguucuuagucuaaaucaucccauccucaacauucuuuca cagccauggccaagcuacauauauugugaaaguauauugaggaugggaugauuuagacaaaaagcucuggguaauacau	-	1004	1253	3.9	4.2	-1.3	uugaggaugggaugauuuaga	uaaaucaucccauccucaaca	ta_iwgsc_2ds_v1_1133030	[illegible]	-99	0.00	645.85
novel-miR0078	ta_iwgsc_4ds_v1_2274320_23450	ugcgcuguugggccggggucuguugggccuugggccgggugcgcuguugggucggggucugcugggcccuaggcuggcuccug gccugccuagcccucgcccgcuugggcuggcuggggugggaccgggucgcggcucuugggccuccugggguguccuaggcccagu cacccuacgagguggauauggccucgaggggauuucccugugcccccuuccacucccgcagccgccaccgcuuccuccgcgcac	+	6344	6593	4.8	1.3	-1.3	uugggccuugggccgggu	cuggccugccuagcc	ta_iwgsc_4ds_v1_2274320	[illegible]	-130.2	858.92	161.46
novel-miR0079	ta_iwgsc_1as_v1_2129014_1024	ccagucgcugcggggcuuuccuuugugcuugaucuaaccaugugguggaacgauggaaacggaacaugguucugucaagcaccgc ggaaagcaccgugcucuccugcagcauggcccgccaccgccgccaccaccgcuggacaccucuccucugcucuggagcaccgcag cccaccaccugccagacccaccucuccc	+	23	272	2.6	2.9	-1.3	uugugcuugaucuaaccaugu	augguucugucaagcaccgcg	ta_iwgsc_1as_v1_2129014	[illegible]	-69.6	1288.38	484.39

Table S4-1 : 7DAP and 14DAP differential expressed target gene known miRNAs

miRNA name	7 DAP	14 DAP	PValue	FDR	log2FC	regulated
bdi-miR319b-3p	2147.31	928.41	0.001076	0.004723	-1.2097	down
gma-miR167g	644.19	5328.27	0	0.000000	3.0481	up
tae-miR1127b-3p	0.00	1170.61	0.000469	0.002384	33.4465	up
tae-miR156	644.19	10091.43	0	0.000000	3.9695	up
tae-miR164	1932.57	4460.41	0.000222	0.001154	1.2067	up
tae-miR167c-5p	3220.96	10434.54	0	0.000000	1.6958	up
tae-miR319	644.19	2179.75	0.000739	0.003469	1.7586	up
tae-miR7757-5p	5797.72	16307.75	0	0.000000	1.4920	up
tae-miR9654b-3p	429.46	5731.93	0	0.000000	3.7384	up
tae-miR9655-3p	429.46	7104.37	0	0.000000	4.0481	up
tae-miR9658-3p	0.00	3390.72	0	0.000000	34.9809	up
tae-miR9662a-3p	3865.15	14309.65	0	0.000000	1.8884	up
tae-miR9662b-3p	3865.15	14309.65	0	0.000000	1.8884	up
tae-miR9663-5p	0.00	888.05	0.00165	0.006679	33.0480	up
tae-miR9668-5p	429.46	2442.13	0.000091	0.000504	2.5075	up
tae-miR9669-5p	2362.04	8961.19	0	0.000000	1.9237	up
tae-miR9670-3p	5797.72	27569.78	0	0.000000	2.2495	up
tae-miR9672b	5153.53	12190.45	0	0.000000	1.2421	up
tae-miR9674b-5p	1932.57	10192.34	0	0.000000	2.3989	up

Table S4-1 : 7DAP and 14DAP differential expressed target gene novel miRNAs

miRNA name	ID	7 DAP	14 DAP	log2FC	PValue	FDR	regulated
novel-miR0079	ta_iwgsc_ 1as_v1_2129014_1024	1288.38	484.39	-1.4113	0.002376	0.008729	down
novel-miR0078	ta_iwgsc_4ds_v1_2274320_23450	858.92	161.46	-2.4113	0.001129	0.004800	down
novel-miR0075	ta_iwgsc_5as_v1_1552033_25658	429.46	3612.73	3.0725	0.000001	0.000007	up
novel-miR0073	ta_iwgsc_2bl_v1_8046566_8407	214.73	13926.17	6.0191	0	0.000000	up
novel-miR0071	ta_iwgsc_5ds_v1_2782660_30778	214.73	48418.67	7.8169	0	0.000000	up
novel-miR0070	ta_iwgsc_2dl_v1_9769407_10602	0.00	787.13	32.8740	0.002585	0.009008	up
novel-miR0060	ta_iwgsc_7dl_v1_3341310_41122	11165.99	787.13	-3.8264	0	0.000000	down
novel-miR005	ta_iwgsc_6bl_v1_2921472_32514	3006.23	6438.33	1.0987	0.000066	0.000372	up
novel-miR0048	ta_iwgsc_7bl_v1_6691063_39345	3006.23	1432.98	-1.0689	0.000662	0.003197	down
novel-miR0046	ta_iwgsc_7bl_v1_571312_38763	214.73	1392.62	2.6972	0.00105	0.004796	up
novel-miR0040	ta_iwgsc_5dl_v1_3183779_28677	0.00	888.05	33.0480	0.00165	0.006679	up
novel-miR0036	ta_iwgsc_4bs_v1_4729575_21772	1717.84	60.55	-4.8264	0	0.000000	down
novel-miR0034	ta_iwgsc_1al_v2_3947972_580	0.00	988.96	33.2033	0.001053	0.004746	up
novel-miR0032	ta_iwgsc_3al_v1_4435088_13419	0.00	2684.32	34.6438	0.000001	0.000007	up
novel-miR0031	ta_iwgsc_5ds_v1_2761097_30588	0.00	1432.98	33.7383	0.000146	0.000790	up
novel-miR0030	ta_iwgsc_5ds_v1_2782660_30771	0.00	1836.64	34.0963	0.000024	0.000144	up
novel-miR0019	ta_iwgsc_6al_v1_5834455_31604	858.92	20.18	-5.4113	0.000021	0.000133	down
novel-miR0018	ta_iwgsc_3b_v1_10775495_16427	2576.77	0.00	-34.5848	0	0.000000	down
novel-miR0010	ta_iwgsc_7bl_v1_6747143_39914	0.00	827.50	32.9461	0.00216	0.008344	up
novel-miR001	ta_iwgsc_2al_v1_6404501_5978	1717.84	15984.82	3.2180	0	0.000000	up

