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CHEN QIAOYAN

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DISSERTATION
BREEDING AND GENETIC BASES OF WINTER WHEAT EAR TRAITS

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/CHEN. QIAOYAN/CHEN QIAOYAN/

Scientific supervisor:Nataliya Kandyba, PhD (Agricultural Sciences), Ass. Professor

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Wheat breeding is one of the key areas of agricultural research aimed at ensuring sustainable food production in the face of global climate change and the growing world population. Winter wheat holds a significant place in global cereal production due to its high yield and grain quality characteristics. One of the critical aspects affecting the productivity and adaptability of winter wheat is the characteristics of the spike and yield. Research focused on understanding the breeding and genetic basis of spike traits in winter wheat allows the identification of genetic mechanisms governing these traits and facilitates the development of new varieties with improved characteristics.

In recent years, significant progress in this field has been achieved through the application of modern molecular genetic technologies, such as marker-assisted selection (MAS) and high-throughput sequencing. MAS significantly accelerates and improves the accuracy of the breeding process by utilizing molecular markers associated with desirable traits. Its application in winter wheat breeding allows for the effective selection of plants with optimal spike traits, ultimately leading to the creation of high-yielding and stress-resistant varieties. High-throughput sequencing («next-generation sequencing») enables the rapid and accurate sequencing of large amounts of DNA and RNA, the study of plant genomes, and the identification of genes and molecular markers associated with important agronomic traits of the wheat spike. This significantly accelerates the breeding process through precise and large-scale genetic data analysis.

This study systematically analyzes the genetic and breeding basis of spike traits in winter wheat, such as the number of grains and the weight of 1000 grains, using marker-assisted selection (MAS) and high-throughput sequencing to analyze genetic

variability and identify molecular markers. Special attention is given to the role of microRNAs in regulating grain development and degradome sequencing (as one of the methods of high-throughput sequencing) for identifying genes targeted by microRNAs and annotating their functions.

For the research consisted of the winter wheat varieties Mexican Large Spike (MLS), Bainong 4199 (BN419), and an F_2 population (145 plants) created by hybridizing Mexican Large Spike × Bainong 4199.

The research results showed that the parent forms significantly differed in spike traits – the number of grains per spike and the weight of 1000 grains, which corresponds to the principle of parental selection when creating a population for QTL mapping. The traits exhibited a normal distribution and bidirectional segregation, indicating quantitative inheritance.

According to the results of the correlation analysis, the number of spikes and the weight of 1000 grains had a highly significant positive correlation with a correlation coefficient of 0.953. A total of 143 pairs of polymorphic markers with distinct differences were tested for polymorphism using 300 pairs of SSR primers. The 143 pairs of polymorphic markers were genotyped, and genetic maps were constructed for the 145 individual plants of the F_2 population.

To construct the linkage map, 145 loci of polymorphic SSR markers were used, covering 19 wheat chromosomes, with a total length of 3128.17 cM. The average distance between markers was 25.23 cM, the maximum distance was 113.85 cM, and the minimum distance was 3.57 cM. The genetic density between some markers exceeded 50 cM, which was mainly due to the low density of molecular markers.

The distribution of 145 polymorphic marker loci across chromosome groups A, B, and D was uneven. There were 77 marker loci present in chromosome group A, 41 in group B, and only 24 in group D, accounting for 54.22%, 28.87%, and 16.9% of the total number of marker loci, respectively. The SSR markers exhibited the highest polymorphism in chromosome group B and the lowest polymorphism in chromosome group D.

Determination and analysis of epistatic QTL loci for number of grains per ear and thousand grain weight showed that nine epistatic QTL loci associated with number of grains per ear and thousand grain weight were identified, which were distributed on chromosomes 1B, 2B, 2D, 3B and 6B, including four associated QTL loci on chromosome 3B, two associated QTL loci on chromosome 6B. and one associated locus each on chromosomes 1B, 2B and 2D. This allows explaining 4.922%~21.1044% of phenotypic variation for the number of grains per ear. QGNS~1B and QGNS~3B2 had large genetic effects and were the main loci explaining 21.1044% and 15.8886% of phenotypic variation. One additive QTL locus controlling thousand grain weight was found at QTGW~3B, which explained 11.4727% of the phenotypic variation.

The effect values for QGNS~2B, QGNS~2D, and QGNS~3B1 were less than zero, so the genetic effect of epistatic QTLs belonging to the recombinant type was greater than that of epistatic QTLs belonging to the parental type, which explained 41.91% of the total phenotypic variation.

Three epistatic QTL loci associated with thousand grain weight were found, mainly on chromosomes 3B and 5A, among which the genetic effect values of QTGW~3B1 and QTGW~3B2 loci were greater than zero and belonged to the paternal type of epistatic QTL loci, and the values of the genetic effect of QTGW~5A loci were less than zero, which belonged to the recombinant type of epistatic QTL loci and accounted for 17.4% of the total variation in the phenotype. The effect values at the QGNS~3B2, QGNS~6B1 and QGNS~6B2 loci were greater than zero for the parental type epistatic QTLs, indicating that the parental type epistatic loci had a higher effect than the recombinant type epistatic loci and accounted for 37.23% of the total phenotypic variation. The effect of epistasis on phenotypic variations of grain size and thousand grain weight was significant in relation to the effect on phenotypic variability of the number of grains in the ear.

Thus, the factors that influence the results of QTL localisation are the choice and size of the population, the type and number of molecular markers, environmental

conditions, statistical methods and the density of markers for genetic linkage mapping. The above factors can be considered in combination for more accurate identification of QTL loci associated with target traits

The results showed that compared to 14 DAP, 7DAP has a broader mRNA-mediated regulation of gene expression. Moreover, more novel microRNAs were expressed in 14 DAP than in 7 DAP. 31 new microRNAs were expressed in two libraries: 1 specific one in the 7 DAP library and 30 specific ones in the 14 DAP library. Most of the new microRNAs had relatively low expression levels. The most common novel microRNAs were novel-m0064_5p, novel-m0492_5p, and novel-m0661_5p.

Non-coding RNAs were identified, and after removing annotated RNAs, the number of unannotated sequences was 2,448,113 (17.46%) in the 7th DAP library and 3,456,209 (33.3%) in the 14 DAP pure read libraries. Of these unannotated sequences, 235.8 79 (10.64%) are reads in 7 DAP libraries and 1 458 680 (43.06%) are reads in 14 DAP libraries, which perfectly corresponds to the shotgun whole-genome sequences (WGS).

The microRNA sequencing samples were used to generate degradome libraries, which were created by ligating polyA-enriched RNAs with a designated RNA adapter containing the 3'-site of Mme I.

The degradome library was created and then subjected to degradome sequencing. A total of 10,620,205 net reads were obtained, including 4,612,965 unique reads. 2,776,485 (60.19%) unique reads were aligned to the reference genome. Using BLASTN searches in GenBank and Rfam databases, structural RNAs (rRNA, tRNA, miRNA and mRNA) were removed and the remaining reads were used to identify potential microRNA targets.

Total RNA lengths ranged from 18 to 30 nt in both libraries, with the majority of them being between 21 and 24 nt, with the most common lengths being 21 nt in the 7 DAP library and 24 nt in the 14 DAP library. The length distributions of pure strand and microRNAs were different in the two libraries: the distribution of mRNA lengths

was 61% and 73.89% between 20~24 nt, respectively, and the percentage of mRNAs with 24 nt sequence (7 DAP was 26.79%, 14 DAP was 40.42%) was higher than that of other sequences. In the library, 7 DAP mRNA with 23 nt was second only to the 24 nt mRNA sequence, and in the library, 14 DAP mRNA with 21 nt.

The comparison of unannotated RNA sequences that perfectly matched the reference genome in miRBase22.0 based on the perfect match criterion resulted in 89 known wheat microRNAs in the two RNA libraries, of which 46 were expressed in the 7 DAP library and 87 in the 14 DAP library. In total, 16 known microRNAs from other plant species were identified in the available two RNA libraries, including 13 expressed in 7 DAP libraries and 16 expressed in 14 DAP libraries.

The base distribution at each position of known small RNAs was calculated, and the results showed that the first base at the 5'-end of microRNAs is most commonly A (adenine) and least commonly G (guanine). This is inconsistent with the existing confirmed microRNA-specific sequences, where the first base at the 5'-end is most commonly U (uracil) and least commonly G (guanine).

It was shown that the expression of known microRNAs, including ata-miR9863a-3p, osa-miR396e-5p, tae-miR9670-3 and tae-miR7757-5p, was the most active, whereas some other known microRNAs such as stae-miR9672b, tae-miR9662a-3p, tae-miR167c-5p ta, e-miR156, tae-miR9777 and tae-miR9669-5p were moderately upregulated. Most of the widely expressed microRNAs are known microRNAs from the wheat microRNA database and are conserved in plant species, such as miR156 and miR396e-5p.

For microRNA identification, 19 known and 20 novel microRNAs were differentially expressed ($r < 0.05$) between 7 and 14 DAP grains, among which 18 known microRNAs and 13 novel microRNAs were up-regulated in 14 DAP grains.

Potential microRNA targets were predicted computationally using software and, starting from 184 total microRNA sequences, a set of 810 potential targets for 25 novel microRNAs, 13 conserved microRNAs and 40 known microRNAs were predicted. Among 88 DEmiRNAs, 477 potential targets for 147 miRNAs were identified.

Among all 810 miRNA targets, 174 (21.48%) could be annotated in the COG database, 605 (74.69%) in the GO database, and 176 (21.73%) in the KEGG database. However, out of 477 DE miRNA targets, only 84 were functionally annotated by COG.

The results showed that the 23 predicted microRNA targets include 3 known and 4 novel microRNAs. In addition, most single microRNAs potentially regulate multiple targets, while some single microRNAs act on only one target gene. Of the 16 targets, 13 have functional annotations. Tae-miR160 targets five genes, including Traes_1AL_147CF243C, Traes_1BL_54CD82AC3, Traes_7AL_E3ADC8C38, Traes_7BL_18D335F08 and Traes_7DL_5. 5ADB3528; tae-miR1119 has three targets, including Traes_6BS_04A3400AF, Traes_6BS_222CE7DA 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 -miR1 56 target Traes_7AS_2084DE83B, Traes_5AL_147EA9565 and Traes_6BS_542961EA4, respectively.

Some of the targets were found to be involved in various biological processes, such as mitotic cell cycle (GO:0000278), nucleation (GO:0000280), cell morphogenesis (GO:0000902), seed development (GO:0048316), embryo development (GO:0009790) (4 targets) For 2 microRNAs), meristem initiation (GO:0010014), leaf development (GO:0048440), cell differentiation (GO:0030154), cell division (GO:0051301), starch metabolism (GO:0005982) and regulation of cell division (GO:0051302). This confirms that microRNAs play an important role in the regulation of grain development.

Keywords:*winter wheat, MAS, high-throughput sequencing, QTL localization, primers, linkage map, 1000 grain weight, growth and development, yield, molecular mechanism, molecular markers, microRNAs, degradome, target gene.*

АНОТАЦІЯ

Чєнь Цяоянь. Селекційно - генетичні основи ознак колосу пшениці озимої. - Кваліфікаційна праця на правах рукопису.

Дисертація на здобуття наукового ступеня доктора філософії за спеціальністю 201 – Агрономія. – Сумський національний аграрний університет. Суми, 2023 р.

Селекція пшениці є однією з ключових областей аграрних досліджень, спрямованих на забезпечення сталого виробництва продовольства в умовах глобальної зміни клімату та зростаючого населення планети. Пшениця озима займає значне місце у світовому виробництві зернових культур завдяки своїй високій урожайності та якісним характеристикам зерна. Одним з критичних аспектів, що впливають на продуктивність та адаптивність пшениці озимої, є ознаки колоса та врожайність. Дослідження, які спрямовані на розуміння селекційно-генетичних основ ознак колоса пшениці озимої дозволяють виявити генетичні механізми цих ознак і сприяють розробці нових сортів з покращеними характеристиками.

В останні роки значних успіхів у цій галузі досягнуто завдяки застосуванню сучасних молекулярно-генетичних технологій, таких як маркерно-асоційована селекція (MAS) і високопродуктивне секвенування. MAS суттєво прискорює і підвищує точність селекційного процесу за рахунок використання молекулярних маркерів, пов'язаних з бажаними ознаками, і її застосування в селекції пшениці озимої дозволяє ефективно відбирати рослини з оптимальними ознаками колоса, що в кінцевому результаті призводить до створення високоврожайних і стресостійких сортів. Високопродуктивне секвенування («секвенування наступного покоління») дозволяє швидко і точно секвенувати великі кількості ДНК і РНК, вивчати геноми рослин, виявляти гени та молекулярні маркери, які пов'язані з важливими агрономічними ознаками

колоса пшениці, і значно прискорювати процес селекції за рахунок точного і масштабного аналізу генетичних даних.

У цьому дослідженні системно проаналізовано генетичні та селекційні основи ознак колоса пшениці озимої, таких як кількість зерен і маса 1000 зерен, із використанням маркерно-асоційованої селекції (MAS) і високопродуктивного секвенування для аналізу генетичної варіабельності та визначення молекулярних маркерів. Особлива увага приділяється ролі мікроРНК у регуляції розвитку зерна та деградомному секвенуванню (як одному з методів високопродуктивного секвенування) для пошуку генів, які є мішенню мікроРНК та анотації їхніх функцій.

Вихідним матеріалом для досліджень стали сорти пшениці озимої Mexican Large Spike (MLS), Bainong 4199 (BN419) та популяція F_2 (145 рослин), створена шляхом гібридизації Mexican Large Spike × Bainong 4199.

Результати досліджень показали, що батьківські форми суттєво відрізнялися за ознаками колоса – за кількістю зерен у колосу та масою 1000 зерен, що відповідає принципу батьківського відбору при створенні популяції для картування QTL. Ознаки мали нормальний розподіл і двонаправлену сегрегацію, що вказує кількісне успадкування. За результатами кореляційного аналізу кількість колосків і маса 1000 зерен мали високозначущу позитивну кореляцію з коефіцієнтом кореляції 0,953.

143 пари поліморфних маркерів з чіткими відмінностями були перевірені на поліморфізм із використанням 300 пар праймерів SSR. Було генотиповано 143 пари поліморфних маркерів і побудовано генетичні карти для 145 окремих рослин популяції F_2.

Для побудови карти генетичного зчеплення було використано 145 локусів поліморфних маркерів SSR, які охоплювали 19 хромосом пшениці, загальною довжиною 3128,17 сМ. Середня відстань між маркерами становила 25,23 сМ, максимальна – 113,85 сМ, мінімальна – 3,57 сМ. Генетична щільність між

деякими маркерами перевищувала 50 cM, що обумовлено переважно малою щільністю молекулярних маркерів.

Розподіл 145 поліморфних маркерних локусів за групами хромосом A, B і D був нерівномірним. 77 маркерних локусів були наявні в хромосомній групі A, 41 - у групі B, і лише 24 - у групі хромосом D, що становило 54,22%, 28,87% і 16,9%від загальної кількості маркерних локусів, відповідно.

Маркери SSR мали найвищий поліморфізм у групі хромосом B та найнижчий поліморфізм групи хромосом D.