Table S5. All targets of miRNAs and their sequences

Target ID	Gene ID	Gene sequence	Target ID	Gene ID	Gene sequence
MIR168-5p	unconservative_ta_iwgsc_6ds_v1_2075519_642817	UCGCUUGGUGCAGAUCGGGAC	MIR1139	tae-miR1139	AGAGUAACAUACACUAGUAACA
MIR9774	tae-miR9774	CAAGAUAUUGGGUAUUUCUGUC	MIR9776	tae-miR9776	UUGGACGAGGAUGUGCAACUG
MIR5384-3p	tae-miR5384-3p	UGAGCGCGCCGCCGUCGAAUG	MIR1120c-5p	tae-miR1120c-5p	UAAUAUAAGAACGUUUUUGAC
MIR1138	tae-miR1138	GCUUAGAUGUGACAUCCUUAAAA	MIR159b-3p.1	tae-miR159b	UUUGGAUUGAAGGGAGCUCUG
MIR9662b-3p	tae-miR9662b-3p	UGAACAUCCCAGAGCCACCGG	MIR9659-3p	tae-miR9659-3p	UCCAAUGGUUGUUCACGGCAUC
MIR9652-5p	tae-miR9652-5p	CCUGUUUGUCAUUAAGUUUCUU	MIR9781	tae-miR9781	UUUUGUCACAUAUAAUACAUA
MIR172b-3p	unconservative_ta_iwgsc_6al_v1_5830987_576438	AGAAUCUUGAUGAUGCUGCAU	MIR9772b-5p	tae-miR9772	UGAGAUGAGAUUACCCCAUAC
MIR1122	tae-miR1122a	UAGAUACAUCCGUAUCUAGA	MIR398g-3p	tae-miR398	UGUGUUCUCAGGUCGCCCCCG
MIR169	tae-miR169	GGGCAAGUCACCCUGGGCUACC	MIR399	tae-miR399	UGCCAAAGGAGAAUUGCCC
MIR160c-5p	tae-miR160	UGCCUGGCUCCCUGUAUGCCA	MIR9782	tae-miR9782	GUAUUAGGUUGGUCAAAUUGACGA
MIR9863b-3p	unconservative_ta_iwgsc_1ds_v1_1896382_84215	UGAGAAGGUAGAUCAUAAUAGC	MIR9663-5p	tae-miR9663-5p	AAGCGUAGUCGAACGAAUCUG
MIR5200-3p	tae-miR5200	UGUAGAUACUCCCUAAGGCUU	MIR9675-3p	tae-miR9675-3p	UUUAUGAUCACUCUCGUUUUG
MIR9664-3p	tae-miR9664-3p	UUGCAGUCCUCGAUGUCGUAG	MIR9656-3p	tae-miR9656-3p	CUUCGAGACUCUGAACAGCGG
MIR9653b	tae-miR9653b	UGGCCAAGGUCUCUUGAGGCU	MIR9674a-5p	tae-miR9674b-5p	AUAGCAUCAUCCAUCCUACCC
MIR531	tae-miR531	CGCUCGCCGGAGCAGCGUGCA	MIR9670-3p	tae-miR9670-3p	AGGUGGAAUACUUGAAGAAGA
MIR9672a-3p	tae-miR9672a-3p	CCACGACUGUCAUUAAGCAUC	MIR166d-3p	unconservative_ta_iwgsc_5bl_v1_10899037_498563	UCGGACCAGGCUUCAUUCCCC
MIR9672-3p	tae-miR9672b	UACCACGACUGUCAUUAAGCA	MIR167f-5p	tae-miR167c-5p	UGAAGCUGCCAGCAUGAUCUGC
MIR9660-5p	tae-miR9660-5p	UUGCGAGCAACGGAUGAAUC	MIR6197-5p	tae-miR6197-5p	UCUGUAAACAAAUGUAGGACG
MIR159b-3p.3	unconservative_ta_iwgsc_3b_v1_10640029_276849	UUUGCAUGACCGAGGAGCCGC	MIR395a	tae-miR395a	GUGAAGUGUUUGGGGGAACUC
MIR9678-3p	tae-miR9678-3p	UCUGGCGAGGGACAUACACUGU	MIR396-5p	tae-miR396-5p	AACUGUGAACUCGCGGGGAUG
MIR9775	tae-miR9775	UGUGCGCAAUAAGAUUUUGCUA	MIR9666b-5p	tae-miR9666b-5p	GCCAUCAUACGUCCAACCGUG
MIR9662a-3p	tae-miR9662a-3p	UUGAACAUCCCAGAGCCACCG	MIR397-5p	tae-miR397-5p	UCACCGGCGCUGCACACAAUG
MIR2275-3p	tae-miR2275-3p	UUUGGUUUCCUCCAAUAUCUCG	MIR9657b-5p	tae-miR9657b-5p	UUCGUCGGAGAAGCAUGUUGC
MIR530b	unconservative_ta_iwgsc_2dl_v1_994825_204770	UGCAUUUGCACCUGCACCUAC	MIR1130a	tae-miR1130a	CCUCCGUCUCGUAAUGUAAGACG
MIR1133	tae-miR1133	CAUAUACUCCCUCCGUCCGAAA	MIR5062-5p	tae-miR5062-5p	UGAACCUUAGGGAACAGCCGCAU
MIR9668-5p	tae-miR9668-5p	CCAAUGACAAGUAUUUUCGGA	MIR167b	tae-miR167b	UGAAGCUGACAGCAUGAUCUA
MIR1122b-3p	tae-miR1122b-3p	AGACUUAUAUGUAGGAACGGA	mir-218-5p	unconservative_ta_iwgsc_1as_v1_2129014_18194	UUGUGCUUGAUCUAACCAUGU
MIR1847-5p	tae-miR1847-5p	ACCUGCAGUUGGGCCAAUGAC	MIR1131	tae-miR1131	UAGUACCGGUUCGUGGCUAACC
MIR156b	tae-miR156	UGACAGAAGAGAGUGAGCACA	MIR1124	tae-miR1124	GCAGGACGUGAAGAGCGAGUCC
MIR1130b-3p	tae-miR1130b-3p	UCUUAUAUUAUGGGACGGAGG	MIR530	tae-miR530	UGCAGUGGCAUAUGCAACUCU
MIR9657a-3p	tae-miR9657a-3p	UGUGCUUCCUCGUCGAACGGU	MIR9779	tae-miR9779	CUUAUGCAACGUCUGAGGAU
MIR9657b-3p	tae-miR9657b-3p	CGUGCUUCCUCGUCGAACGGU	MIR1136	tae-miR1136	UUGUCGCAGGUAUGGAUGUAUCUA
MIR444a	tae-miR444b	UUGCUGCCUCAAGCUUGCUGC	MIR9777	tae-miR9777	AGCAAACAUAUCUGAGCACA

Table S5(continued)

Target ID	Gene ID	Gene sequence	Target ID	Gene ID	Gene sequence
MIR5175-5p	tae-miR5175-5p	UUCCAAAUUACUCGUCGUGGU	MIR1134	tae-miR1134	CAACAACAACAAGAAGAAGAAGAU
MIR7757-5p	tae-miR7757-5p	AUAAAACCUUCAGCUAUCCAUC	MIR1123	tae-miR1123	UCCGUGAGACCUGGUCUCAUAGA
MIR9655-3p	tae-miR9655-3p	CAAGGGAAGGAAGUAGCCAAC	MIR1127a	tae-miR1127a	UCCUUCCGUUCGGAAUUAC
MIR9674a-5p	tae-miR9674a-5p	GCAUCAUCCAUCCUACCAUUC	MIR9661-5p	tae-miR9661-5p	UGAAGUAGAGCAGGGACCUCA
MIR1137a	tae-miR1137a	UAGUACAAAGUUGAGUCAUC	MIR408	tae-miR408	CUGCACUGCCUCUUCCCUGGC
MIR1125	tae-miR1125	AACCAACGAGACCAACUGCGGCGG	MIR9666a-3p	tae-miR9666a-3p	CGGUAGGGCUGUAUGAUGGCGA
MIR9667-5p	tae-miR9667-5p	AAAUAUGGCAAACAAUGAAUG	MIR5048-5p	tae-miR5048-5p	UUUGCAGGUUUUAGGUCUAAGU
MIR164c-5p	tae-miR164	UGGAGAAGCAGGGCACGUGCA	MIR1120b-3p	tae-miR1120b-3p	UUCUUAUAUUGUGGGACAGAG
MIR171c	unconservative_ta_iwgsc_1bl_v1_3820936_34131	UGAUUGAGCCGCGCCAAUAUC	MIR1117	tae-miR1117	UAGUACCGGUUCGUGGCACGAACC
MIR171c-3p	tae-miR171b	UUGAGCCGUGCCAAUAUCACG	MIR1135	tae-miR1135	CUGCGACAAGUAAUUCCGAACGGA
MIR167d-5p	unconservative_ta_iwgsc_5ds_v1_2769792_560237	UGAAGCUGCCAGCAUGAUCUGA	MIR9669-5p	tae-miR9669-5p	UACUGUGGGCACUUAUUUGAC
MIR9780	tae-miR9780	CGGGUCGGCGCUGCACGCGGC	MIR9677-3p	tae-miR9677a	UGGCCGUUGGUAGAGUAGGAGA
MIR5084	tae-miR5084	AUACAGUACUGCAGAGGAUCCUAA	MIR5049-3p	tae-miR5049-3p	AAUAUGGAUCGGAGGGAGUAC
MIR9778	tae-miR9778	UGCAUCAUCUCGAACUCGUCG	MIR319-3p	tae-miR319	UUGGACUGAAGGGAGCUCCCU
MIR5086	tae-miR5086	ACAUUGGUGGAAGGCGUGGUA	MIR9676-5p	tae-miR9676-5p	UGGAUGUCAUCGUGGCCGUACA
MIR1122c-3p	tae-miR1122c-3p	UCUAAUAUUAUGGGACGGAGG	MIR1120a	tae-miR1120a	ACAUUCUUAUAUUAUGAGACGGAG
MIR9679-5p	tae-miR9679-5p	CAGAACCAGAAUGAGUAGCUC	MIR5085	tae-miR5085	AAGGACAUUUUUUGUGGCCUG
MIR9783	tae-miR9783	AUAAGCACCGGUGCUUAAGAA	MIR1137b-5p	tae-miR1137b-5p	UCCGUUCCAGAAUAGAUGACC
MIR9674b-3p	unconservative_ta_iwgsc_3b_v1_10686187_281121	UGAAUUUGUCCAUAGCAUCAG	MIR9665-3p	tae-miR9665-3p	GCUAGCAGUGUAAACUCAAAUCA
MIR1119	tae-miR1119	UGGCACGGCGUGAUGCUGAGUCAG	MIR171j	tae-miR171a	UGAUUGAGCCGUGCCAAUAUC
MIR1118	tae-miR1118	CACUACAUUAUGGAAUGGAGGGA	MIR1121	tae-miR1121	AGUAGUGAUCUAAACGCUCUUA
MIR9677b	tae-miR9677b	CAGGGCGGGGAACAGGUGGCC	MIR396e-5p	unconservative_ta_iwgsc_7as_v1_4208248_676353	UUCCACAGCUUUCUUGAACUG
MIR9666b-3p	tae-miR9666b-3p	CGGUUGGGCUGUAUGAUGGCGA	MIR396b-5p	unconservative_ta_iwgsc_2bl_v1_7931332_135085	UCCACAGGCUUUCUUGAACUG
MIR1127b-3p	tae-miR1127b-3p	ACAAGUAUUUCUGGACGGAGG	MIR9653a-3p	tae-miR9653a-3p	UUUGAGACUUUGGCCAUGGCC
MIR9773	tae-miR9773	UUUGUUUUUAUGUUAUUUUGUGAA	MIR395c-3p	tae-miR395b	UGAAGUGUUUGGGGGAACUC
MIR9658-3p	tae-miR9658-3p	AUCGUUCUGGGUGAAUAGGCC	MIR1128	tae-miR1128	UACUACUCCCUCCGUCCGAAA
MIR1129	tae-miR1129	CAGCGAGCCAGCGGAGACCGGCAG	MIR9652-3p	tae-miR9652-3p	AAGCUUAAUGAGAACAUGUG
MIR393-5p	unconservative_ta_iwgsc_2bl_v1_8036763_148264	UUCCAAAGGGAUCGCAUUGAU	MIR5050	tae-miR5050	UUGAACGACCUCACCAUGUCG
MIR9654a-3p	tae-miR9654a-3p	UUCUGAAAGGCUUGAAGCGAAU	MIR167e-5p	tae-miR167a	UGAAGCUGCCAGCAUGAUCUA
MIR9654b-3p	tae-miR9654b-3p	UUCCGAAAGGCUUGAAGCGAAU	MIR9671-5p	tae-miR9671-5p	UGACUUUACACAACUGUCCGGC
MIR9673-5p	tae-miR9673-5p	UAAGAAGCAAAUAGCACAUG	MIR6201-5p	tae-miR6201	UGACCCUGAGGCACUCAUACCG