Визначення та аналіз епістатичних локусів QTL для кількості зерен на колос та маси тисяч зерен показали, що було ідентифіковано дев'ять епістатичних локусів QTL, пов'язаних з кількістю зерен на колос та масою тисячі зерен, які були розподілені на хромосомах 1B, 2B, 2D, 3B і 6B, серед яких чотири асоційовані локуси QTL - на хромосомі 3B, два асоційованих локуси QTL - на хромосомі 6B. і по одному асоційованому локусу - на хромосомах 1B, 2B та 2D. Це дозволяє пояснити 4,922%~21,1044% фенотипних варіацій за ознакою кількості зерен на колос. QGNS~1B та QGNS~3B2 мали великі генетичні ефекти та були основними локусами, пояснюючи 21,1044% та 15,8886% фенотипічних варіацій. Один адитивний локус QTL, який контролював масу тисячі зерен, було виявлено на QTGW~3B, що пояснювало 11,4727% мінливості фенотипу.

Значення ефекту для локусів QGNS~2B, QGNS~2D та QGNS~3B1 були менше за нуль, отже, генетичний ефект епістатичних локусів QTL, які належали до рекомбінантного типу, був більшим, ніж у епістатичних локусів QTL, які належали до батьківського типу, що пояснює 41,91% загальної фенотипічної мінливості.

Виявлено три епістатичні локуси QTL, які пов'язані з масою тисячі зерен, переважно на хромосомах 3B та 5A, серед яких значення генетичного ефекту локусів QTGW~3B1 і QTGW~3B2 були більшими за нуль і належало до батьківського типу епістатичного типу QTL-локусів, а значення генетичного ефекту локусів QTGW~5A були меншими за нуль, що належало до

рекомбінантного типу епістатичних локусів QTL і складало 17,4% від загальної варіації фенотипу. Значення ефекту в локусах QGNS~3B2, QGNS~6B1 та QGNS~6B2 були більшими за нуль для епістатичних локусів QTL батьківського типу, що вказувало на те, що епістатичні локуси батьківського типу мали вищий ефект, ніж епістатичні локуси рекомбінантного типу і складали 37,23% від загальної фенотипічної мінливості. Вплив епістазу на фенотипові варіації розміру зерна та маси тисячі зерен був значним щодо впливу на фенотипічну мінливість кількості зерен у колосі.

Отже, факторами, які впливають на результати локалізації QTL є вибір і розмір популяції, тип і кількість молекулярних маркерів, умови навколишнього середовища, статистичні методи і щільність маркерів картування генетичного зчеплення. Вищезазначені фактори можна розглядати комплексно для більш точної ідентифікації локусів QTL, які пов'язані з цільовими ознаками.

Зчитування послідовності міток (тегів) бібліотек кДНК дало результат у вигляді чистих зчитувань (тегів) після відпадіння адаптерів та низькоякісних зчитувань.

Мітки довжиною 18 – 30 нуклеотидів (нк) порівнювалися з послідовностями у Rfam, GenBank та RepBasedatabases, щоб відрізнити їх від рРНК, scRNA, snoRNA, snRNA і тРНК. Відомі мікроРНК і нові мікроРНК були ідентифіковані за допомогою наведених вище некодуючих тегів з деякими модифікаціями.

При спостереженні за розвитком зерна пшениці було встановлено, що зростання маси та об’єму зерна було відносно повільним на початку (до 11 діб), збільшувалося між 11 і 14 добою і продовжувало збільшуватися приблизно до 26 доби. Для визначення зв’язку між цими змінами та великою кількістю мікроРНК під час раннього розвитку зерна використовувалися невеликі бібліотеки РНК і деградомів з 7DAP і 14DAP.

З двох бібліотек було отримано 43,18 мільйона необроблених читань (міток) і 24,4 МБ чистих читань. Загалом із бібліотек 7DAP і 14DAP було

отримано 14 020 684 (86,25%) і 14 212 862 (87,44%) послідовностей відповідно. Це еквівалентно 1 806 597 (51,66%) і 1 981 099 (56,65%) унікальних послідовностей мРНК з бібліотек 7DAP і 14DAP відповідно), а загальна кількість унікальних послідовностей у бібліотеці 7DAP була нижчою, ніж у бібліотеці 14DAP.

Результати показали, що порівняно з 14 DAP 7DAP має ширшу регуляцію експресії генів, опосередковану мРНК. При чому, більше нових мікроРНК було експресовано в 14 DAP, ніж у 7 DAP. 31 нова мікроРНК експресувалася у двох бібліотеках: 1 спеціальна в бібліотеці 7 DAP і 30 спеціальних у бібліотеці 14 DAP. Більшість нових мікроРНК мали відносно низькі рівні експресії. Найпоширенішими новими мікроРНК були novel-m0064_5p, novel-m0492_5p, andnovel-m0661_5p.

Були ідентифіковані некодувальні РНК і після видалення анотованих РНК кількість неанотованих послідовностей становила 2 448 113 (17,46 %) у 7-й бібліотеці DAP і 3 456 209 (33,3 %) у 14 DAP-бібліотеках чистих прочитань. З названих неанотованих послідовностей 235,8 79 (10,64%) читаються в 7 DAPLibrary і 1 458 680 (43,06%) читаються в 14 DAPLibrary, що ідеально відповідає послідовностям повногеномного збирання дробовика (WGS).

Зразки секвенування мікроРНК були використані для створення бібліотек деградома, які були створені шляхом лігування РНК, збагачених поліА, з призначеним адаптером РНК, що містить 3'-сайт MmeI.

Була створена бібліотека деградомів, а потім піддана деградомному секвенуванню. Загалом було отримано 10 620 205 чистих читань, зокрема 4 612 965 унікальних читань Усього 2 776 485 (60,19%) унікальних прочитань було зіставлено з еталонним геномом. Використовуючи пошук BLASTN у базах даних GenBank і Rfam, структурні РНК (рРНК, тРНК, м'яРНК і мРНК) видалили, а читання, що залишилися, використовували для виявлення потенційних мішеней мікроРНК.

Довжина чистої РНК коливалася від 18 до 30 нт в обох бібліотеках, водночас довжина більшості з них становила від 21 до 24 нт, причому найпоширеніша довжина становила 21 нт у бібліотеці 7 DAP і 24 нт у бібліотеці 14 DAP. У двох бібліотеках розподіл довжин чистих рядів і мікроРНК різний: розподіл довжин мРНК склав 61% і 73,89% між 20~24 нт відповідно, а відсоток мРНК з послідовністю 24 нт (7 DAP становив 26,79%, 14 DAP становив 40,42%) був вищим, ніж в інших послідовностей. У бібліотеці 7DAP-РНК з 23нт поступалася тільки послідовності 24-нтРНК, а в бібліотеці 14DAP-РНК з 21нт.

У результаті порівняння неаннотованих послідовностей РНК, що ідеально відповідали еталонному геному в miRBase22.0 на основі критерію ідеальної відповідності, у двох бібліотеках РНК було виявлено 89 відомих мікроРНК пшениці, з яких 46 експресувалися в бібліотеці 7 DAP і 87 - у бібліотеці 14 DAP. Усього в наявних двох бібліотеках РНК було ідентифіковано 16 відомих мікроРНК з інших видів рослин, зокрема 13, що експресуються в 7 бібліотеках DAP, і 16, що експресуються в 14 бібліотеках DAP.

Було розраховано розподіл основ у кожному положенні відомих малих РНК і результати показано, що перша основа на 5'-кінці мікроРНК найбільш поширена A (аденін), найменш поширена – G (гуанін). Це не узгоджується з наявними підтвердженими специфічними для мікроРНК послідовностями, де перша основа на 5'-кінці найпоширеніша U (урацил) і менше за G (гуанін).

Було показано, що експресія відомих мікроРНК, включно з ata-miR9863a-3p, osa-miR396e-5p, tae-miR9670-3 та tae-miR7757-5p, відбувалася найактивніше, у той час як деякі інші відомі мікроРНК, такі як stae-miR9672b, tae-miR9662a-3p, tae-miR167c-5p та, e-miR156, tae-miR9777 та tae-miR9669-5p - помірно. Більшість мікроРНК, що широко експресуються є відомими мікроРНК з бази даних мікроРНК пшениці та консервативними у видів рослин, такими як miR156 і miR396e-5p.

Для ідентифікації мікроРНК було диференційно експресовано 19 відомих і 20 нових мікроРНК (r <0,05) між 7 і 14 зернами DAP, серед яких 18 відомих мікроРНК і 13 нових мікроРНК мали підвищену експресію в 14 зернах DAP.

Потенційні мішені мікроРНК були передбачені обчислювальним шляхом з використанням програмного забезпечення і, починаючи зі 184 загальних послідовностей мікроРНК, було передбачено набір із 810 потенційних мішеней для 25 нових мікроРНК, 13 консервативних мікроРНК і 40 відомих мікроРНК. Серед 88 DEmiRNAs було ідентифіковано 477 потенційних мішеней для 147 miRNA.

Серед усіх 810 мішеней мікроРНК 174 (21,48%) можна було анотувати в базі даних COG, 605 (74,69%) - у базі даних GO і 176 (21,73%) - у базі даних KEGG. Однак, із 477 мішеней DEmiRNA тільки 84 отримали функціональну анотацію COG.

За результатами 23 передбачені мішені мікроРНК включали 3 відомі та 4 нові мікроРНК. Крім того, більшість поодиноких мікроРНК потенційно регулюють кілька мішеней, тоді як деякі окремі мікроРНК діють тільки на один ген-мішень. Із 16 цілей 13 мають функціональні анотації. Tae-miR160 націлений на п'ять генів, включно з Traes_1AL_147CF243C, Traes_1BL_54CD82AC3, Traes_7AL_E3ADC8C38, Traes_7BL_18D335F08 і Traes_7DL_5. 5АДБ3528; tae-miR1119 має три цілі, включно з Traes_6BS_04A3400AF, Traes_6BS_222CE7DA і Traes_6BS_4D03398A8; роман-miP0012 націлений на Traes_3DS_2F5F2C276, Traes_3B_8824DBB56 і Traes_3AS_7EEF1386F, а Novel-miP0011 націлений на Traes_3DS_C6D17D438 і Traes_3B_E7D2E8720. Novel-miR0036, novel-miR0075 і tae -miR1 56 націленіна Traes_7AS_2084DE83B, Traes_5AL_147EA9565 і Traes_6BS_542961EA4, відповідно.

Встановлено, що деякі з мішеней беруть участь у різних біологічних процесах, таких як мітотичний клітинний цикл (GO:0000278), поділ ядра (GO:0000280), морфогенез клітин (GO:0000902), розвиток насіння (GO:0048316), розвиток ембріона (GO:0009790) (4 мішені). для 2 мікроРНК),

ініціація меристеми (GO:0010014), розвиток листка (GO:0048440), диференціювання клітин (GO:0030154), ділення клітин (GO:0051301), процес метаболізму крохмалю (GO:0005982) і регуляція поділу клітин. (GO:0051302). Це підтвержує, що мікроРНК відігравати важливу роль у регуляції розвитку зерна.

Ключові слова: *пшениця озима, MAS, високопродуктивне секвенування, локалізація QTL, праймери, карта зчеплення, маса 1000 зерен, ріст і розвиток, урожайність, молекулярний механізм, молекулярні маркери, мікроРНК, деградом, ген-мішень.*

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CONTENT

ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis ofvariance
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 oforthologous groups
CTAB	Cetyltriammonium bromide
ddH_2O	Distilled and deionized water
DH	Double haploid
DAP	Days after pollination
EDTA	Ethylenediaminetetra acetic acid
FSS	Fertile spikelet number per spike
GO	Geneontology
GNS	Grain number per spike
ICIM	Inclusive composite interval mapping
KEGG	Kyoto Encyclopedia ofGenes 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

Actuality of theme. Winter wheat is one of the world's three major grain crops, and China ranks first in the world in both acreage and total wheat production. The world's population is projected to increase to 9 billion by 2050, and food production will have to increase by more than 30% to meet people's growing consumer demand. Therefore, growing new varieties of the three major food crops—high-yielding and sustainable wheat, rice, and corn—is an effective way to meet future human food needs. Using traditional breeding methods requires a lot of time and resources, but 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 for studying the genetics of quantitative traits. Yield is a quantitative trait controlled by multiple genes, has a complex genetic basis, and is easily influenced by the environment.

Yield consists of the number of spikelets per unit area, the number of grains per spikelet and the weight of a thousand grains. When the number of spikelets per unit area and thousand grains is limited, increasing the number of grains is the key to increasing yield

Connectionofworkwithscientificprograms,plans,themes. The dissertation was completed as part of the scientific plan of research work at the Henan Institute of Science and Technology. In addition, in accordance with the thematic plans and within the framework of the state scientific theme of the Sumy National Agrarian University for 2019–2024, «Improving the elements of technologies for growing grain and cereal crops, taking into account the optimization of agrotechnical measures and agrobiological control of plant growth and development in the conditions of the northeastern Forest Steppe of Ukraine» (state registration number 0119U103779).

The purpose and objectives of the study. The purpose of the study was to identify the molecular genetic basis of winter wheat productivity traits, in particular, according to the structure of the ear, as well as the development and implementation of

marker selection methods to increase yield and improve other agronomic characteristics of winter wheat.

The set goal involved solving the following tasks:

1. To analyze the genetic mechanism of wheat ear traits such as the number of grains and the weight of one thousand grains, using SSR molecular markers.

2. To build a wheat genetic linkage map that contains loci of molecular markers.

3. Perform a QTL (quantitative trait loci mapping) analysis associated with the studied traits.

4. Identify types of microRNAs and their targets, which play a significant role in the development of wheat grain.

5. To find out the molecular mechanisms underlying grain development and to propose molecular improvements for wheat breeding.

6. To confirm the perspective of marker selection and its effectiveness in selection work with other crops.

7. To introduce into breeding practice selections based on molecular markers linked to target genes to improve wheat productivity traits.

Object ofstudy **-**genetic composition of three winter wheat yield factors and miRNA regulation in wheat grain development.

Subject of study is genetic loci that regulate the number of spikelets, mechanisms of mi RNA that regulates growth and development.

Observational method: is the selection of samples that meet the requirements of the study.

Field method - selection of the test site, establishment of the experiment, sowing and care of plants, evaluation of signs, selection and preservation of the best genotypes, use of the results.

Genetic analysis - characteristics of a wheat ear, the number of grains and the weight of a thousand grains.

Experimental method - scientific data by DNA extraction, polymerase chain reaction (PCR) results, polyacrylamide gel electrophoresis.

Statistical method: QTL localization software - Excel, SAS, DPS, SPSS, etc.; for QTL analysis - MAPMAKER/QTL1.1, QTLcartgrapher2.5, QTLNetwork2.0, IciMapping4.0, JoinMap4.0; for prediction of miRNA targets psRNA Target (version 12).

Inductive method: according to measured data, statistical analysis, etc., comprehensive analysis and comparison of results.

Literary method: analyze and understand the progress of research on the stated topic in order to identify the molecular genetic basis of the productivity traits of winter wheat, in particular, the structure of the ear, methods of marker selection to increase yield and improve other agronomic indicators of winter wheat, as well as ensure the accuracy of the experimental results by reading and collecting literature

The scientific novelty:

The practicability of the topic: the topic of research is closely related to the actual problems of improving the breeding process of wheat when applying the methods of molecular biology, genetic analysis to achieve specific breeding goals to ensure dynamic growth of yield.

The comprehensiveness of the experimental design: the selection of SSR markers for plant genotyping, polymorphism testing using a set of primers, creation of genetic maps based on the obtained genotyping data, determination of the location of markers on chromosomes, identification of microRNA varieties as a regulatory system of gene expression that ensures the manifestation of morphological traits of the ear was complete and comprehensive, which ensures the accuracy of the experimental results.