Table S7: 7 DAPvs 14 DAP differential expressed target gene list

Target ID	FDR	Regulated*	Target ID	FDR	Regulated*	Target ID	FDR	Regulated*	Target ID	FDR	Regulated*
Traes_1AL_08A2F5FA4	0.00675272	down	Traes_1DL_74C5155C71	0.003427875	up	Traes_2BL_5320E59A6	0.00675272	down	Traes_3AS_2984B96FF	0.006881614	down
Traes_1AL_0BE456AC0	0	up	Traes_1DL_7AC3AE1EB	0.007343798	up	Traes_2BL_621DAFE84	0.007330092	up	Traes_3AS_2A0765E10	0.002915577	up
Traes_1AL_3F7B8E94D	0.00688161	down	Traes_1DL_8F7356527	0.002915577	up	Traes_2BL_6AEE8AC28	0	up	Traes_3AS_5320E59A6	0.00675272	down
Traes_1AL_655726D7B	0.00675272	down	Traes_1DL_9344DD117	0	up	Traes_2BL_6FB991299	0.003304355	down	Traes_3AS_6CB1DBD28	0	up
Traes_1AL_8475A65E8	0.0073438	up	Traes_1DL_DE87A275A	0.007343798	up	Traes_2BL_7D700A166	0	up	Traes_3AS_9A9B26465	0.00675272	down
Traes_1AL_9A9B26465	0.00675272	down	Traes_1DL_E50A6EE94	0.00675272	down	Traes_2BL_85B3DD81F	0.003304355	down	Traes_3AS_B852F4AE7	0	up
Traes_1AL_B17E6CD28	0.00330435	down	Traes_1DS_05B16509E	0	up	Traes_2BL_DE9A7327E	0	up	Traes_3AS_E462B8D6F	0	up
Traes_1AL_B6931117F	0	up	Traes_1DS_0A4757864	8.66E-05	up	Traes_2BS_257C5B099	0	up	Traes_3B_12726B04B	0	up
Traes_1AL_C66F70A34	0.00675272	down	Traes_1DS_26F1546F6	7.39E-06	up	Traes_2BS_257C5B099	0	up	Traes_3B_224A6B7E5	0	up
Traes_1AL_EEF9D88DA	0	up	Traes_1DS_52539E9F6	0	up	Traes_2BS_273971066	0.003304355	down	Traes_3B_240E7DC49	0.003304355	down
Traes_1AS_0F7CF1F34	0	up	Traes_1DS_5320E59A6	0.00675272	down	Traes_2BS_47D142DD2	0.00675272	down	Traes_3B_312EF888A	0.006881614	down
Traes_1AS_17306388F	0.00291558	up	Traes_1DS_5852D4AB6	0.003304355	down	Traes_2BS_47E2D6444	7.39E-06	up	Traes_3B_472072804	0.003304355	down
Traes_1AS_3C84E3ADE	8.66E-05	up	Traes_1DS_5D3ACE9A3	8.66E-05	up	Traes_2BS_5320E59A6	0.00675272	down	Traes_3B_5320E59A6	0.00675272	down
Traes_1AS_45E7AEA09	0	up	Traes_1DS_610553C1F	0	up	Traes_2BS_74269A4C5	0	up	Traes_3B_5320E59A61	0.00675272	down
Traes_1AS_488596E82	0.00330435	down	Traes_1DS_85171B27B	0	up	Traes_2BS_80474BB5B	0	up	Traes_3B_5320E59A62	0.00675272	down
Traes_1AS_4D4B7A137	0	up	Traes_1DS_9A9B26465	0.00675272	down	Traes_2BS_926E8FE94	8.66E-05	up	Traes_3B_5320E59A63	0.00675272	down
Traes_1AS_5317928F1	8.66E-05	up	Traes_1DS_9A9B264651	0.00675272	down	Traes_2BS_9A9B26465	0.00675272	down	Traes_3B_5CCE0BB6B	0.006881614	down
Traes_1AS_5320E59A6	0.00675272	down	Traes_1DS_9D0A687D5	8.66E-05	up	Traes_2BS_CCA9B58A3	0	up	Traes_3B_74D1769AF	0.003304355	down
Traes_1AS_6EDAD815E	0	up	Traes_1DS_ACCFFA791	8.66E-05	up	Traes_2BS_D26800667	0.007330092	up	Traes_3B_7FFFF7BCF	0	up
Traes_1AS_81CF30171	0	up	Traes_1DS_BDACE1560	0	up	Traes_2BS_DDF26C784	0	up	Traes_3B_BA8E1E7ED	0.003304355	down
Traes_1AS_91A304B6B	0.00330435	down	Traes_1DS_DE3675D591	8.66E-05	up	Traes_2BS_E1C6C866F	0.002915577	up	Traes_3B_BE203E9B7	0	up
Traes_1AS_92A698B19	8.66E-05	up	Traes_1DS_EF905F915	8.66E-05	up	Traes_2BS_EA063BB91	0.003304355	down	Traes_3B_BFCCD3CD5	0	up
Traes_1AS_AEF846E0A	0	up	Traes_1DS_EFA1BC727	0	up	Traes_2BS_EAED42A93	0.00675272	down	Traes_3B_D2DA34BB5	1.39E-05	up
Traes_1BL_1E61EBE23	0.0073438	up	Traes_1DS_F3DE3946F	8.66E-05	up	Traes_2DL_092D759DB	0.00675272	down	Traes_3B_EC4229279	0.003304355	down
Traes_1BL_29B4E633D	0	up	Traes_1DS_F957D1B2C	8.66E-05	up	Traes_2DL_0A7F757AB	0.003304355	down	Traes_3B_FAA7BF6EB	0.005795692	up
Traes_1BL_30CF86D20	0.00330435	down	Traes_1DS_FD33F6FB81	0.006881614	down	Traes_2DL_0D2359A4B	0.00675272	down	Traes_3DL_266169C52	0	up
Traes_1BL_32F5519AA	7.39E-06	up	Traes_2AL_060BD6D85	0.002915577	up	Traes_2DL_3B4943F11	0	up	Traes_3DL_2BAC94A99	0.003304355	down
Traes_1BL_43408C9B0	0	up	Traes_2AL_07B1977B8	0	up	Traes_2DL_6523CB829	0	up	Traes_3DL_5320E59A6	0.00675272	down
Traes_1BL_5320E59A6	0.00675272	down	Traes_2AL_21B8BCA9C	0.003304355	down	Traes_2DL_6523CB829	0	up	Traes_3DL_557CF2133	0	up
Traes_1BL_68FE6F389	0.00128768	up	Traes_2AL_24A1BF00C	7.39E-06	up	Traes_2DL_7788247EE	0.002915577	up	Traes_3DL_AE21424BF	0.005795692	up
Traes_1BL_80EEC6A4B	0	up	Traes_2AL_2FE59A2DC	0	up	Traes_2DL_8616F1BE0	0.002915577	up	Traes_3DL_E4BCD7E0F	0	up
Traes_1BL_894270AB6	0	up	Traes_2AL_5320E59A6	0.00675272	down	Traes_2DL_A51AE44DB	0.001820934	up	Traes_3DS_5A7B6610B	0.003304355	down
Traes_1BL_D3435B186	0.00330435	down	Traes_2AL_57BF37A86	0	up	Traes_2DL_B985E7A31	0.003304355	down	Traes_3DS_642F82187	0	up
Traes_1BS_0201F2E08	0	up	Traes_2AL_6061FA883	0	up	Traes_2DL_BE754F0C1	0	up	Traes_3DS_698693E7B	0.00675272	down
Traes_1BS_039F236C8	0	up	Traes_2AL_63B2E5BC2	0	up	Traes_2DL_DECB6FBDB	0.003304355	down	Traes_3DS_A3E61DE40	0.006881614	down
Traes_1BS_2C0997E92	8.66E-05	up	Traes_2AL_9A9B26465	0.00675272	down	Traes_2DS_1A728B7C8	0	up	Traes_4AL_01B51B9A6	0	up
Traes_1BS_34551F337	0	up	Traes_2AL_9F9CB4FC5	0.003304355	down	Traes_2DS_3C9BB1430	0.003593411	up	Traes_4AL_364F56EDB	0.005795692	up
Traes_1BS_5320E59A6	0.00675272	down	Traes_2AL_CF41123D7	0.007330092	up	Traes_2DS_3F4E4E00D	0.003304355	down	Traes_4AL_3A009739E	0	up
Traes_1BS_566CEE7F6	0	up	Traes_2AL_DB39E5DF0	0.001820934	up	Traes_2DS_5320E59A6	0.00675272	down	Traes_4AL_41BB996C2	0.003304355	down
Traes_1BS_743E7D07B	8.66E-05	up	Traes_2AS_38949B685	8.66E-05	up	Traes_2DS_6764E73B1	0.007330092	up	Traes_4AL_5320E59A6	0.00675272	down
Traes_1BS_9495B49F3	8.66E-05	up	Traes_2AS_4074D3013	8.66E-05	up	Traes_2DS_7A8332C11	8.66E-05	up	Traes_4AL_5320E59A61	0.00675272	down
Traes_1BS_9A9B26465	0.00675272	down	Traes_2AS_7EC007005	0	up	Traes_2DS_851973269	0	up	Traes_4AL_5320E59A62	0.00675272	down
Traes_1BS_A04AAC3F4	0	up	Traes_2AS_861B2054C	0.00675272	down	Traes_2DS_C70D4FD30	0.007330092	up	Traes_4AL_6752FAFB9	0.002915577	up
Traes_1BS_B64557385	0.00688161	down	Traes_2AS_9A9B26465	0.00675272	down	Traes_2DS_EED4D856B	0.002915577	up	Traes_4AL_8C19008DD	0.00675272	down
Traes_1BS_BA046E212	0.00330435	down	Traes_2AS_BCEF3FFED	0.002915577	up	Traes_3AL_0B934C4F9	0.005795692	up	Traes_4AL_9B1541282	0.003304355	down
Traes_1BS_DDC038FDC	0	up	Traes_2AS_EC7E6EAF0	0	up	Traes_3AL_0FF2B47AC	0.003304355	down	Traes_4AL_A4B73CC6A1	0	up
Traes_1DL_23506ECCE	0	up	Traes_2BL_094926B66	0.001820934	up	Traes_3AL_5320E59A6	0.00675272	down	Traes_4AL_A8CBB811B	0	up
Traes_1DL_3C82599EB	0.00330435	down	Traes_2BL_0CCE42A38	0.005795692	up	Traes_3AL_723E36E27	0	up	Traes_4AL_ACA5DBB90	0.006474875	up
Traes_1DL_430D91CBE	0.00291558	up	Traes_2BL_173580E45	0	up	Traes_3AL_9A9B26465	0.00675272	down	Traes_4AL_AE051A2FF	0	up
Traes_1DL_74C5155C7	0.00342788	up	Traes_2BL_177A50B76	0.002915577	up	Traes_3AL_CBDA73B13	0	up	Traes_4AL_B7076F544	0.003304355	down

Table S7(continued)

Target ID	FDR	Regulated*	Target ID	FDR	Regulated*	Target ID	FDR	Regulated*	Target ID	FDR	Regulated*
Traes_4AL_EE269B066	0.00647488	up	Traes_5AL_B4E8A3115	0	up	Traes_5DS_1BDB9837E	0.003304355	down	Traes_6BS_7F1846171	0	up
Traes_4AS_3698CBB71	0.00330435	down	Traes_5AL_E3F2E3C4C	0.006881614	down	Traes_5DS_50846FD0C	0	up	Traes_6BS_82F5BF11F	0	up
Traes_4AS_5320E59A6	0.00675272	down	Traes_5AL_EBED2DA42	0.002915577	up	Traes_5DS_511F35A45	0	up	Traes_6BS_843505CB3	0	up
Traes_4AS_61277E38D	0.00291558	up	Traes_5AS_268D29356	0	up	Traes_5DS_8DB03B7DA	0	up	Traes_6BS_9A9B26465	0.00675272	down
Traes_4AS_8610A0310	0.00330435	down	Traes_5AS_5320E59A6	0.00675272	down	Traes_5DS_9A9B26465	0.00675272	down	Traes_6BS_B0E9CDCB7	0.003304355	down
Traes_4AS_9E5A45F1C	0.00688161	down	Traes_5AS_BDFB08E23	0	up	Traes_5DS_9A9B26465	0.00675272	down	Traes_6BS_B59D85BB7	0.003304355	down
Traes_4AS_AF545C909	0	up	Traes_5AS_E5FD82E36	0	up	Traes_5DS_9F4A5AE9F	0	up	Traes_6BS_C3247654A	0	up
Traes_4AS_B726A789E	0.00579569	up	Traes_5BL_0212ACBD9	0.005795692	up	Traes_5DS_C87840EBA	0	up	Traes_6BS_C921AEBA2	0.006881614	down
Traes_4AS_CBBAA0282	0.00291558	up	Traes_5BL_1E751EF1F	0	up	Traes_5DS_CBE009155	0.00675272	down	Traes_6BS_CBD931158	0	up
Traes_4AS_FFF5B1ECA	0.00675272	down	Traes_5BL_27DA4F6E1	0	up	Traes_6AL_4A505E21A	0	up	Traes_6BS_E71FD70EE1	0	up
Traes_4BL_217ED47D4	0.00579569	up	Traes_5BL_3AD291756	0.005795692	up	Traes_6AL_5320E59A6	0.00675272	down	Traes_6BS_EE1BBAFD1	0	up
Traes_4BL_29D8171E0	0	up	Traes_5BL_48A35287E	0.006009053	up	Traes_6AL_EEA5F4238	0	up	Traes_6BS_F50663DF7	0	up
Traes_4BL_3874C14AB	0	up	Traes_5BL_5320E59A6	0.00675272	down	Traes_6AL_F5BFFCA3E	0	up	Traes_6BS_F8176B2AA	0	up
Traes_4BL_4D49C13C4	0.00291558	up	Traes_5BL_58BAC1B31	0.003304355	down	Traes_6AS_026FEB41A	0.00675272	down	Traes_6DL_239151DA0	0	up
Traes_4BL_5320E59A6	0.00675272	down	Traes_5BL_60427EB5A	0	up	Traes_6AS_0A204AEC9	0	up	Traes_6DL_249028825	0.00675272	down
Traes_4BL_5DBDE54F9	0	up	Traes_5BL_6164B21A3	0.003304355	down	Traes_6AS_1496A7825	0	up	Traes_6DL_2490288251	0.00675272	down
Traes_4BL_65AB61144	0.00330435	down	Traes_5BL_6216A6108	0	up	Traes_6AS_15C1E6F18	0	up	Traes_6DL_426E71A31	0.003304355	down
Traes_4BL_84706BB7B	0	up	Traes_5BL_76435E271	0.003304355	down	Traes_6AS_2BDC1663A	0	up	Traes_6DL_4C3F04219	0	up
Traes_4BL_9A9B26465	0.00675272	down	Traes_5BL_7F0FD1538	0	up	Traes_6AS_328702561	0.003304355	down	Traes_6DL_6CE682354	0	up
Traes_4BL_F91DC939A	0.00688161	down	Traes_5BL_7FB30012A	0.006881614	down	Traes_6AS_32A35158A	0	up	Traes_6DL_B07415A76	0	up
Traes_4BL_F91DC939A1	0.00688161	down	Traes_5BL_8740ABFAA	0.003304355	down	Traes_6AS_3641B9C87	0	up	Traes_6DS_0E84AAF77	0	up
Traes_4BS_257C5B099	0	up	Traes_5BL_96D5EA4EE	0	up	Traes_6AS_36EAEADDD	0	up	Traes_6DS_0F1335DA2	0	up
Traes_4BS_276BA2B3E	0	up	Traes_5BL_96FDFC152	0	up	Traes_6AS_3AFC735F7	0	up	Traes_6DS_1BEF46735	0	up
Traes_4BS_375EF1D15	0	up	Traes_5BL_9774FCB1A	0.003304355	down	Traes_6AS_3DD96BAE3	0	up	Traes_6DS_2D4EFFF3E	0	up
Traes_4BS_4FB4784F0	0.00330435	down	Traes_5BL_A832F6F2B	0.003304355	down	Traes_6AS_45D1CB908	0.003304355	down	Traes_6DS_2F77E1434	0.007330092	up
Traes_4BS_5320E59A6	0.00675272	down	Traes_5BL_B47117D0F	0	up	Traes_6AS_48B701BB4	0	up	Traes_6DS_3F194116E	0	up
Traes_4BS_95683BBEB	0.00647488	up	Traes_5BL_DD4C9F446	0	up	Traes_6AS_5320E59A6	0.00675272	down	Traes_6DS_3FC2ADA87	0	up
Traes_4BS_AAE1439D4	0	up	Traes_5BL_E63627BC8	0.002915577	up	Traes_6AS_6612DF8D7	0	up	Traes_6DS_422644406	0	up
Traes_4BS_CAEB4EC73	0.00647488	up	Traes_5BS_360DD5644	0	up	Traes_6AS_8920C1490	0.006881614	down	Traes_6DS_5320E59A6	0.00675272	down
Traes_4BS_FB4E1F982	0.00675272	down	Traes_5BS_5320E59A6	0.00675272	down	Traes_6AS_99C6A3B0B	0	up	Traes_6DS_5878FA87F	0	up
Traes_4DL_2C636B5DB	0	up	Traes_5BS_5320E59A61	0.00675272	down	Traes_6AS_A2EAE01C1	0	up	Traes_6DS_7876A591C	0	up
Traes_4DL_30D9B0865	0.00330435	down	Traes_5BS_9A9B26465	0.00675272	down	Traes_6AS_B24138E62	0	up	Traes_6DS_7979B425D	0	up
Traes_4DL_3AF08121F	0.00688161	down	Traes_5BS_9F043AE17	0	up	Traes_6AS_CFFBE97AE	0	up	Traes_6DS_7B1596298	0	up
Traes_4DL_44AB91D93	0	up	Traes_5BS_BCEA0112C	0	up	Traes_6AS_D5A6B2CDD	0	up	Traes_6DS_7C389C178	0	up
Traes_4DL_5320E59A6	0.00675272	down	Traes_5BS_E10756355	0.005795692	up	Traes_6AS_EBADF37C7	0	up	Traes_6DS_893F318A9	0	up
Traes_4DL_964466BEC	0.00330435	down	Traes_5BS_F5AFB24CD	0	up	Traes_6AS_EE73C7654	0	up	Traes_6DS_8AB083B251	0	up
Traes_4DL_A7AFE1359	0.00579569	up	Traes_5DL_1272B4BAC	0	up	Traes_6AS_EE8BA0A57	0	up	Traes_6DS_90DF8DF85	0	up
Traes_4DL_AEF5AB905	0.00330435	down	Traes_5DL_1733FB4DA	0	up	Traes_6AS_FBBCFBCB0	0	up	Traes_6DS_93C7DA226	0	up
Traes_4DL_C56ABA3D6	0.00291558	up	Traes_5DL_3480D5338	0.007330092	up	Traes_6BL_066E7881C	7.39E-06	up	Traes_6DS_93C7DA2261	0	up
Traes_4DL_F064084A9	0	up	Traes_5DL_51EE2478D	0.005795692	up	Traes_6BL_5320E59A6	0.00675272	down	Traes_6DS_9A9B26465	0.00675272	down
Traes_4DS_261E93270	0	up	Traes_5DL_5E1EF029C	0.002915577	up	Traes_6BS_0159CBF63	0.0025484	up	Traes_6DS_9DF272FBB	0.006881614	down
Traes_4DS_477CA2A2F	0.00291558	up	Traes_5DL_61539C61A	0.00675272	down	Traes_6BS_02E6D8921	0	up	Traes_6DS_A2E0A29DE	0	up
Traes_4DS_4836E882E	0.00647488	up	Traes_5DL_6A730A96A	7.39E-06	up	Traes_6BS_1FF560649	0	up	Traes_6DS_A490DF33E	0	up
Traes_4DS_5320E59A6	0.00675272	down	Traes_5DL_7643B2ADB	0	up	Traes_6BS_28F0D2955	0	up	Traes_6DS_A80BF9FB0	0	up
Traes_4DS_9509649D6	0.00647488	up	Traes_5DL_8F7AC72B0	0	up	Traes_6BS_2AC6A2287	0	up	Traes_6DS_C4D93239C	0	up
Traes_4DS_C08BF9DB2	0	up	Traes_5DL_90BD9F306	0.005795692	up	Traes_6BS_2F108C5BB	0	up	Traes_6DS_CD0257751	0.003304355	down
Traes_4DS_F6F01D11D	0.00733009	up	Traes_5DL_9B467A4B5	0.002915577	up	Traes_6BS_3A00751B5	0	up	Traes_6DS_CF627A1BC	0	up
Traes_5AL_46A8C76DD	0.00675272	down	Traes_5DL_A9969D596	0	up	Traes_6BS_46641470F	0	up	Traes_6DS_D12396896	0.006881614	down
Traes_5AL_5320E59A6	0.00675272	down	Traes_5DL_B888E7A8A	0.00675272	down	Traes_6BS_4E6E57FEB	0	up	Traes_6DS_DAD8BEF41	0	up
Traes_5AL_8453D8DEB	7.39E-06	up, down	Traes_5DL_E23A44A1A	0.003304355	down	Traes_6BS_508C2BEA2	0	up	Traes_6DS_E352D66A4	0	up
Traes_5AL_8992A0E53	0	up	Traes_5DS_0479C6E0F	0	up	Traes_6BS_5320E59A6	0.00675272	down	Traes_6DS_E65BDAC1F	0	up
Traes_5AL_A5234EC05	0	up	Traes_5DS_094A03C13	0	up	Traes_6BS_542961EA4	0	up	Traes_6DS_E95930B7D	0	up