The benefit of the research results:the research results determined the genetic mechanism of the number of grains in the ear of wheat using the extreme difference in the number of grains in the ear of two parents (Mexico Large Spike and Bainong 419). A map of wheat genetic linkage was created containing 143 molecular marker loci covering 19 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.

105 known and 79 novel microRNAs were identified, including 46 known and 32 novel microRNAs from the 7 DAP library and 87 known and 78 novel microRNAs from the 14 DAP library, respectively. The identification of genes and markers associated with important agronomic traits can be of significant benefit for more efficient selection of plants with desirable characteristics and for accelerating breeding. Improvements in traits such as number of grains per ear and thousand grain weight have a direct impact on overall grain yield and quality and will allow farmers to obtain more yield at lower costs.

The practical significance of the results. Based on the conducted research, the results were obtained that can be used in the breeding practice of soft winter wheat. Thus, the genetic control of the grain number per ear trait in the multigrain variety 'Megaclus Mexicani' will allow us to study the main molecular regulatory pathways of genes that determine the number of grains per ear, which will contribute to the future study of the relevant mechanisms and regulatory networks, as well as provide theoretical guidance for future innovations of multigrain wheat germplasm. After cloning the target gene and verifying its function, at least three generations of wheat can be produced in combination with a vernalisation chamber, an artificial climate chamber, an intelligent daylight greenhouse and a large cold greenhouse and other auxiliary facilities. This is conducive to the sustainable development of agriculture, and this research has been recognised by the Department of Education and the Department of Science and Technology of Henan Province (Appendix A). The results of the experiment have been incorporated into the educational process of the Sumy National Academy of Sciences (Appendix B).

The personal contribution of the applicant. This research is determined by the applicant and the scientific supervisor according to the applicant's situation and previous research work, the applicant has conducted the implementation of research, sampling, data analysis, review of the topic, and the writing of papers.

Approbation of dissertation results. Main provisions, research results and work conclusions during 2019-2023. were reported and discussed at the meetings of

the Department of Breeding and Seed Production named after M.D. Goncharova of the Sumy National Agrarian University also at the International Scientific and Practical Conference: «Interaction of fundamental and applied sciences in the paradigm of post-industrial society» (April 24, 2020, Barcelona, Spain); at the 9th International Scientific and Practical Conference «Scientific Achievements of Modern Society» (April 28-30, 2020, Liverpool, Great Britain); at the 9th International Scientific and Practical Conference «Dynamics of World Science Development» (May 13-15, 2020, Vancouver, Canada); at the IV International Scientific and Practical Conference (Budapest, February 9-12, 2021); at the 5th International Scientific and Practical Conference «Theoretical and Scientific Foundations of the Development of Scientific Thought» (February 16-19, 2021, Rome, Italy); at the International Scientific and Practical Conferences «Honcharivski Chytannya» (2019, 2020)**.**

Publications. Based on the results of the research, 12 scientific papers were published, including: 2 articles in the Scopus scientometric databases, 3 articles in scientific specialized publications of Ukraine, 7 abstracts of reports in collections of conference materials.

The structure and scope of the dissertation. The dissertation contains abstracts, a list of abbreviations, introduction, three chapters, conclusions, a list of references, and appendixes. The volume of the dissertation is 213 pages of computer text, includes 15 tables and 15 figures.

CHAPTER1
OVERVIEW OF QTL MAPPING METHODS, MOLECULAR DNA MARKERS AND MECHANISM OF ACTION

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, providing about 30% of food production and more than 20% of the calories and protein in the daily human diet. today [3, 4]. According to the forecast, the world population will increase from the current 7 billion to 9 billion by 2050, and grain yields will need to be increased by more than 30% to meet growing human demand, so breeding new high-yielding and stable wheat varieties is an effective way to meet future demand. person for food [5]. The yield mainly depends on three factors: the number of ears per unit area, the number of ears per unit area, among them the number of ears per unit area is determined by the characteristics of the variety itself, the number of ears and the weight of one thousand grains depend on the last development of ears, and the number of ears and weight of one thousand grains are negatively correlated [6, 7], and at the stage of physiological ripeness y ieldincrease ultimately depends on the grain of spikelet No. [8-10], which is confirmed by the practice of breeding high-yielding wheat varieties in recent years.

Yield is a quantitative trait that is controlled by several genes, has a complex genetic basis and is sensitive to environmental influences [11]. Traditional breeding methods require a lot of time and resources. However, with the development of molecular biology and biostatistics, quantitative trait loci (QTL) mapping methods for quantitative trait loci based on genetic mapping of molecular markers have provided an effective technical tool for studying the genetics of quantitative traits [12]. With the help of DNA markers and QTL mapping, complex quantitative traits can be located on chromosomes using advanced genetics [13].This study aims 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 use of molecular markers can be traced back to the early 1990s, when restriction fragment polymorphism (RFLP) markers were used for gene localization, cultivar identification, identification of wheat-rye combines, and homologous chromosome arms in wheat. 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 to construct wheat linkage groups has been studied previously, it has not become an ideal marker due to its low frequency in bread wheat and the expensive and time-consuming method. This led researchers to use PCR-based molecular markers; the two main categories include random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR). Only a few examples of RAPD markers are used to locate important QTL and are converted to more authentic sequence markers (STS) or signature amplification region (SCAR) markers. le, including QTL for Russian wheat aphid (Dn), Lr24 and Lr28. PCR-RFLP is a simple and rapid method for detecting codon mutations based on another restriction endonuclease site caused by codon mutations [17]. Identification of the DNA sequences encoding γ-alcoglysin and non-coding γ-alcoglysin of different alleles of common wheat 15 using restriction fragment polymorphism (RFLP) revealed seven alleles of the Gli B1-encoded γ-alcoglysin variant[18].

Genotyping using simple sequence repeat (SSR) markers has been one of the most common methods for identifying individuals for decades. Because SSR markers are highly informative, codominant, and multiallelic, they can be experimentally reproduced within and between laboratories and are often transferable between related species [19]. Although SSRs are often used for gene and marker mapping, they have limited potential for use in real plant breeding for the following four reasons. First, obtaining accurate information about multiple perlocus alleles is a challenge; Second,

it is difficult to integrate or compare SSR data from different platforms or groups; thirdly, the number of SSR motifs in the genome is limited and unevenly distributed; Fourth, gel-based SSR analysis requires low genotyping time. Therefore, it is imperative to create simple, accurate and high-throughput breeding platforms using molecular markers [20].In wheat, SSRs based on salt-responsive genes were developed and phylogenetic analysis identified four taxa. Salt-sensitive genotypes were mainly distributed in taxa I and III, while the remaining two taxa had moderate and high salt tolerance genotypes [21]. A new apple genotyping kit (named Ap17) was developed using simple sequence repeats (SSRs) for fast, convenient and accurate apple genotype identification [22].SNPs are abundant in crop genomes and are ideal markers for genetic discovery and molecular breeding research. Similarly, SNPs derived from genome-wide linkage and association analysis using an array-based platform complemented by genotype sequencing can develop a powerful toolkit for breeding array- or sequencing-based typing, target function polymorphisms based on a single economic trait, and provide desirable predictive accuracy for quantitative traits and is commonly used across a wide range of genetic backgrounds in crops. The development of such a platform faces both serious challenges at the technical level due to inefficient cost and a serious challenge for the level of knowledge due to large genotype gaps in cultures heat9KiSelectSNParray、IlluminaWheat 90KiSelect SNP genotyping array、Wheat15K SNP array、Axiom®Wheat 660K SNP array、 Wheat 55K SNP, theAxiom® HD wheat genotyping array (820K), Wheat Breeders 35K Axiom array and Wheat 50K Triticum TraitBreedarray [16, 23-27]. The Wheat660KSNP array has the highest percentage (99.05%) of genome-specific SNPs and its physical location is reliable. SNP density analysis showed that SNPs were almost evenly distributed throughout the genome. In addition, 229,266 SNPSinwheat660KSNParray were located in 66,834 annotated genes or promoters [28].

1.2Principlesandmethodsof QTLmapping

A QTL lists the 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 more and more reports of QTL localization[29]. Molecular markers have been used to map QTL, detect the relationship between molecular markers and QTL, determine the location of QTL by calculating the rate of exchange between unknown and known markers, and estimate the effect of QTL, in order to perform QTL localization, the following conditions must be met.

Construction of a cartographic collection. First, we need to define the population-based segregationphenotypic data of quantitative traits of QTL localization. When constructing a segregated population, we need to consider both parental selection, population type, and population size.

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

1. Genetic differences between parents. In constructing this segregated population, the genetic differences between the two parents should be neither too large nor too small. Evolutionary relationships, phenotypic differences, and geographic differences in the distribution of parental plants were used as criteria for determining relatedness. Tomato is extremely poor and requires the use of offspring from different species to create populations [30, 31].

2. Extremely high standards of purity between parents.

3. Due consideration should be given to whether their hybrid offspring can reproduce normally. If the parents are distantly related, most pairings and combinations between chromosomes do not occur properly for hybrid individuals in meiosis, resulting in low rates of recombination between linked loci and biased segregation. In more severe cases, the phenomenon of cross-incompatibility can occur and the fertility of the offspring can be reduced, even leading to failure and the inability to continue producing hybrid offspring.

4. Because chromosome exchange problems, translocations, and other variations may occur, parental material and its hybrid individuals should also be identified and analyzed at the cellular level to avoid affecting the efficiency of genetic linkage mapping.

Selection of individual types of population. Based on their genetic stability, QTL localization populations can be divided into two categories:

1. Temporary separation of groups. It mainly includes F_2 and F_3 populations, as well as backcross (BC) populations, etc. The advantages of this type of population are: simple population construction, rich genetic information, and additive and dominant effects that can be estimated; the disadvantage is that there is separation between individuals, which does not allow for long-term preservation and use by many generations.

2. Permanently separated populations. Such a population of recombinant line (RIL), double haploid line (DH) and near isogenic line (NIL). The population is re-classified by strain and can be subjected to a repeated block study, distinguishing between blocking effects, repeated effects and random errors, thus increasing the accuracy of QTL detection; however, it takes a long time to establish a population and is vulnerable to environmental and human factors.

The F_2 population is one of the earliest and most widely used quantitative trait locus mapping populations [32-34]. It is obtained by crossing parents to obtain F_1, and then independent crossing. F_2 populations are easy to construct to have a variety of segregation types, provide a large amount of genetic information, and both recessive and dominant effects can be assessed. There are also two disadvantages, one of which is the variety of segregation types and the existence of heterozygous genotypes, which leads to the inability to identify dominant pure and heterozygous genotypes by phenotype. The second is that it is not easy to maintain for a long time, because the genetic structure of the population changes after one generation of sexual reproduction.

A backcross population is formed by multiple crosses between the F_1 and one of the parents. A population has only two genotypes at a locus, which directly reflects the

proportion of cells in the F_1 generation. Therefore, the localization accuracy is higher than that of the F_2 population, and it is not easy to maintain for a long time like the F_2population. In addition, it is not easy to establish ger populations for some crops that are difficult to hybridize by hand and are prone to false hybrids.

A Recombinant inbred population line (RIL) is produced by self-crossing several generations of hybrid progeny, usually generated from F_2 by replacing the single-grain transmission method. Since the population consists of several generations of self-crossing and the line of plants in the population is pure, the RIL population is a permanent population that can be used and tested over a long period of time. The population also has the disadvantage that there is self-pollination in heterogeneously pollinated plants, which makes it difficult to construct RIL populations; gertime for construction compared to the F_2 population [35, 36].

The Double haploid (DH) population is first formed by chromosome doubling of F_1 heterozygotes, and the formation of haploid plants is induced by another culture method, and then the DH strain is formed by chromosome doubling haploid plants. DH plants are homozygous lines produced by self-crossing and continuous segregation. Some plants are very difficult to obtain a DH population, which causes limitations in the application of the DH population; Second, the flowering ability of plants is related to genotypes, and the process of flower breeding can cause selection effects on pollen of different gene types, which can disrupt the genetic structure of DH populations and cause serious segregation bias, thus affecting the accuracy of QTL localization [37, 38].

Near isogenic lines (NLI) is obtained by repeated backcrossing. Targeted donor strains have introduced chromosome fragments and other genomic components compatible with the incarnate parents. NLI recalculates the breakdown of several QTs into individual GJ Mendelian factors on the same genetic background to transform quantitative traits into qualitative ones, which allows accurate localization and cloning on the map [39]. Compared to another segregating population, several genetic factors

of whole genomes are segregating at the same time, leading to interference Q Results of TLlocalization depend on the genetic background and affect localization accuracy.

In addition to the above mapping populations, there is also a nested association mapping population (NAM) and a multiparental forward generation cross (MAGIC) [40, 41]. Below in Table 1.1 is a comparison of different typical QTL locations.

Table 1.1.

Comparison of several population in common use for QTL mapping

Populationtype	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 the genetic background	Big	Big	Big	Big	Small
Amount of information	Richest	Rich	Rich	Rich	Rich

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

*Determining the size of the population***.** In addition to population type, the accuracy of QTL localization is also highly dependent on population size. The larger the population, the higher the localization accuracy. However, the population is too large, which not only increases the experimental burden but also increases the cost. Therefore, it is important to determine the optimal population size. In real research, the population size is determined by the purpose of the experiment, and how small a population can be selected if only a molecular genetic map is constructed; for QTL-localization of agronomic traits (quantitative traits), larger populations should be selected. The size of these breeding populations also varies between population types. For example, F_2 populations have more genotype types in the progeny separation than other mapping populations, and it is necessary to obtain a large enough sample size that all genotypes are likely to occur in order to make the accuracy of mapping the two comparable. Order of required population size of F_2, RIL, BC1, and DH for comparable mapping accuracy.

QTL localizationmethods. QTL localization methods fall into two main categories depending on the basis of individual clustering. (1) Grouping phenotypic data based on quantitative traits is called trait-based analysis (TBA). This is mixing the

DNA of two extreme individuals to detect genetic polymorphism between two DNA pools [42]. Molecular markers that show differences between the two pools are considered to be associated with a QTL, also called Based Segregation Analysis (BSA). This method is widely used in qualitative features [43, 44]. It can greatly reduce the number of DNA samples, reduce the cost of marker analysis, which is suitable for locating some resistance genes, but it can only be used to locate QTL of individual traits, and the sensitivity and accuracy are low, and only QTL with large effects can be detected. Consequently, this type of method is currently rarely used for QTL localization. They are not described in detail in this document.

Another type of grouping is based on marker genotypes, called marker-based analysis (MBA) [45], which includes Single Marker Analysis (SMA), Interval Mapping (IM), CompositeInterval Mapping (CIM), Inclusive Composite Interval Mapping (ICIM) and MixedompositeInterval Mapping (MCIM). Interval Mapping, ICIM) and Mixedomposite Interval Mapping (MCIM) based on a hybrid linear model. A comparison of the characteristics of five different QTL localization methods that are commonly used is summarized (Table 1.2).

Table 1.2.

Comparison ofthe five analytical methods for QTLmapping

Analytical methods	SMA	IM	CIM	ICIM	MCIM
Number of markers	1	2	Multiple	Multiple	Multiple
Accuracy	Low		High	High	High
Additive effect	+	+	+	+	+
Dominan teffect	+	+	+	+	+
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. Single Marker Mapping (SMA) is a method of indicating the mean difference of phenotypic traits corresponding to different marker genotypes. If a marker is associated with a quantitative trait, a significant difference in mean value is required.