Table S7(continued)

Target ID	FDR	Regulated*	Target ID	FDR	Regulated*	Target ID	FDR	Regulated*
Traes_6DS_EF75D5C67	0	up	Traes_7BL_3185B27FC	0.002915577	up	Traes_7DL_6E5F4B350	0	up
Traes_6DS_F6C7E588F	0	up	Traes_7BL_3998DF2E9	0	up	Traes_7DL_8D1D4C6B9	0	up
Traes_6DS_FB434BB3C	0	up	Traes_7BL_5320E59A6	0.00675272	down	Traes_7DL_91CFE828F	0	up
Traes_7AL_5320E59A6	0.00675272	down	Traes_7BL_545257543	0	up	Traes_7DL_AB7C59D49	7.39E-06	up
Traes_7AL_628A69311	0	up	Traes_7BL_5E48E79E9	0.003304355	down	Traes_7DL_B3F28F4C5	0.002915577	up
Traes_7AL_8B2703BF9	0.00182093	up	Traes_7BL_6EB8A9854	0.002915577	up	Traes_7DL_C9F7DA32F	0.002915577	up
Traes_7AL_BDB97667C	0.00291558	up	Traes_7BL_83E236266	0.003304355	down	Traes_7DS_08353C1F8	0	up
Traes_7AL_BE3253C8F	0	up	Traes_7BL_CEB031295	0	up	Traes_7DS_234E9CF89	0.002915577	up
Traes_7AL_CA0186AF1	0	up	Traes_7BL_EDD1A215E	0.002915577	up	Traes_7DS_2B1FE04A6	0	up
Traes_7AS_1589E2272	0.00330435	down	Traes_7BL_F1A0270C6	0	up	Traes_7DS_2C77A229F	0.006881614	down
Traes_7AS_451D041F9	0	up	Traes_7BS_43035700A	0	up	Traes_7DS_344FF0A7C	0	up
Traes_7AS_46C76AD7C	0.00675272	down	Traes_7BS_5320E59A6	0.00675272	down	Traes_7DS_3A130381E	0.002915577	up
Traes_7AS_5320E59A6	0.00675272	down	Traes_7BS_598310C32	0	up	Traes_7DS_45E7AEA09	0	up
Traes_7AS_61114500F	0.00128768	up	Traes_7BS_600DF224D	0.006881614	down	Traes_7DS_5B1210681	0	up
Traes_7AS_7D807D401	0.00291558	up	Traes_7BS_B8F3E8F3D	0.006881614	down	Traes_7DS_6AE21AA6B	0.006881614	down
Traes_7AS_8336BE8C1	0.00688161	down	Traes_7BS_F3F4F9276	0	up	Traes_7DS_87E0FB885	0.006881614	down
Traes_7AS_B17BC0FC2	0.00579569	up	Traes_7DL_1E343B6E6	0	up	Traes_7DS_88B703F52	0.00675272	down
Traes_7AS_BBE386C09	0.00330435	down	Traes_7DL_208961165	0.003304355	down	Traes_7DS_9C9AA098D	0	up
Traes_7AS_BD1F4F597	0	up	Traes_7DL_31349FE57	0.003304355	down	Traes_7DS_A21E1E053	0.006881614	down
Traes_7AS_BDE4D9057	0	up	Traes_7DL_35A4FFEFD	0.001820934	up	Traes_7DS_C01D6468D	0	up
Traes_7AS_CFAF9635F	0.00291558	up	Traes_7DL_5320E59A6	0.00675272	down	Traes_7DS_D16B7A9E5	0.002915577	up
Traes_7AS_D114D49AD	0.00688161	down	Traes_7DL_5320E59A61	0.00675272	down	Traes_7DS_DAC187F5E	0	up
Traes_7AS_FE67F9E86	0	up	Traes_7DL_574A91F0E	0.001820934	up	Traes_7DS_DBB29C7CD	0	up

Table S10. Summary of KEEG pathway of differentially expressed miRNA targets.