Since this method does not require the construction of a complete genetic linkage map, early QTL localization is widely used [46-48]. In addition, this method has many disadvantages:a) it is impossible to determine whether a marker is associated with one or more traits;b) it can only determine the linkage with this particular marker, but cannot know the exact location in the chromosome;c) genetic effects can be underestimated, and some false positives can be easily detected;d)the detection efficiency is lower, but a larger sample size is required.

2. Interval Display (IM)based on the principle of mapping one marker, it was proposed to use the method of interval mapping with two adjacent molecular markers [49]. The main statistical methods and mathematical models used are regression analysis, the likelihood ratio method, or the least squares method because the approximate position on the chromosome is known; in the presence of only one marker chromosome, the position estimates and effect values are asymptotically unshifted; detection performance is improved compared to single-marker assays, but the required population sample size is reduced. Since this method compensates to some extent for some of the defects and shortcomings of the SMA method, it is more widely used in the genetic study of quantitative traits.

3. Complex Interval Mapping (CIM) - the composite interval mapping method, which can combine multiple linear regressions with interval mapping, was first proposed in 1994[50]. A model is found for a given interval of markers and combined with all markers associated with another QTL, which in turn controls for the genetic effect of background. If all QTL loci are free from epistatic effects and genotypes and environment also interact, then maximum likelihood estimates of each parameter are obtained using interval mapping methods, likelihood profiles are drawn, and QTL are located at possible marker intervals based on significant differences between likelihood ratio statistics. Its main advantages are: a) although only one interval is detected in time, it can turn into a multi-dimensional scan from many to a one-dimensional scan; b) marker information about the whole genome can be reused; c) the influence of genetic background on detection results can be greatly minimized,

thus increasing the accuracy and efficiency of mapping. However, this method cannot analyze complex genetic problems, such as epistasis and environmental interactions, and is prone to effect localization and estimation.

4. Inclusive Composite Interval Mapping (ICIM) - the full interval mapping is derived from the composite interval mapping [51]. There are two main processes: first, based on information about known molecular markers, some important marker variables were selected by stepwise regression analysis and their genetic effect values were estimated. Corresponding phenotypic data values were adjusted using a linear model obtained by stepwise regression, and univariate and multivariate responses were performed.In addition, full interval mapping analysis software was successfully applied to detect and analyze nested association mapping populations [52].

5.Mixed Composite Interval Mapping (MCIM) - To estimate epistasis between QTL locations and environmental interaction effects, a method of composite mapping of intervals based on a mixed linear model was proposed [53]. This method allows the analysis of additive QTL loci, different types of epistatic QTL, and hod genotype-environment interaction effects, this method can avoid the influence of selected markers on the QTL effect analysis, and unbiasedly analyze the QTLanden environment interaction effect. The composite interval mapping method based on a mixed linear model can be extended to analyze QTL with additive × additive, additive × dominant, dominant × dominant epistasis and their interaction effects with the environment. These effect estimates can be used to predict total heterosis based on QTL main effects, reciprocal heterosis based on QTL-environment interactions, and to directly estimate breeding values of individuals, thereby increasing breeding efficiency.

Common analysis software for QTL localization. Statistical analysis software widely used for QTL localization include Excel, SAS, DPS, SPSS, etc.; QTL analysis software includes MAPMAKER/QTL1.1, QTLcartgrapher2.5, QTLNetwork2.0, IciMapping4.0, JoinMap4.0, etc. Among them, QTLNetwork2.0 and Ici Mapping 4.0 can analyze the interaction between genes and the 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 of phenotypic variation 5 software [54].

1.3. Domestic and international research progress

Grain number per spike. The wheat inflorescence is a spike, which is an unbranched inflorescence, and the axillary meristem, distinct from the inflorescence meristem, develops directly into a spike, with each spike meristem producing several flowers. Inflorescence meristems cannot multiply indefinitely, forming a limited amount of spike meristem lateral tissues and terminal spike meristem. The sign of the amount of grain in an ear is determined by such signs as the length of an ear, the number of ears in an ear, the number of sterile ears and the density of an ear, and is a complex quantitative sign [55 - 57].

A recombinant inbredline (RIL) population combining HG28 and HG67 was used to determine the major quantitative trait locus qGN4.1 for grain number in the ear of rice genotype Pusa1266. Overexpression of the nal1(LOC_Os04g52479) gene is unlikely to cause high spike number in these QTL-NILs. In contrast, another closely related gene, nal1(LOC_Os04g52590), encoding the structural domain of a protein kinase, is consistently overexpressed in high-grain cells[5]. 8]. A high-density genetic map was generated for the Kechengmai1/Chuanmai 42 double haploid population, and the total number of $F_2$7 qtl associated with total number of spikelets per spike (TSN) and number of fertile spikelets per spike (FSN) was 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 ear [59]. A total of 27 SNS QTLs were detected in a population of recombinant inbred lines (RILs) of a common spikelet and a multi-spike wheat line (with an additional spike with more canonically oriented apical spikelets). was positively correlated with spike length (SL), growth date (AD), and number of grains per ear (GNS) [60]. No QTL was detected on chromosome 2 for the total number of

spikelets and fertile spikelets, among them the GNI-A1 gene was identified, which increases the number of grains due to the increase of fertile flower spikelets [61]. A high-resolution genetic map was developed by promoter capture analysis with exon combination assumptions in 2019, aQTL was localized for reliable spike (SNS) on the long arm of chromosome 7, this gene is homologous to the trait gene APO1 and may be the best candidate for SNS [62].

*Thousand grain weight.*Thousand-grain weight is also a component of yield and is not controlled by many genes, including environmental and genetic factors. DH linkage mapping was constructed using SparkxRialto, the QTL locus for 1000-grain weight was located on chromosome 6A [7]. Construction of hybrid combinant inbred lines between common wheat Nongda 3331 and Tibetan semi-wild wheat accession Zang 1817 identified a total of 15qtlon TGW distributed on 11 chromosomes; Among them, the main QTL (QTgw-4D), located in the Xbarc 1118 – Xbarc 105 locus interval of chromosome 4D, explained 25.08% of the phenotype variation [63]. Using the nongda 3338 (ND3338) / Jingdong6 (JD6) double haploid population, major QTL of TGW 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 1000-grain weight by 5.16-27.48% compared to ND 3338[64]. QTL localization of 60 RILs (Zhongmai 871 and Zhongmai 895) using the 660KSNP array, four genetic regions on chromosomes 1AL, 2BS, 3AL, and 5B were found to have significant effects on TGW-related traits [65], tag for TGW [66]. ND3338 and JD6 were crossed to obtain 203DH populations by in vitro cultivation and thirteen stable TGWQTL located on chromosomes 2A, 2D, 4A, 4B, 5A, 6A and 7A, favorable alleles were obtained from biparental ND3338 and JD6[67].

*Yield***.** Some scholars have started their related studies directly from yieldtraits and also detected many QTL loci related to yield. Three yield QTLwere detected on chromosome 2H, and one QTL on 7H.2 and3H.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[56, 70]. The F1-DH lines of the cross between «UI Platinum» and «LCS Star» were used for QTL detection, only one QTLlocus, Qgy[71]. Genetic analysis of yield traits was carriedout using114 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 toyield traits [72].

1.4. Application of QTL localization in crop genetic breeding

The application of molecular marker technology to facilitate the genetic improvement of crop varieties is called marker-assisted selection (MAS). The principle is to identify molecular markers closely associated with the target QTL, and then simply identify the associated markers at the molecular level to find out whether they carry the target QTL S does not require many years of breeding experience, does not depend on constraints environment and stages of crop development, monitoring good alleles effectively reduces or prevents the introduction of unfavorable genes [73, 74]. In recent years, QTLincommon research has developed rapidly, and QTL localization methods have played a major role in the extraction of new gene sources and genetic breeding of crops. MAS first came from Tanksley for tomatoes. Unlike conventional breeding methods, which require backcrossing more than a hundred individual plants over six generations, the selection was successful through the use of a molecular marker strategy using only a few dozen individual plants backcrossing over three generations[75]. Introducing the major QTLqHSR1 for resistance to the head mutation using MAS into highly susceptible material, the resistance of the improved self-fertilized lines was greatly improved and the combinations were still highly resistant to the disease[76]. The Opaque2 gene significantly increased the lysine content of corn endosperm protein. Successful introduction of the opaque2 gene into

the best maize variety based on the MAS strategy resulted in the consistent production of high-quality protein maize (QPM)[77]. KRN4 , an important regulator controlling the UB3 gene for female spikelet development in maize, was successfully cloned using a genome-wide association study (GWAS) combined with map cloning [78].

The first genetic linkage map of a wheat RFLP marker based on a genetic linkage map generated 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]. VRT2 and SVP1 mutants were identified from the Kronos and Cadenza mutant libraries that could increase the number of spikelets, and the Cadenza mutant was transferred to Kronos, and the study was carried out after several backcrosses using transgenic overexpression of VRT2, lemma and pallium of transgenic lines with high by VRT2 expression longer, and spike meristem was transformed into inflorescence meristem

VRT2 was crossed with vrn/ful2 and young spikelets of vrt2/vrn1/ful2, VRT2/vrn1/ful2 at the same period were compared by scanning electron microscopy (SEM). We could clearly see that the spikelet phenotype of vrt2/Vrn1/ful2 tended to be normal compared to VRT2/Vrn1/ful2 plants, but significantly less ter spikelet lemmas and palea andalarge reduction in spikelet lemmas[80]. Two populations of recombinant inbred lines (RILs) were crossed between the highly resistant and tolerant varieties «Blizzard» and «Bonneville» and the disease-sensitive variety «Rainer». seasons, QTLs for resistance to head blight are located on chromosomes 1AL, 1BS, 7AL and 7DS[81] PM resistance loci in the F_2 population of a Korean cucumber cross using powdery mildew resistant and powdery mildew susceptible inbred lines were identified, and two QTLs 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 found to be associated with rice blast in an F_6 recombinant line (RIL) population from a cross between Yugu5 and Jigu31[83].

1.5. Origin and effect mechanism of mi RNA

*Origin of mi RNA.*MicroRNAs are a class of small non-coding RNAs with important regulatory functions that are commonly found in ukaryotes, and their mature sequence is typically only 20–24 nucleotides in size [84]. mRNA is produced by the principle of complementary base pairing. The functions of miRNAs are very diverse and include plant development, protein degradation, signal transduction, response to adverse stress, and regulation of its own metabolism. [85-87]. Currently, RNA research is becoming increasingly popular throughout the world.

In 1993, the professor and his team, studying the genetic analysis of the nematode Caenorhabditieselegans, discovered an RNA they called lineage-4[88]. It has been shown not to encode any protein and to regulate nematode cell development by complementary mating of lineage-4 and lineage-14 to complete the developmental transition. In 2000, researchers identified another gene similar to lineage-4 from the nematode -let-7, which regulates nematode development and completes the transition from larva to adult [89]. That same year, researchers identified let-7 homologs in humans, Drosophila, and other animals[90]. Since then, the study of mi RNA has become a hot topic. In 2001, the laboratories of Bartel, Tuschl, and Ambros identified a number of small molecule RNAs with potential regulatory functions in Cryptobacterium showyeri, invertebrates, and vertebrates, respectively, and formally named them miRNAs [91–93].

In 2002, a breakthrough occurred in the study of microRNAs in plants; information was obtained on the biosynthesis of plant microRNAs, mechanisms of action, and biological functions [94, 95]. Currently, a large number of miRNAs have been found not only in model plants such as Arabidopsis and rice, but also a significant number of miRNAs have been discovered and confirmed in other plants [96–98]. Number and diversity of plant miRNAs Plant miRNA may not only be involved in its own temporal developmental regulation processes, but its functions may include all aspects of plant growth and development and play important roles in many physiological activities. As the number of miRNAs discovered has increased, miRNA

libraries have been generated internationally (http://www.mirbase.org/). As of December 2021, mi RBASE 22 has been released, with a total of $F_2$4521mi precursor RNA sequences and 30424 mature miRNA sequences registered. These include 6150 micrometer long RNA precursor sequences and 7390 micrometer long mature RNA sequences in plants.

*miRNAs effect mechanism.*Mature miRNA strands are selected for integration into RISC to form silencing complexes. The entire cell is scanned for parallel selection of complementary nucleic acids to make them functional. Due to the difference in the degree of complementation between the mi RNA and the target gene, the mi RNA and different binding regions also act differently. There are three main types of miRNA effects based on their complementary relationships: miRNAs are fully or early fully complementary when paired with target gene mRNA, and most miRNAs exert regulatory functions by shifting target genes [99]; in general, miRNAs cleave the RNA of the target gene at 10–11 bases [100, 101]. Inhibition of mRNA translation. mi RNA is usually capable of incomplete complementation with several recognition sites. at the 3' end of the untranslated region of the RNA of the target gene, this interferes with the translation of ribosomes, which in turn can inhibit the expression levels of their proteins [102]. Although the detailed process of this mode of action is not yet fully understood, the vast majority of animal miRNA functions are achieved through this mode of action [103]. Very little RNA has been found in yeast and plants. suppress the transcription of target genes, triggering their methylation in the genome. 104]. DNA methylation is one of the first genetic modification pathways discovered. Numerous studies have shown that DNA methylation can induce changes in DNA conformation, DNA stability, DNA-protein interaction patterns, and chromatin structure, thereby regulating gene expression [105, 106].

1.6. Progress of mi RNA biological function research

Many studies have shown that the synthesis of mi RNA inplantsisstrictly regulated,otherwise,variousdevelopmentaldefectswilloccur,suchasplantsize,anthesisd

ate,andfertility,etc.ItshowsthatmiRNA 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].

*miRNAs are involved in plant growthanddevelopment.*Plants undergo many growth and development processes throughout their lives, mainly containing conidia that form embryos, seed germination, morphogenesis, and the formation of flowers, fruits, and seeds [108]. These stages have distinct morphological features or the development of new organs, and one of the transition stages is the transition from nutrition to reproductive growth. Plants require suitable conditions and time to complete flowering and set seeds, so entering the stage at the appropriate time is critical for plants [109]. Normal expression of mi RNAs is essential for normal plant growth and development, and they directly or indirectly regulate key transcription factors associated with cell growth and differentiation involved in the process of plant growth and development. For example, young leaves develop into mature leaves, changes in nutritional growth maintain productive growth, inflorescence differentiation develops into the growth of floral organs and the establishment of organ polarity. Currently, miRNAs have been widely reported in rice, maize, barley, and cabbage [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 which may be involved in the regulation of starch accumulation or grain formation based on predicted target genes. associated with seed filling [115]. Expression of the targets miR396, miR164, miR156 and miR319 is relatively high during early stages of seed development but decreases sharply at later stages, representing an amiRNA-mediated transition from rapid cell proliferation to grain filling in wheat grains, and it was hypothesized that 86 of these conserved miRNAs may be involved in the regulation of wheat grain filling, and 18 novel miRNAs may play an important role in wheat grain ripening [114]. Nine cultivars were selected and developing seeds were collected at 10, 20 and 30 days after anthesis to

check miR396 gene expression during grain development. Eleven of the 18 target genes were found to be growth regulatory factor (GRF) genes, and miR396 is involved in seed development by regulating the expression of GRF genes (GRF1, GRF6 and GRF9) during grain filling in wheat [117]. Fruit enlargement is an important and complex biological process. Using high-throughput sequencing, the author identified 1253 known miRNAs and 1269 novel miRNAs from 9 small cucumber fruit RNA libraries. Using pollination RNA libraries, a total of 105 highly differentially expressed miRNAs were identified in fruits 5 days after anthesis. Based on the functional prediction of miRNAs and target genes, our results indicate that miRNAs have a potential regulatory role in cucumber fruit enlargement by focusing their target genes [118].

Plant miRNA is involved in regulating embryo development and root growth. Lateral root formation, meristem development, apical organ separation, silica and vascular cell development, as well as cell wall synthesis and cellulose development in Arabidopsis are all associated with miRNAs [119, 120]. Small RNAs and their targets were identified during embryogenesis in cotton somatic cells, and the hypocotyl and embryogenic callus of YZ1 cotton seedlings were compared. A total of 36 known miRNA families were found to be differentially expressed, with 19 miRNA families represented by 29 precursors. The expression profiles of miR156, miR167 and miR3476 are upregulated from the globular stage of the somatic embryo to the embryogenic callus. Although the expression level of miR164 was downregulated in embryogenic callus to somatic embryo at the cotyledon stage (CE), it suggests that these five semiRNAs may be involved in the differentiation process of somatic embryogenesis in cotton [121].

*miRNAs are involved in the regulation of plant stem and leaf development.*The expression of mi RNA and its potential target genes was assessed in the stem tips of «Changfu2» and the thorny shoot mutation «Yanfu 6» [122]. A total of 700 mature miRNAs were identified, including 202 known apple RNAs and 498 potential novel miRNA candidates.

Apparently, they regulate the growth of the shoot apical meristem. mi RNA159, mi RNA167, mi RNA396 and their potential targets, as well as related phytohormones, appear to regulate cell division and internode length. In Arabidopsis, miR171c influences stem branching through negative regulation [123]. miR396 slows cell proliferation by inhibiting growth regulatory factor (GRF) activity, thereby regulating leaf cell number and leaf size [124, 125].

miRNAs 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 MIR172families. 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 miR172 resulted in reduced petals and conversion of sepals to carpels, while overexpression of target genes resistant to mi R172 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].

miRNAs response in plantadversity. The former mainly include pests and diseases, while the latter mainly include high temperature, hot and dry wind, drought, low temperature, salt stress, cadmium stress, etc. We identified 186 known mi-RNAs in cultivated grapes and 427 known mi-RNAs in cultivated grapes. -RNA in Beibinghong. In addition, 105 and 129 new RNAs were identified in Cabernet Sauvignon and Beibinghong, respectively. the expression of some microRNAs in Cabernet Sauvignon and Beibinhong was associated with low temperature stress [130]. To better understand the molecular mechanisms of powdery mildew resistance (PM resistance) in cucumbers, we sequenced RNA and degradase libraries generated from PM-infected leaves of 48 treated with D8 and SSSL508-28 and corresponding

uninfected controls. Using comparative analysis, 32 and 6PM-sensitive differentially expressed mi RNAs were identified in D8 and SSSL508-28, respectively. More differential RNAs were identified when comparing between ID (PM-inoculated D8) and NID (D8 uninoculated), while comparison between IS (PM inoculated SSSL508-28) and NIS (SSSL508-28 uninoculated) showed that expression levels miRNAs can vary significantly depending on the strain/cultivar [131]. The expression of seven miRNAs, miR156a, 159b, 166e-3p, 394, 396c-3p, 812 and 827, was reduced in disease resistance in rice. These seven miRNAs have previously been shown to respond to pathogenic infection through incompatible interactions that can lead to the activation of multiple defense responses. Our data indicate that these even differentially expressed miRNAs may be involved in the regulation of rice blastin [132].Four treatment groups were created: control, 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). Multi-omics analysis of the RNAome, transcriptome, and degradome was performed to construct an RNA-mRNA network influencing starch synthesis, protein metabolism, and other seed characteristics. , while miR156/157, miR159/319, miR164, miR394 and miR398 were disabled. regulated under low temperature stress in Arabidopsis [134]. mi R414, mi R415, miR837-5p and miR10546-akr regulate expression under high temperature stress [135]. mi RNA is also involved in the stress response to microelements. Studies have shown that mi R398, miR397, mi R408 and mi R857 are induced to express under low copper stress and maintain copper homeostasis in plants [136, 137].

1.7. Research methods ofplant mi RNAs

*High-throughput sequencing method.*High-throughput sequencing, also known as «next generation» sequencing technology, is based on Sanger sequencing and single-molecule deep sequencing [138, 139]. High-throughput sequencing has now become one of the most efficient and accurate methods for identifying plant miRNAs, and many papers devoted to the identification of plant miRNAs are published every

year [140, 141]. This technology has the advantages of large sequencing data, high accuracy and low cost, so it is widely used in life sciences, agriculture, medicine, etc. To identify microRNAs of two maize hybrid lines (PH6WC and PH4CV) and their action targets under seed development, two mRNA libraries and two degradase libraries were constructed, and the results showed that miRNAs associated with reproductive development (miR156, miR171, miR396 and miR444) could be differentially expressed in seed development [142]. The expression of 19 conserved miRNAs and 13 novel candidate miRNAs was analyzed by high-throughput sequencing. The expression of miRNAs varied greatly between young leaves, stems and flowers. six new miRNAs (miRC1, miRC14, miRC16, miRC112, miRC179 and miRC181) did not undergo significant changes in different tissues [143]. According to the principle of high-throughput small RNA sequencing, at 5°C and -10°C, 30 and 29mi RNAs were detected, respectively, which were mainly involved in transcription regulation, metabolism, stress response and signal transduction based on target gene prediction and functional analysis [116]. A total of 54 novel conserved miRNAs and 8 novel miRNAs were identified in Brassica Campestris ssp pollen. Chinensis, eighteen of these miRNAs differed in expression between buds of male sterile and fertile lines by more than 2-fold, and qRT-PCR analysis showed that the majority of differentially expressed miRNAs were predominantly expressed in buds of male sterile lines [144].

Degradome Sequencing. Degradomic sequencing is a recently emerging method for detecting miRNA target genes [145]. By high-throughput sequencing of degraded fragments generated by mi-RNA-mediated shifts of target mRNAs in cells or tissues, coupled with bioinformatics analysis, mi-RNA target genes are accurately and efficiently verified, 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 volume compared with previous methods for identifying target RNA genes, and the advantages of three high-throughput sequencing technologies, bioinformatics analysis and rapid amplification of cDNA ends. siRNAs that can translocate and degrade target mRNAs. The predicted and validated targets of these

developmental miRNAs are involved in various cellular responses and metabolic processes, including cell proliferation, growth hormone signaling, nutrient metabolism, and gene expression [116] . Sequencing analysis of miRNA-degrading enzymes revealed significant changes in the expression of miR-164d, which regulates genes encoding antioxidant enzymes in tetraploid rice, resulting in decreased miRNA-mediated degradation of target genes [146]. Expression of phytohormone-related miRNAs may play an important role in ovule development, providing evidence for cross-talk between sporophytic tissues and females. Gametophytes[147]. Several erythropopathogen-interacting target genes were identified by degradome sequencing analysis, and candidate RNAs were also identified by qRT-PCR, indicating that the expression of most miRNAs is negatively correlated with the expression of their targets [96].

1.8. Purpose and significance of the research

Wheat is one of the most important food crops in the world. As the world's population grows, the problem of food shortages will become more acute. However, wheat breeding has reached a new stage at the current level. Relies heavily on people's understanding of the key genes and molecular regulatory mechanisms that control wheat growth and development [148]. miRNA is a class of small endogenous non-coding single-stranded RNA molecules widely present in animals and plants, it influences virtually all biological processes and represents a new level of regulation of gene expression. Thus, studying the regulatory role of mi RNAs in wheat growth and development will greatly contribute to research on genetic improvement of wheat.

The early stage of grain development is an important period that determines grain yield and quality characteristics of the wheat crop. Grain development indicates that wheat is entering the reproductive growth stage, which is the period that determines grain weight, and miRNAs have become a hot research topic in the field of rain development. To identify miRNAs involved in wheat grain development, in the present study, wheat grains were 7 days post pollination (DAP) and 14 DAP, which

represent two early phases of wheat grain development, were isolated. High-throughput small RNA sequencing and degradome sequencing were performed to study miRNAs and their target genes that may be involved in the regulation of early grain development.

Conclusions to Chapter 1

Therefore, SSR analysis provides insight into the importance of creating simple, accurate and high-throughput selection platforms using molecular markers. In addition, analysis of markers and different types of interval mapping, as well as comparison of mixed intervals, can determine the accuracy of QTL localisation. MicroRNAs also regulate early grain development and embryo development. Expression of miR396, miR164, miR156 and miR319 targets is relatively high during early seed development but significantly decreases at later stages, implying that this mediates the transition from rapid cell polypheresis to grain filling in wheat seeds. It has been proposed that conserved microRNAs may regulate wheat grain filling and novel microRNAs may regulate wheat grain maturation.

CHAPTER 2
CONDITIONS, MATERIALS AND METHODS OF RESEARCH

2.1. Experimental materials

2.1.1. Trial plant materials

Parent material: Mexican Large Spike (MLS), Bainong419-9 (BN419); Mexican Big Spike originally originated from the Mexican wheat variety «Tanori» and was later introduced into China through systematic breeding of wheat into a new variety called «Mexican Big Spike». Bainong 4199 is a semi-winter wheat variety developed by Professor Ru Zhengan of the Henan Institute Science and Technology grouped with Bainong Highlight 3709 F_2 and Bainong AiKang 58, Henan Provincial Crop Variety Certification Committee (approved it in April 2017). The F_2 population was created by crossing Mexican Large Spike with Bainong 4199, which contains 145 individual plants. All of the above materials are stored and created in our laboratory. Morphological characteristics and characteristics of grains of whole plants and ears of parent plants are shown in Fig. 2.1.

Fig.2.1.Mexican Large Spike and Bainong 4199:
A - the characters of plant, B - spike, C - grains.

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 ofwheat A, B, and D. A total of 200 pairs of SSR primers were collected and synthesized by Shanghai Biotechnology Synthesis.

2.2. Experimental method

2.2.1. Field trials

The F_2 population and parents were planted in 2018-2019 in the experimental field of Henan Institute of Science and Technology with row length of 2 m and row spacing of 0.2 m, 20 rows of F_2 population and 5 rows of each parent, with traditional cultivation management.

2.2.2. Investigation of agronomic traits

At maturity, 10 randomly selected plants of the parent material and single plants of the F_2 population were examined for agronomic traits such as spike length, number of spikelets, spikelet and spikelet density according to the method of [149], which took into account the average value of each trait of the parent material.

2.2.3. DNA extraction and reagent configuration DNA extraction

The parents (Mexican Large Spike and Bainong 4199) 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 μL 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 20min in 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 800μL 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 (480μL) the volume of supernatant in isopropanol (4℃ refrigerator) and shake well (if flocculent 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 500μL 70% ethanol twice, place it on the ultra-clean table and blow dry.

8. Add 200μL ddH_2O 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 ng·μL^{-1} with sterile water. DNA-labeled SSR 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 μL 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（μL）
1	Former primer	0.5
2	Reverse primer	0.5
3	DNA	1
4	2×Es Taq MasterMix (Dye)	5
5	ddH_2O	3

*Amplification procedure***.** Pre-denaturation at 95 ℃ for 5 min, denaturation at 95℃ for 30s, annealing at 60℃ for 30s (annealing temperature depends on primers), extension at 72℃ for 30s, 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 μL of 10% ammonium persulfate and 40 μLTEMED procoagulant in turn, and gently shake the three solutions well.

3. Filling glue: fill the configured glue slowly along the concave plate, 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, 40Wpower pre-electrophoresis for more than 20min to clear the air bubbles.

6. Spot sample: 2 μL ofPCR product was added to each well and electrophoresiswasperformed at 180 W power 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-Na_2 to dissolve, and finally use distilled water to fix the volume to 1L.

3. Configure 1L of8% 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% $AgNO_3$ 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 pipettesEppendorf

0.1-2.5 μL 、0.5-10μL、 2.0-20μL、 20-200μL、 100-1000μL

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 QTLICI MappingVersion 4.2 software (https://www.isbreeding.net/). Based on information on the distribution of SSR marker loci on chromosomes published in GrainGenes 2.0 (http://wheat.pw.usda.gov), polymorphic markers were first sorted using the ANCHOR command so that each locus could be located on the appropriate chromosome. The Grouping command was used to assign all classified markers to corresponding chromosome positions by setting the LOD value to 2.5; then default values and algorithms were used for ordering and copying, and the window size was set to 5; finally, the results were output using the Output command to display the results.

2.2.7. Analysis of phenotypic data

The analysis of normality, ANOVA and correlation between differenttraits was performed for parental and F_2 population spike traits using Excel 2021 and SPSS 22.0 software.

2.2.8 . Additive QTL detection and analysis

QTL analysis was performed using a full composite interval mapping method based on the entire process of QTL ICIMapping software version 4.2 [152]. The LOD threshold was 2.5, the progression interval was 1.0 cm, and the probability of stepwise regression was $P < 0.001$ for QTL detection [153]. Favorable alleles were estimated based on parental coding at the time of QTL mapping, and if the additive effect was positive, this indicated that the potentiation effect came from the parent coding 2; if it was negative, it came from the encoding parent [150].

2.2.9. Super ordination QTL detection and analysis

QTLICIMapping software version 4.2 was also used for super natural revelation of gender. Specific parameters were set as a LOD threshold of 5, a progression

interval of 5.0 cm, and a stepwise regression probability of $P < 0.0001$. The magnitude of the epistatic effect between the parental and recombinant types can be judged by the magnitude of the positive or negative influence of the phenotypic characteristics of the epistatic location. If the effect value is greater than zero, this indicates that epistatic loci belonging to the parental type have a higher effect than loci of the recombinant type; effect than the parent type.

2.2.10. Naming of QTL

QTL naming was then carried out using the formula QTL + trait + chromosome name [154]. QTLs were denoted by the letter Q, traits were denoted by abbreviations, chromosomes were denoted by the wheat chromosome name, and QTLs for the same trait located on the same chromosome were denoted by numbers 1, 2, etc., after the chromosome.

2.3. Results and analysis

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

ANOVA results showed that the two parents differed at highly significant levels in two spikelet phenotypic traits in number of grains per spikelet and thousand grain weight (Table 2.2 and Figure 2.2), consistent with the principle of parental selection in constructing the population for QTL mapping .

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
	MLS	Bainong 4199	Mean	Max	Min	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 ofdifferences (*, p< 0.05; **, p<0.01).