#Pathway	ko_ID	Unigene	gene	Unigene_all	gene_all		
Starch and sucrose metabolism	ko00500	1	1	101	145	Traes_4DS_C08BF9DB2	K00696
Protein processing in endoplasmic reticulum	ko04141	2	5	101	145	Traes_1AS_488596E82;Traes_1DS_5852D4AB6	K09517+K09517
Porphyrin and chlorophyll metabolism	ko00860	5	5	101	145	Traes_2BL_094926B66;Traes_2DL_A51AE44DB;Traes_7AL_8B2703BF9;Traes_7DL_35A4FFEFD;Traes_7DL_574A91F0E	K00510+K00510+K00510+K00510+K00510
Purine metabolism	ko00230	4	9	101	145	Traes_1AL_8475A65E8;Traes_1BL_1E61EBE23;Traes_1DL_7AC3AE1EB;Traes_1DL_DE87A275A	K01952+K01952+K01952+K01952
Cysteine and methionine metabolism	ko00270	1	1	101	145	Traes_5BL_9774FCB1A	K00558
Histidine metabolism	ko00340	1	1	101	145	Traes_1AL_3F7B8E94D	K01814
mRNA surveillance pathway	ko03015	2	7	101	145	Traes_4AL_6752FAFB9;Traes_4DS_477CA2A2F	K03265+K03265
Photosynthesis	ko00195	76	76	101	145	Traes_1AL_08A2F5FA4;Traes_1AL_655726D7B;Traes_1AL_9A9B26465;Traes_1AL_C66F70A34;Traes_1AS_5320E59A6;Traes_1BL_5320E59A6;Traes_1BS_5320E59A6;Traes_1BS_9A9B26465;Traes_1DL_E50A6EE94;Traes_1DS_5320E59A6;Traes_1DS_9A9B26465;Traes_1DS_9A9B264651;Traes_2AL_5320E59A6;Traes_2AL_9A9B26465;Traes_2AS_861B2054C;Traes_2AS_9A9B26465;Traes_2BL_5320E59A6;Traes_2BS_47D142DD2;Traes_2BS_5320E59A6;Traes_2BS_9A9B26465;Traes_2BS_EAED42A93;Traes_2DL_092D759DB;Traes_2DL_0D2359A4B;Traes_2DS_5320E59A6;Traes_3AL_5320E59A6;Traes_3AL_9A9B26465;Traes_3AS_5320E59A6;Traes_3AS_9A9B26465;Traes_3B_5320E59A6;Traes_3B_5320E59A61;Traes_3B_5320E59A62;Traes_3B_5320E59A63;Traes_3DL_5320E59A6;Traes_3DS_698693E7B;Traes_4AL_5320E59A6;Traes_4AL_5320E59A61;Traes_4AL_5320E59A62;Traes_4AL_8C19008DD;Traes_4AS_5320E59A6;Traes_4AS_FFF5B1ECA;Traes_4BL_5320E59A6;Traes_4BL_9A9B26465;Traes_4BS_5320E59A6;Traes_4BS_FB4E1F982;Traes_4DL_5320E59A6;Traes_4DS_5320E59A6;Traes_5AL_46A8C76DD;Traes_5AL_5320E59A6;Traes_5AS_5320E59A6;Traes_5BL_5320E59A6;Traes_5BS_5320E59A6;Traes_5BS_5320E59A61;Traes_5BS_9A9B26465;Traes_5DL_61539C61A;Traes_5DL_B888E7A8A;Traes_5DS_9A9B26465;Traes_5DS_9A9B264651;Traes_5DS_CBE009155;Traes_6AL_5320E59A6;Traes_6AS_026FEB41A;Traes_6AS_5320E59A6;Traes_6BL_5320E59A6;Traes_6BS_5320E59A6;Traes_6BS_9A9B26465;Traes_6DL_249028825;Traes_6DL_2490288251;Traes_6DS_5320E59A6;Traes_6DS_9A9B26465;Traes_7AL_5320E59A6;Traes_7AS_46C76AD7C;Traes_7AS_5320E59A6;Traes_7BL_5320E59A6;Traes_7BS_5320E59A6;Traes_7DL_5320E59A6;Traes_7DL_5320E59A61;Traes_7DS_88B703F52	K02707+K02707
Phenylpropanoid biosynthesis	ko00940	1	2	101	145	Traes_1BS_BA046E212	K00430
Phenylalanine metabolism	ko00360	1	2	101	145	Traes_1BS_BA046E212	K00430
RNA transport	ko03013	3	6	101	145	Traes_2AS_EC7E6EAF0;Traes_2BS_80474BB5B;Traes_2DS_1A728B7C8	K03260+K03260+K03260
Ribosome biogenesis in eukaryotes	ko03008	1	1	101	145	Traes_7DL_208961165	K07178
Spliceosome	ko03040	4	8	101	145	Traes_4AS_9E5A45F1C;Traes_4BL_F91DC939A;Traes_4BL_F91DC939A1;Traes_4DL_3AF08121F	K12820+K12820+K12820+K12820

Table S11: Sequencing data of degradome library from early developing grains

RNA name or ID	RNA name or ID	Target	AlignmentScore	AlignmentRange	CleavageSite	Category	P-value	Duplicate?
novel-miR0012	ta_iwgsc_7ds_v1_384317_42212	Traes_3DS_2F5F2C276	6.5	32-54	45	1	0.041077513	1
novel-miR0012	ta_iwgsc_7ds_v1_384317_42212	Traes_3B_8824DBB56	6.5	494-516	507	1	0.041077513	1
novel-miR0012	ta_iwgsc_7ds_v1_384317_42212	Traes_3AS_7EEF1386F	6.5	497-519	510	1	0.041077513	1
novel-miR0011	ta_iwgsc_5dl_v1_4495350_28881	Traes_3DS_C6D17D438	6	55-79	70	0	0.012371827	1
novel-miR0011	ta_iwgsc_5dl_v1_4495350_28881	Traes_3B_E7D2E8720	6	55-79	16	0	0.012371827	1
novel-miR0075	ta_iwgsc_5as_v1_1552033_25658	Traes_5AL_147EA9565	6.5	160-181	172	0	0.043959217	0
novel-miR0036	ta_iwgsc_4bs_v1_4729575_21772	Traes_7AS_2084DE83B	7	558-579	570	0	0.03365536	0
tae-miR160	tae-miR160	Traes_7DL_55ADB3528	1	714-734	725	0	0.030978321	0
tae-miR160	tae-miR160	Traes_7BL_18D335F08	1	171-191	182	0	0.030978321	0
tae-miR160	tae-miR160	Traes_7AL_E3ADC8C38	1	399-419	410	0	0.030978321	0
tae-miR160	tae-miR160	Traes_1BL_54CD82AC3	1	642-662	653	0	0.030978321	0
tae-miR160	tae-miR160	Traes_1AL_147CF243C	1	642-662	653	0	0.030978321	0
tae-miR156	tae-miR156	Traes_6BS_542961EA4	2	349-369	360	1	0.045091293	0
tae-miR1119	tae-miR1119	Traes_6BS_4D03398A8	6.5	348-372	362	0	0.035324752	0
tae-miR1119	tae-miR1119	Traes_6BS_222CE7DA7	6.5	348-372	362	0	0.035324752	0
tae-miR1119	tae-miR1119	Traes_6BS_04A3400AF	6.5	348-372	362	0	0.035324752	0

Table S14-1: Detailed information of the differentially expressed known miRNAs between 7DAP and 14DAP grains

miRNA name	7 DAP	14 DAP	PValue	FDR	log2FC	regulated
bdi-miR319b-3p	2147.31	928.41	0.001076	0.004723221	-1.21	down
gma-miR167g	644.19	5328.27	0	0	3.05	up
tae-miR1127b-3p	0.00	1170.61	0.000469	0.002383789	33.45	up
tae-miR156	644.19	10091.43	0	0	3.97	up
tae-miR164	1932.57	4460.41	0.000222	0.0011544	1.21	up
tae-miR167c-5p	3220.96	10434.54	0	0	1.70	up
tae-miR319	644.19	2179.75	0.000739	0.003469194	1.76	up
tae-miR7757-5p	5797.72	16307.75	0	0	1.49	up
tae-miR9654b-3p	429.46	5731.93	0	0	3.74	up
tae-miR9655-3p	429.46	7104.37	0	0	4.05	up
tae-miR9658-3p	0.00	3390.72	0	0	34.98	up
tae-miR9662a-3p	3865.15	14309.65	0	0	1.89	up
tae-miR9662b-3p	3865.15	14309.65	0	0	1.89	up
tae-miR9663-5p	0.00	888.05	0.00165	0.006679042	33.05	up
tae-miR9668-5p	429.46	2442.13	0.000091	0.00050423	2.51	up
tae-miR9669-5p	2362.04	8961.19	0	0	1.92	up
tae-miR9670-3p	5797.72	27569.78	0	0	2.25	up
tae-miR9672b	5153.53	12190.45	0	0	1.24	up
tae-miR9674b-5p	1932.57	10192.34	0	0	2.40	up