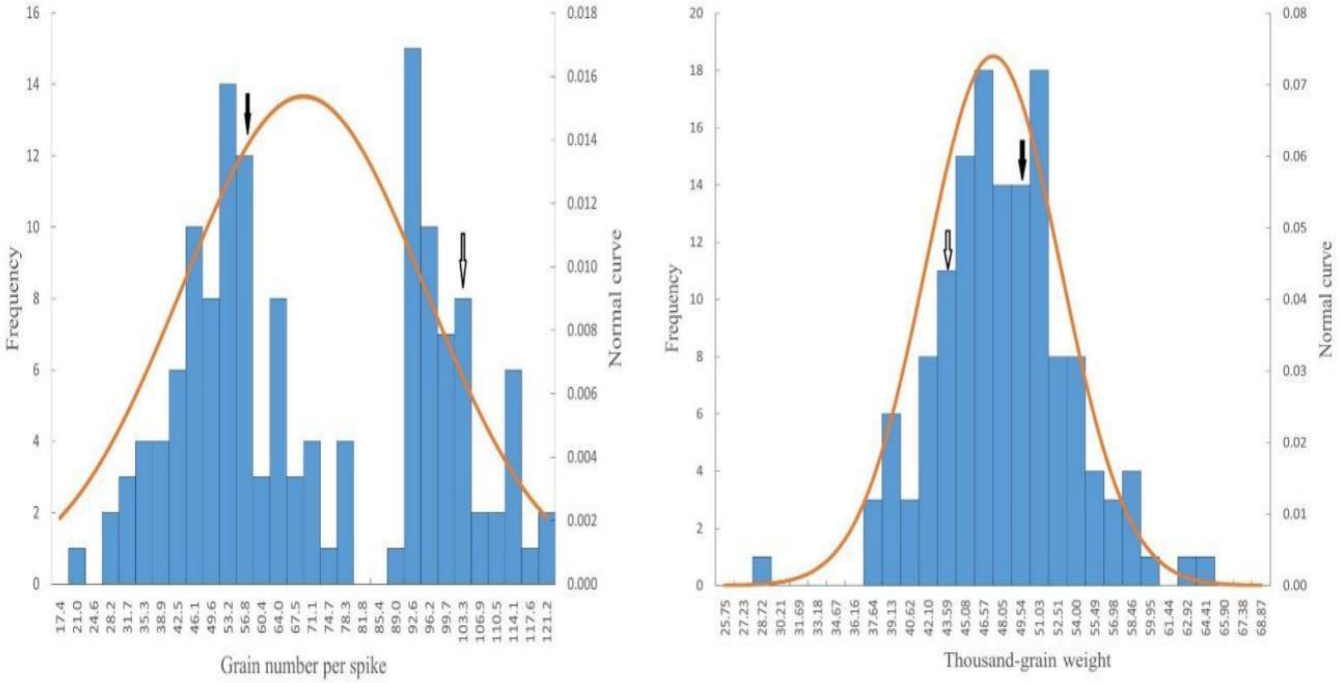

Fig. 2.2. Distribution of variation in spikelet-related traits in 145 plantsderived from the cross Mexican large spikelet × Bainong 4199. denotes Mexican large spikelet, denotes Bainong 4199.

The rainfall per kernel weight of the maternal Mexican Big Spike was a phenotypically high-value parent, while the thousand-kernel weight of the paternal Bainong 4199 was a phenotypically high-value parent. Results from population normality analysis showed that the two traits were largely normally distributed, and

there was bidirectional superparental segregation, indicating that both are quantitative traits inherited [155]. Correlation analysis showed that the number of spikelets and thousand grain weight had a highly significant positive correlation with a correlation coefficient of 0.953.

2.3.2. Polymorphism screening of SSR markers and amplification of polymorphism in F_2 population

A total 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 F_1 cross between the parents Mexican Large Spike and Bainong 4199 and the two parents. The 143 pairs of polymorphic markers were further genotyped and genetic maps were constructed for 145 individual plants of the F_2 population. The amplified bands of some polymorphic markers in the parental and F_2 populations are shown in Fig. 2.3, 2.4.

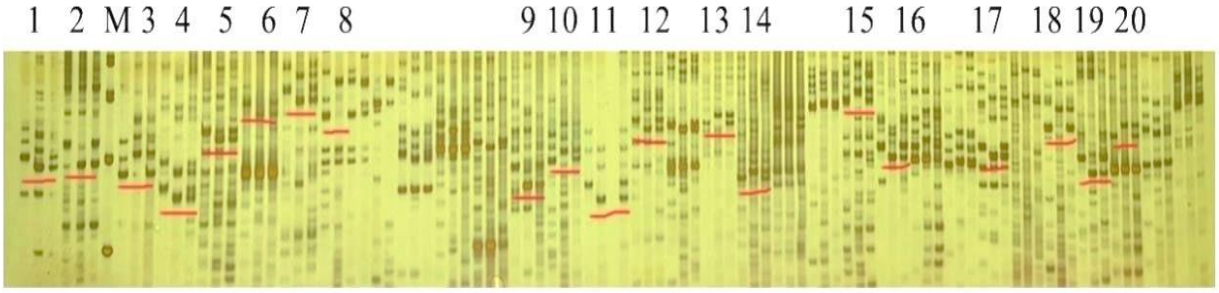

Fig. 2.3. Amplification results of some SSR polymorphic markers between parents and F_1

(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)

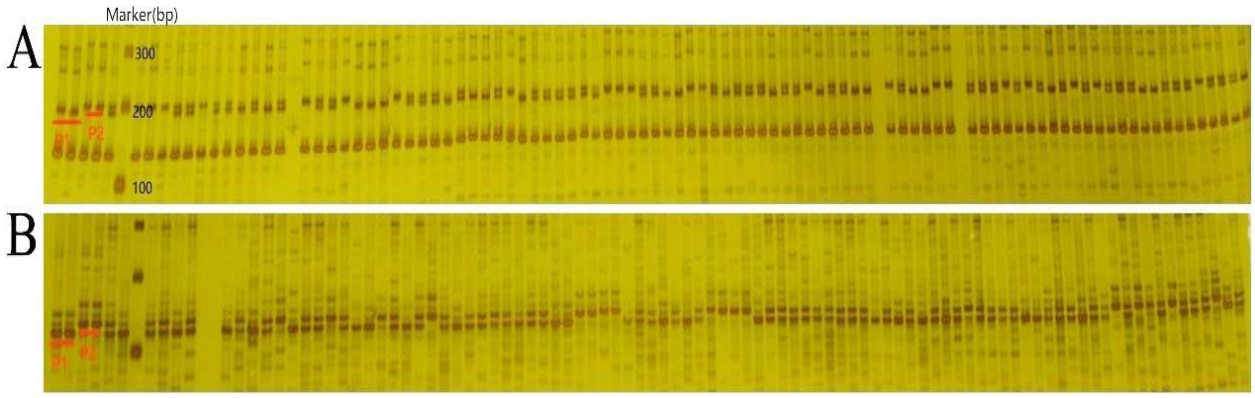

Fig.2.4. Segregation of some polymorphic molecular markers in the F_2population
(A: yzu113903; B: yzu129560; P1:BN4199; P2: MLS)

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 (Fig. 2.5) with a total length of 3128.17 cM using the F_2 population of «Mexican Large Spike/Bainong 4199» as the mapping population, and 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 3.57cM, 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.

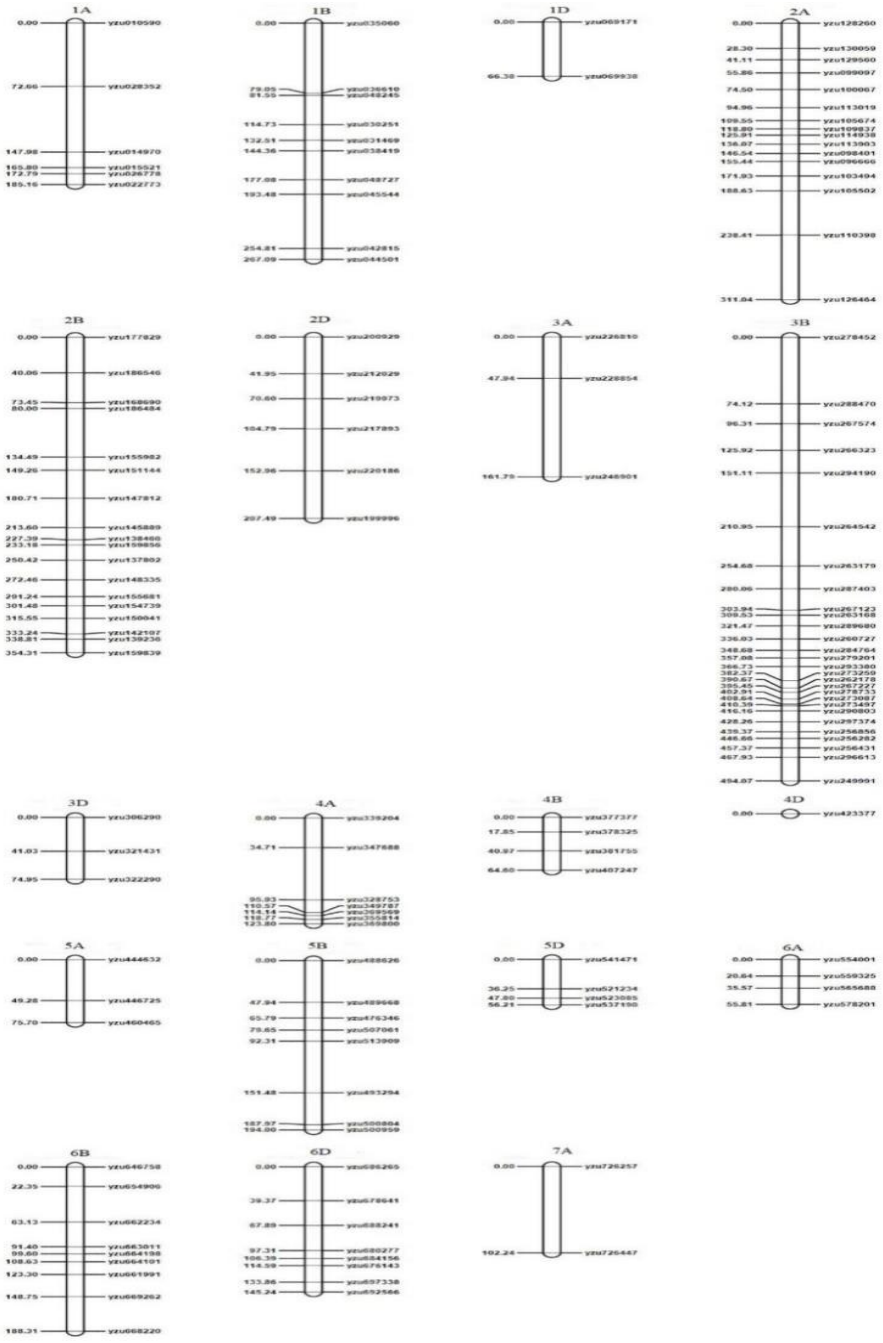

Fig. 2.5. Genetic linkage map constructed for the F_2 population based onMLS/ BN4199.

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.

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, a total of 10 additive QTL related to grain number per spike and thousand grain weight were detected, which could explain 4.922%~21.1044% of the 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 (%)
	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
Grain number per spike	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 in grain number per spike. On three loci QGNS~1B, QGNS~2B and QGNS~2D,.

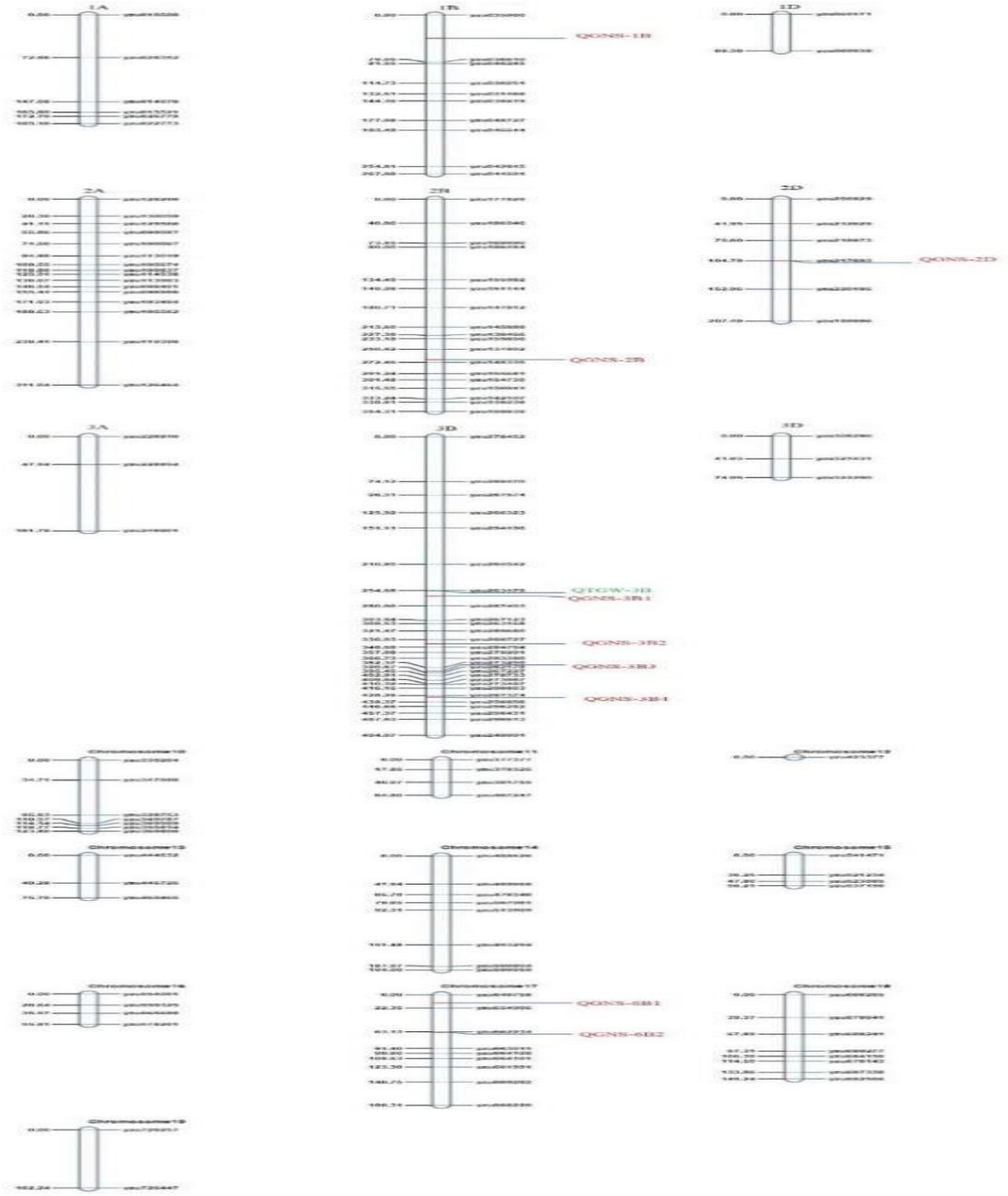

Fig.2.6. Position of additive grain number per spike and thousandgrain weight related traits in wheat

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.

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	Flankingmarker	LODvalue	Additive effect	PVE(%)
Grainnumber 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. Mapping population and molecular markers

The first prerequisite for QTL localization is the creation of a genetic linkage map, and to construct a genetic linkage map, it is necessary to select suitable parental combinations to create mappable populations. In the early days of quantitative trait research, transient segregating populations such as backcross and F_2 populations were commonly used, and with the development of molecular biotechnology, permanent segregating populations such as DH populations and RIL populations have replaced transient segregating populations [156 , 157]. Permanent segregating populations can be reared in trials in the same year and different locations, or in different years and locations, to develop specific environmentally responsive traits to study the effects of

environmental conditions and genotype interactions. Compared to transiently segregating populations, permanently segregating populations are genetically stable and can be used for a long time. Temporary segregating populations are still widely used due to their ease of creation. creation and abundance of genetic types [37, 158].

In crop QTL localization studies, some use interspecific hybrid populations [159], which are more distantly related, have high parental polymorphism, and can provide rich variation when constructing genetic linkage maps, but still use interspecific hybrid populations to be close to selection purposes[160]. In this study, an F_2 population generated between Mexican Large Spike and Bainong 419, which differed greatly in spine characteristics, was used as a mapping population to initially localize the QTL for spine number and thousand grain weight and F2 population.

Common wheat is a heterozygous hexaploid species (2n=6x=42; AABBDD) witha genomesize of 17,000 Mb(Megabase),whichisquite large and complex, and more than 80% ofthe DNAis repetitive sequencesSSR markers are basic repeating units in the genome, consisting of 1-6 nucleotides. Basic repeat units of the same microsatellite type can be distributed at different positions throughout the genome. Site polymorphism is formed by varying numbers of repetitions of the basic unit. Compared with other types of molecular markers, primers have the advantages of codominance, high polymorphism, good stability, easy operation, low dosage, random distribution in the genome, etc., especially in wheat. Thus, SSR markers are considered an ideal tool for genetic research in wheat [161]. In this study, using 300 primer pairs distributed across chromosomes A, B and D, parents and their segregating populations were analyzed, and 143 primer pairs were polymorphic among parents, accounting for 47.67%, and SSR markers were highly polymorphic among parents. which was associated with large genetic differences between the two parents [162].

2.4.1. Construction of genetic linkage maps

In this study, the F_2 population of a Mexican cultivar with a very large ear, a long ear and a large number of grains was selected as the mapping population, and the

sample size of the population was 145.143 SSR molecular markers with polymorphism among parents were used to construct a genetic linkage map of wheat, based on the results of these molecular markers were unevenly distributed among wheat chromosome groups A and Band D. Among them, chromosome group B had the largest number of marker loci - 77 loci, which accounted for 54.22% of the total number of marker loci. The B chromosome group had the highest number of markers: 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 markers; and the D chromosome group had the least number of markers, with a total of 24 markers, accounting for 16.9% of the total number of markers.

In the present study, SSR markers had the highest polymorphism in chromosome group B and the lowest polymorphism in 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 arises, which may be due to the absence or low polymorphism of individual chromosomal segments of both parents, as well as differences in the probability of exchange between different chromosome segments. For example, regions at the ends of chromosomes have a higher turnover rate than those near mitotic sites. This problem can be solved by choosing different combinations. Large gaps exist between multiple markers greater than 50 cm, mainly due to the low density of molecular markers.

2.4.2. Localization of QTL for related traits

In this study, QTLICIMappingV4.0 software with full interval complex mapping method was used to detect and analyze additive and epistatic QTL loci associated with various 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 between 4.922% and 21.1044% of the phenotypic variation in spike number. The results of the study were not consistent with those of others, likely

due to the small number of molecular markers used and the large genetic distance between markers; small population sample, which reduced the efficiency of QTL detection; and differences in environmental conditions. Therefore, the next study should increase the number of molecular markers and population samples, encrypt the genetic linkage map, construct persistent segregating populations, conduct longitudinal and multisite studies, and conduct more in-depth genetic studies of QTL loci for spike traits.

Epistatic crossbreeding refers to the fact that some random regions of the chromosome do not directly affect the phenotype, but these localities influence phenotypic traits by interacting with each other. There are three types of epistatic crossbreeding: crossbreeding between two additive QTL loci, crossbreeding between additive loci of random loci, and crossbreeding between random loci and random loci. QTL analysis of epistatic traits of ear length and some other ear characteristics showed that nine epistatic QTL loci associated with the number of spikelets and thousand grain weight were found 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 showed that epistatic influence on the phenotypic variation of both spikelet number and thousand grain weight. The above data showed that the effect of epistasis on phenotypic variations in grain size and thousand grain weight was significant.

Five genomic regions affecting only the TGW or GNS were identified using 191 recombinant self-incompatible lines on chromosomes 1B, 3A, 3B, 5Bor 7A and chromosome 6A, affecting both the TGW and another GNS region [166]. 5AC, 5BL, 6A, 7AL and 7BL, of which 11 loci increased TK alleles from parent week 8425B, but Chinese spring contributed two positive alleles. The number of spike grains 11QTL for GNS on chromosomes 1BS, 2AL, 2B(2), 2D, 3AL, 3B, 4AL, 4BL, 6BL and 7BS has been identified. alleles for increasing KNS on chromosomes 2AL, 2B(2), 2D, 3B, 4A and 4BL were provided by Zhou 8425B, and chromosomes 1BS, 3AL,6BL and 7BS alleles were from Chinese Spring[167]. Nine stable QTLs located on chromosomes 2B, 4A, 5A, 6B and 7A were identified. The genomic region on chromosomes 2B and 4A

spanned two stable QTLs for GNS (QGns.cau-2B.4 and QGns.cau-4A.4), of which all dominant alleles were from ND3338; 13 stable QTL for TGW were located on chromosomes 2A, 2D, 4A, 4B, 5A, 6A and 7A, with the favorable alleles being biparental ND3338 and JD6 [67]. Sixteen QTL for GNS were identified on chromosomes 1BS, 1BL (2), 3DL, 4AL, BS(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 explain 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 «Opata85» alleles at TGW contributed positively to T.G.W. The remaining 14 alleles that positively affected 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 differences in sample populations and molecular markers.

Conclusions to Chapter 2

Factors affecting the results of QTL localisation are mainly the choice of the dividing population, population size, type and number of molecular markers, environmental conditions, statistical methods and density of genetic linkage mapping markers. The above factors can be considered together to more accurately and precisely identify QTL loci associated with target traits.

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 Phredand 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 RepBasedatabases to distinguish rRNA, scRNA[173], 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) orwheatgenome 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 hairpinsecondary structure whenanalyzed 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 using methods described by Chu et al. [175]. The identified miRNAs were compared with wheat sequence databases. The miRNA targets were predicted using Target Finder (https://github.com/carringtonlab/TargetFinder?). BLASTN hits with fewer than four mismatches were selected as potential targets; for unpredictable miRNAs, psRNA Target software (http://plantgrn.noble.org/psRNATarget/) (version 12) was used to predict targets in wheat transcripts with prediction score cutoff = 3.0 length for complementation assessment. = 20 and target availability = 25.

To obtain degradome sequencing data, 20–21 nucleotide sequences were collected for subsequent analysis. Unique reads that perfectly matched the wheat expressed sequence tag (EST) database from NCBI or contig sequences from the WGS assembly were retained. Approximately 15 nt upstream and downstream of wheat 5′ EST sequences mapped using degradome reads were extracted to generate 31 target signatures as «t-signatures» [17]. Cleavel and the pipe line were used to identify

miRNA targets [170]. Potential targets were considered matches with scores up to four, where G:U pairs scored 0.5 and no mismatches were found in the site between the 10th and 11th nucleotides of the corresponding miRNAs. Matching mRNAs obtained by both methods were chosen as targets for microRNAs. To understand their functions, the putative target genes of these miRNAs were subjected to BLASTX and 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 10miRNAs and 8 target genes were selected randomly. RNA was extracted from three independent biological samples of 7 DAP and 14DAP grains, individually, and used for transcription (RT)reactions.The One Step Primer ScriptmiRNAcDNASynthesisKit (Takara) and PrimeScript®RT reagent Kit with gDNA Eraser (Perfect Real Time;Takara) were used for theRT reactions of miRNAs and targets according to the manufacturer's instructions, respectively. RT-PCR was performed in the Bio-RadIQ5Real-TimePCRDetectionSystem (BIO-RAD, Hercules, CA, USA) using 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®PremixEx Taq™ (Perfect Real Time, Takara) and 8.5 µl of nuclease-free water]. All reactions were first performed at 95°C for 30 s, followed by 40 cycles (95°C for 5 s, 61°C for 30 s and 72°C for 30 s). All reactions were carried out three times. Wheat U6[GenBank: X63066] cnRNA and actigene (GenBank: AB181991) were used as endogenous controls for miRNAs and target genes. The relative amount of each miRNA was calculated by comparative analysis (ΔΔCT) using the formula 2-ΔΔΔCT [178]. The miRNA and target samples in the 0 DAP library with CT value were selected as calibrator and the expression level was set as 1.0. The expression levels of the miRNAs and targets themselves were normalised by comparison. All primers used in this study are summarised in Appendix C.

3.4. Results

3.4.1. Phenotypic analysis of developing grain

Wheat grain development was assessed by observing the pattern of increase in grain weight and volume. Here, growth in grain weight and volume was relatively slow early (up to 11 DAP), increased sharply between 11 and 14 DAP, and continued to increase until approximately 26 DAP (Figures 3.1.A and 3.1.B). The appearance of developing grains at some representative time points is shown in Fig. 1C. Based on this pattern, we focused primarily on the transition phases 7DAP and 14DAP, key periods of early grain development. To identify the relationship between these changes and miRNA abundance during early grain development, we next used small RNA and degradome libraries from 7DAP and 14DAP grains to compare miRNA expression and analyze possible targetfunctions.

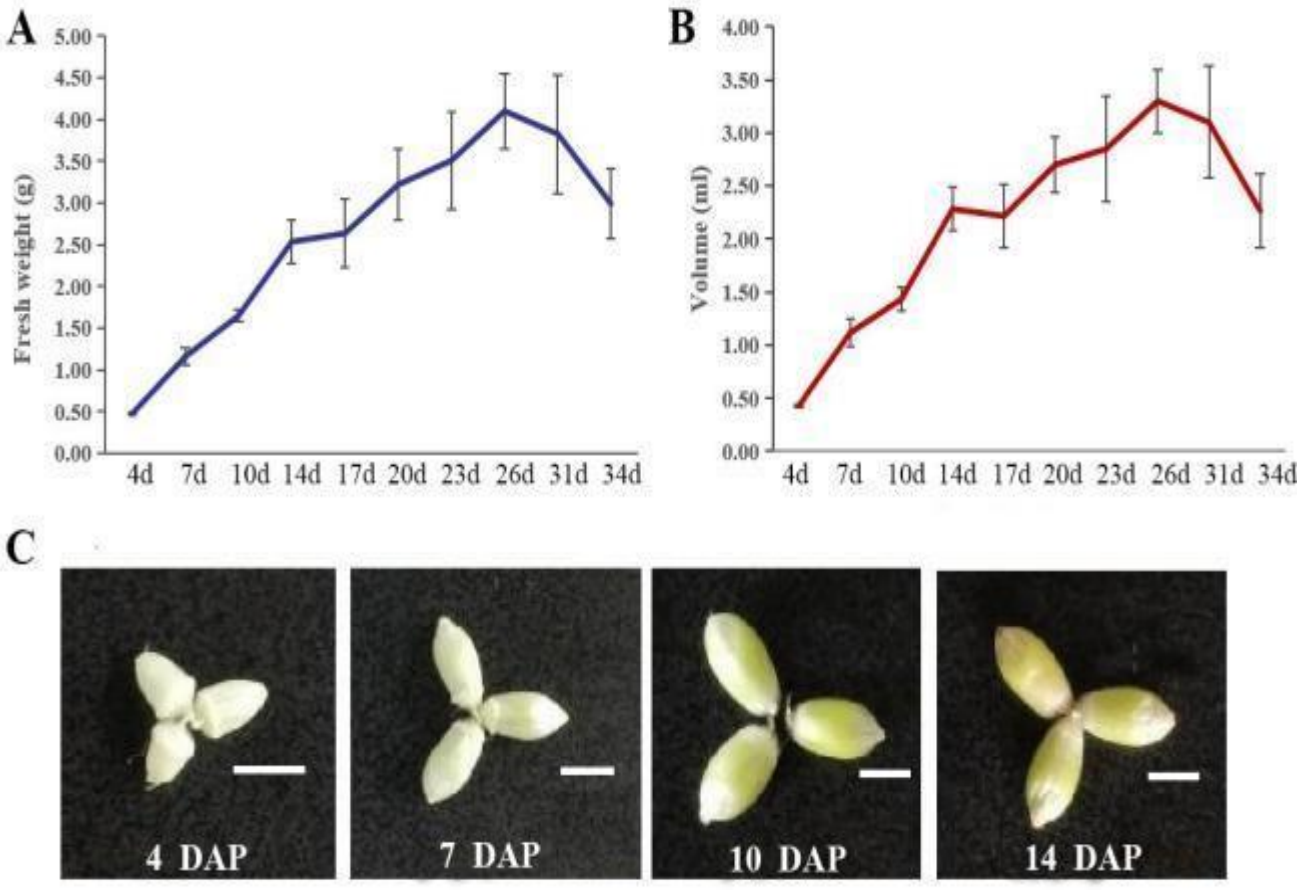

Fig.3.1. Phenotypic changes ofdeveloping grain at differentdays after pollination

A: Fresh weight changes; B: Volume changes; C: Grain appearances at 0,4, 7, 10and14 DAP. Scale bars represent 1.0 cm. DAP: Days after pollination.

To uncover the role of miRNAs in early post-pollination wheat grain development, 7DAP and 14 DAP grains of the cultivar «Bainong 4199» were used to generate two small RNA libraries, which were then sequenced. The raw sequencing fragments obtained from the base identification analysis of high-throughput sequencing results are raw reads. Raw reads are filtered to remove low-quality adapters and N content greater than 10% of these sequencing fragments are clean reads. A total of 43.18 million raw reads and 24.4 MB of 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 7DAP and 14DAP libraries, respectively. This is equivalent to 1,806,597 (51.66%) and 1,981,099 (56.65%) Uniquesrna sequences from the 7 DAP and 14 DAP libraries, respectively) unique mRNA, and the total number of unique sequences in the 7 DAP library is less than in the 14 DAP library (Table 3.1). The results indicate that, compared with 14 DAP, 7DAP has broader sRNA-mediated regulation of gene expression.

Table 3.1

Unique and total sRNA sequences between 7 and14DAP

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

Non-coding RNAs, such as astRNA, rRNA, small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and small cytoplasmic RNA (scRNA), were identified based on the Rfam (http://rfam.xfam.org) and RepBase (http ://www.girinst.org). /repbase/).After removing annotated RNAs (tRNA, rRNA, Repbase and snoRNA), the number of remaining unannotated sequences was 2,448,113 (17.46%) in the 7 DAP Library and 3,456,209 (33.3%) in the 14 DAP libraries of clean reads (Table .3.2).

Table 3.2.

Annotation and distribution of small RNAs in the libraries from 7 DAP and 14DAP grains

Category	7 DAP		14DAP	
	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
cleanreads	14020684	100	10379004	100

Of these unannotated sequences, 235.8 79 (10.64%) reads in 7 DAPLibrary and 1,458,680 (43.06%) reads in 14DAPLibrary, which perfectly match 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 3.3.

Sequencing data of small RNA libraries derived from grains at 7 and 14 DAP mappd 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

Reports show that the distribution of mRNA lengths generally reflects species or tissue specificity [113, 179]. Pure RNA length ranged from 18 to 30 nt in both libraries, with most being between 21 and 24 nt in length, the most common length being 21 nt in the 7 DAP library and 24 nt in the 14 DAP library (Figure 3.2). . In the two libraries, the length distribution of clean reads and miRNAs are different: the mRNA length distribution was 61% and 73.89% between 20~24 nt, respectively, and

the percentage of mRNA with 24 nt sequence (7 DAP was 26.79%, 14 DAP was 40. 42%) and was higher than that of other sequences.