Table S14-2: Detailed information of the differentially expressed novel miRNAs between 7DAP and 14DAP grains

miRNA name	ID	7 DAP	14 DAP	log2FC	PValue	FDR	regulated
novel-miR0079	ta_iwgsc_ 1as_v1_2129014_ 1024	1288.38	484.39	-1.41	0.002376	0.008729217	down
novel-miR0078	ta_iwgsc_4ds_v1_2274320_23450	858.92	161.46	-2.41	0.001129	0.004800025	down
novel-miR0075	ta_iwgsc_5as_v1_ 1552033_25658	429.46	3612.73	3.07	0.000001	0.00000683	up
novel-miR0073	ta_iwgsc_2bl_v1_8046566_8407	214.73	13926.17	6.02	0	0	up
novel-miR0071	ta_iwgsc_5ds_v1_2782660_30778	214.73	48418.67	7.82	0	0	up
novel-miR0070	ta_iwgsc_2dl_v1_9769407_ 10602	0.00	787.13	32.87	0.002585	0.009007526	up
novel-miR0060	ta_iwgsc_7dl_v1_3341310_41122	11165.99	787.13	-3.83	0	0	down
novel-miR005	ta_iwgsc_6bl_v1_2921472_32514	3006.23	6438.33	1.10	0.000066	0.0003718	up
novel-miR0048	ta_iwgsc_7bl_v1_6691063_39345	3006.23	1432.98	-1.07	0.000662	0.003196514	down
novel-miR0046	ta_iwgsc_7bl_v1_571312_38763	214.73	1392.62	2.70	0.00105	0.004795946	up
novel-miR0040	ta_iwgsc_5dl_v1_3183779_28677	0.00	888.05	33.05	0.00165	0.006679042	up
novel-miR0036	ta_iwgsc_4bs_v1_4729575_21772	1717.84	60.55	-4.83	0	0	down
novel-miR0034	ta_iwgsc_ 1al_v2_3947972_580	0.00	988.96	33.20	0.001053	0.00474552	up
novel-miR0032	ta_iwgsc_3al_v1_4435088_ 13419	0.00	2684.32	34.64	0.000001	0.00000683	up
novel-miR0031	ta_iwgsc_5ds_v1_2761097_30588	0.00	1432.98	33.74	0.000146	0.000789568	up
novel-miR0030	ta_iwgsc_5ds_v1_2782660_30771	0.00	1836.64	34.10	0.000024	0.000143575	up
novel-miR0019	ta_iwgsc_6al_v1_5834455_31604	858.92	20.18	-5.41	0.000021	0.000132673	down
novel-miR0018	ta_iwgsc_3b_v1_10775495_ 16427	2576.77	0.00	-34.58	0	0	down
novel-miR0010	ta_iwgsc_7bl_v1_6747143_39914	0.00	827.50	32.95	0.00216	0.008343771	up
novel-miR001	ta_iwgsc_2al_v1_6404501_5978	1717.84	15984.82	3.22	0	0	up

Table S15: Primer sequences used in this study for qRT-PCR

target	miRNA name	primer	sequence
Traes_1BL_54CD82AC3	tae-miR160	L	GCGTCTCTTGCTTGTTGACA
		R	AGTCATCTGCTGCTCCGTAA
Traes_3AS_6CB1DBD28	tae-miR9674b-5p	L	GTTGCATTAGTGAGGCGAGG
		R	CATCAGCCTCAAGACCAAGC
Traes_6BS_7F1846171	tae-miR9662b-3p	L	CAGCAATGAGAAGATCGCCG
		R	CTGTATGTGAGCAATGCCGG
Traes_3B_7FFFF7BCF	tae-miR7757-5p	L	GCATGTGTGGCTCGTACTTA
		R	GGCCAATTTCTCGTTTGCAC
Traes_5AS_E5FD82E36	tae-miR164	L	ATAACAGTGCCAGCAACAGC
		R	CTTGTCACAGTTCATGGCGG
Traes_1AL_D7A903FD2	novel-miR009-2	L	GTAGCTGGTGGTGGAGGATT
		R	GCCATCTCCATCCTCATCGA
Traes_2BL_621DAFE84	novel-miR0026-3	L	TTTCTAGCCTCCGGTCGTTT
		R	CGGCAAGGAAGACACAACAA
Traes_3B_325EA6658	novel-miR0031-1	L	ATTCTTTCCCTCCGCTACCC
		R	GGGCTTGTCTGAACTCAACC

Fig S1: Most of miRNA sequences showed a strong bias for a uridine at position 1 and the majority of 24 nucleotide-long siRNAs have an apparent preference for 5' adenosine

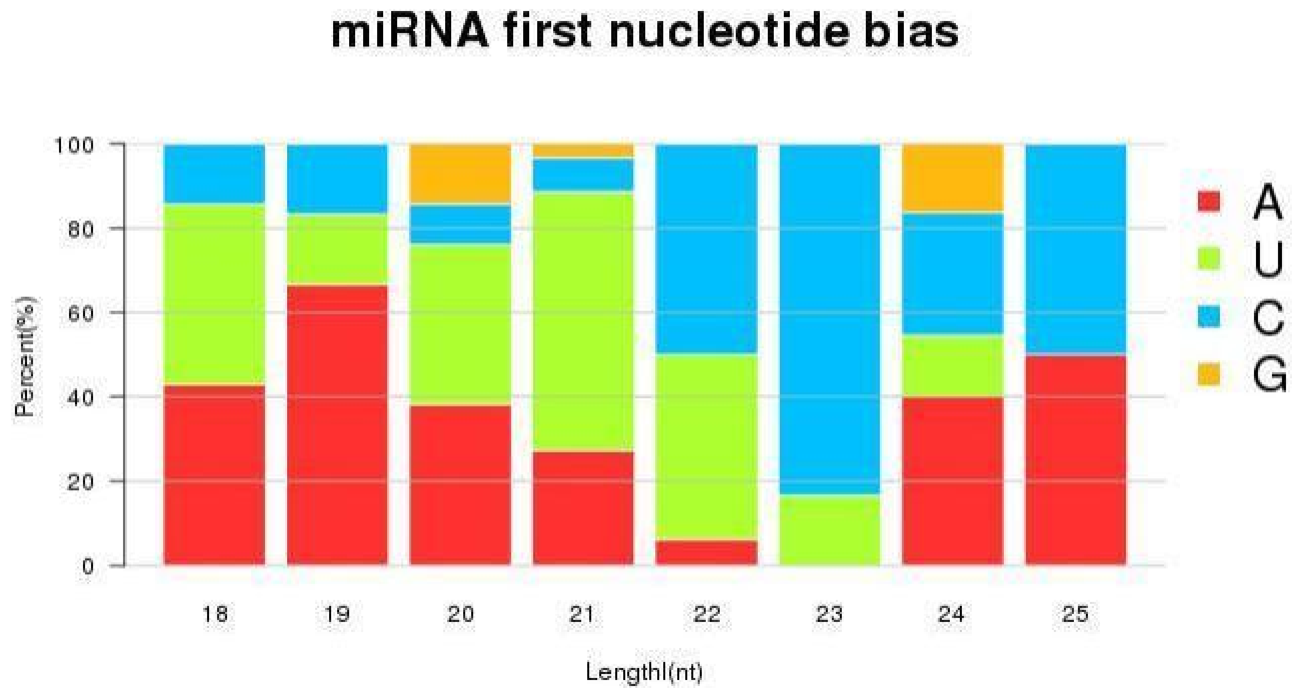

Fig S4: Degraded sites of 13 annotated target genes predicted by degradome sequencing