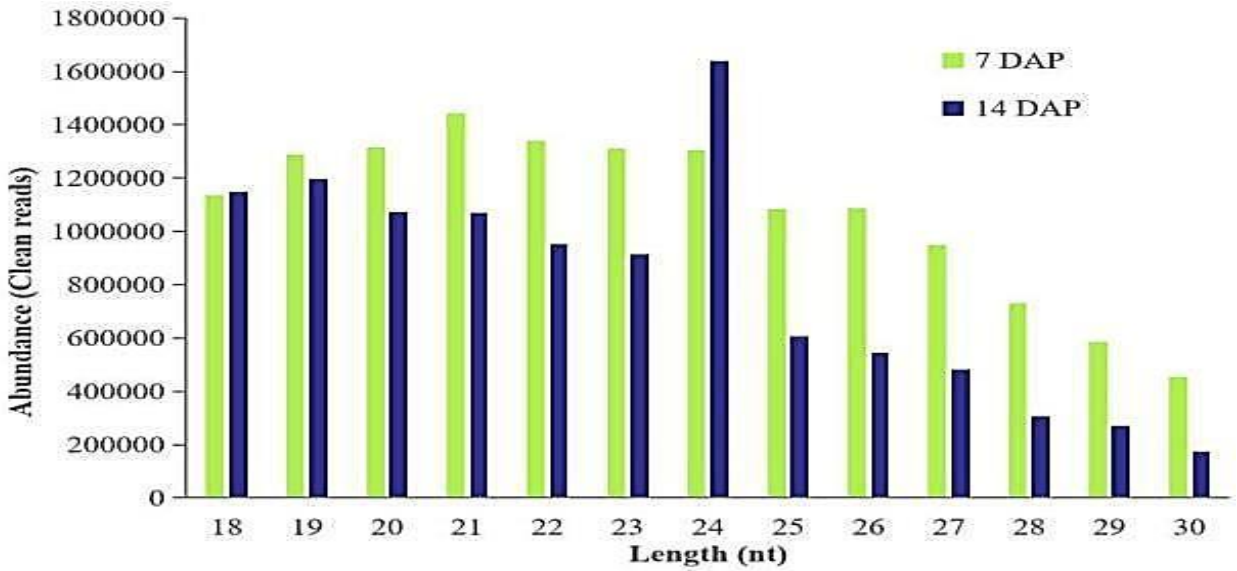

Fig.3.2. Length distributions ofsRNAs in developing grains at 7 DAP and14 DAP

Length 24nt. In the library, 7-DAP-RNA with 23 nt is inferior only to the sequence of 24-ntRNA, and in the library, 14-DAP-RNA with 21 nt (Table 3.4)

Table 3.4.

Summary ofthe length distribution of clean reads and miRNA by sequencing

Length	7 DAP		14DAP	
	Clean(%)	miRNA(%)	Clean(%)	miRNA(%)
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(40.42)
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(100.00)

To investigate whether there is a base preference for small RNAs, the distribution of bases at each position of known small RNAs was calculated. The results showed that the first base at the 5' end of the miRNA is most abundant in A and least abundant in G. This is not consistent with the reported validated miRNA-specific sequences, where the first base at the 5' end is most abundant in U and least abundant in G [180] (Appendix D). Deep sequencing of miRNAs was analyzed using the miRDeep2 v2.0.5 software [181], and miRNA precursor sequences with hairpin structure and folded RNA structures of each candidate miRNA were listed in Appendix E and Appendix F.

3.4.2. Identification of known miRNAs and prediction of new miRNAs

To identify known miRNAs, we compared unannotated RNA sequences that perfectly matched the reference genome in miRBase22.0 (http://www.mirbase.org) based on the perfect match criterion. A total of 89 known miRNAs from wheat were detected in miRBase/Triticumaestivum in the two RNA libraries, of which 46 were expressed in the 7 DAP library and 87 were expressed in the 14 DAP library. For known miRNAs from other plant species, their secondary structure and miRNA* were predicted based on RNA folding (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi), and these semiRNAs were predicted using MIREAP (http://sourceforge.net/ A total of 16 known miRNAs from other plant species were identified in the present two RNA libraries, including 13 expressed in 7 DAP libraries and 16 expressed in 14 DAP libraries (Table 3.5., Appendix E).

Table 3.5.

The number of known miRNAs, novel miRNAs and targetsidentified from 7 DAP and 14 DAP grains

Library	Known miRNAs	KnownothermiRNAs	NovelmiRNAs	Total
7DAP	46	13	32	91
14 DAP	87	16	78	181
Total miRNAs	89	16	79	184
MiRNAswithtarget	41	13	25	79
Target genes	266	152	258	676

Regarding the expression of known miRNAs, including ata-miR9863a-3p, osa-miR396e-5p, tae-miR9670-3 and tae-miR7757-5p, they were expressed most actively, while some other known miRNAs such as stae- miR9672b, tae-miR9662a-3p, tae-miR167c-5p ta, e-miR156, tae-miR9777 and tae-miR9669-5p were in moderate abundance (Fig. 3.3). Most of the abundantly expressed miRNAs were known miRNAs from the wheat miRNA database and conserved between plant species, such as miR156 and miR396e-5p.

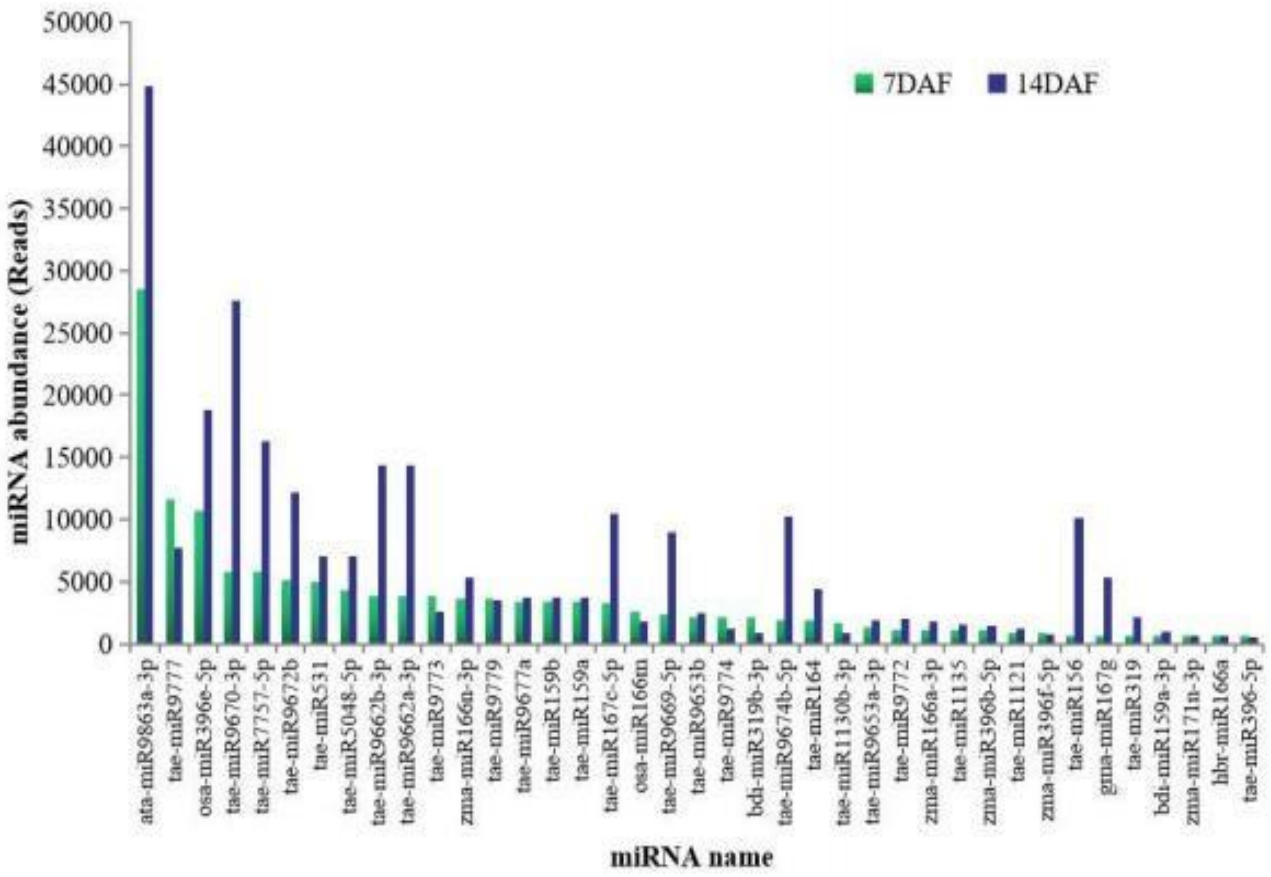

Fig.3.3. Most abundantly expressed known mi RNAs in 7 DAP and 14 DAP grains.

(Horizontal axis: The most abundant known mi RNAs among all identified known mi RNAs; Vertical axis: The reads abundances corresponding to mi RNAs.)

To predict novel miRNAs, unannotated mRNAs were analyzed based on pre-miRNA sequences that can form a canonical hairpin stem-loop structure [182]. A total of 79 novel miRNAs were predicted, of which 32 were expressed in the 7 DAP library and 78 were expressed in the 14DAP library (Table 3.6.). The minimum free

energy of pre-miRNA 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 hairpin structures.

More novel miRNAs were expressed in 14 DAP than in 7 DAP, and 31 novel miRNAs were commonly expressed in the two libraries: 1 special in the 7 DAP library and 47 special in the 14 DAP library. Most of the new miRNAs had relatively low expression levels. The most abundant novel miRNAs were novel-m0064_5p, novel-m0492_5p, and novel-m0661_5p (Appendix F).

Table 3.6.

Summary ofmiRNA and target gene number from 7 DAP and 14 DAP

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

3.4.3. Differentially expressed miRNAs between 7 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 7and 14 DAP grains, among which 18 known miRNAs and 13 novelmiRNAs were up-regulated in 14 DAP grains (Appendix G).

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 withthose from deep-sequencing,which indicate that sRNA sequencing is reliable (Fig. 3.4).

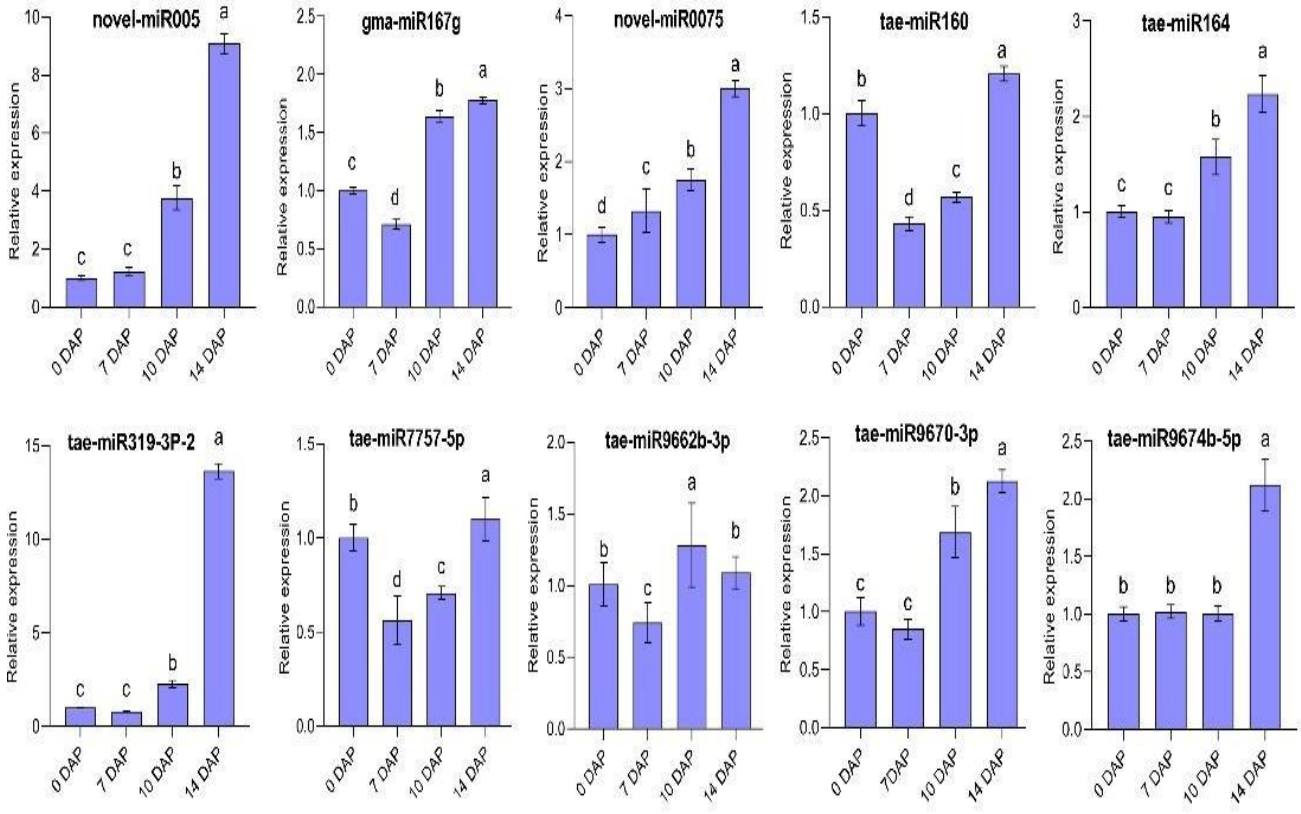

Fig.3.4. Validating of partial differentially expressed miRNAs by qRT-PCR

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 randomlyselected miRNAs (2-ΔΔct values).

3.4.4. Putative target genes involved in seeddevelopment

Plant microRNAs have an almost perfect pairing with their target genes and function through direct target cleavage or, in some cases, through translational repression. Therefore, identifying the targeting of miRNAs is key to understanding their functions [183, 184]. Potential miRNA targets were predicted computationally using Target Finder v1.6script software [185]. Starting with 184 common miRNA sequences, a set of 810 potential targets was predicted for 25 novel miRNAs, 13

conserved miRNAs, and 40 known miRNAs (Table 3.6). Among the 88 DEmiRNAs, 477 potential targets of 147 miRNAs were identified. Sequence details of the newly identified targets for all miRNAs and DEmiRNAs are listed in AppendixesH, I, J.

Table 3.7.

Summary of annotated all and differentially expressed miRNATargets

Annotated data base all	miRNA target 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 generally show strong preference for their target genes with important functions [186], the predicted target sequences of all miRNAs and DEmiRNAs were massively searched in the GO, COG, KEGG, Swiss-Prot and NR databases, 810 targets from the total miRNAs and 477 DEmiRNA targets were able to successfully obtain annotated functional information (Table 3.7, Fig. 3.5). Among all 810 miRNA targets, 174 (21.48%) could be annotated in the COG database, 605 (74.69%) in the GO database, and 176 (21.73%) in the KEGG database. However, of the 477 DEmiRNA targets, only 84 received functional COG annotation. Both of these targets were found to include global function prediction only and global function prediction only (17.46%), replication, recombination and repair (11.9%), transcription and signal transduction mechanisms (8.73%), post-translational modification , protein turnover, chaperones (5.56%), cell wall/membrane/envelope biogenesis (4.76%), translation, structure and biogenesis of the ribosome (3.97%), defense mechanisms, transport and metabolism of amino acids and transport and metabolism of nucleotides (3.17%).

Transport and metabolism of lipids (2.38%), intracellular transport, secretion and vesicular transport. Transport and metabolism of inorganic ions and biosynthesis, transport and biosynthesis of secondary metabolites. catabolism (1.59%), RNA

processing and modification, cell cycle control, cell division, chromosome separation, energy production and conversion (0.79%) and biological process (31.99%) (Fig. 3.6).

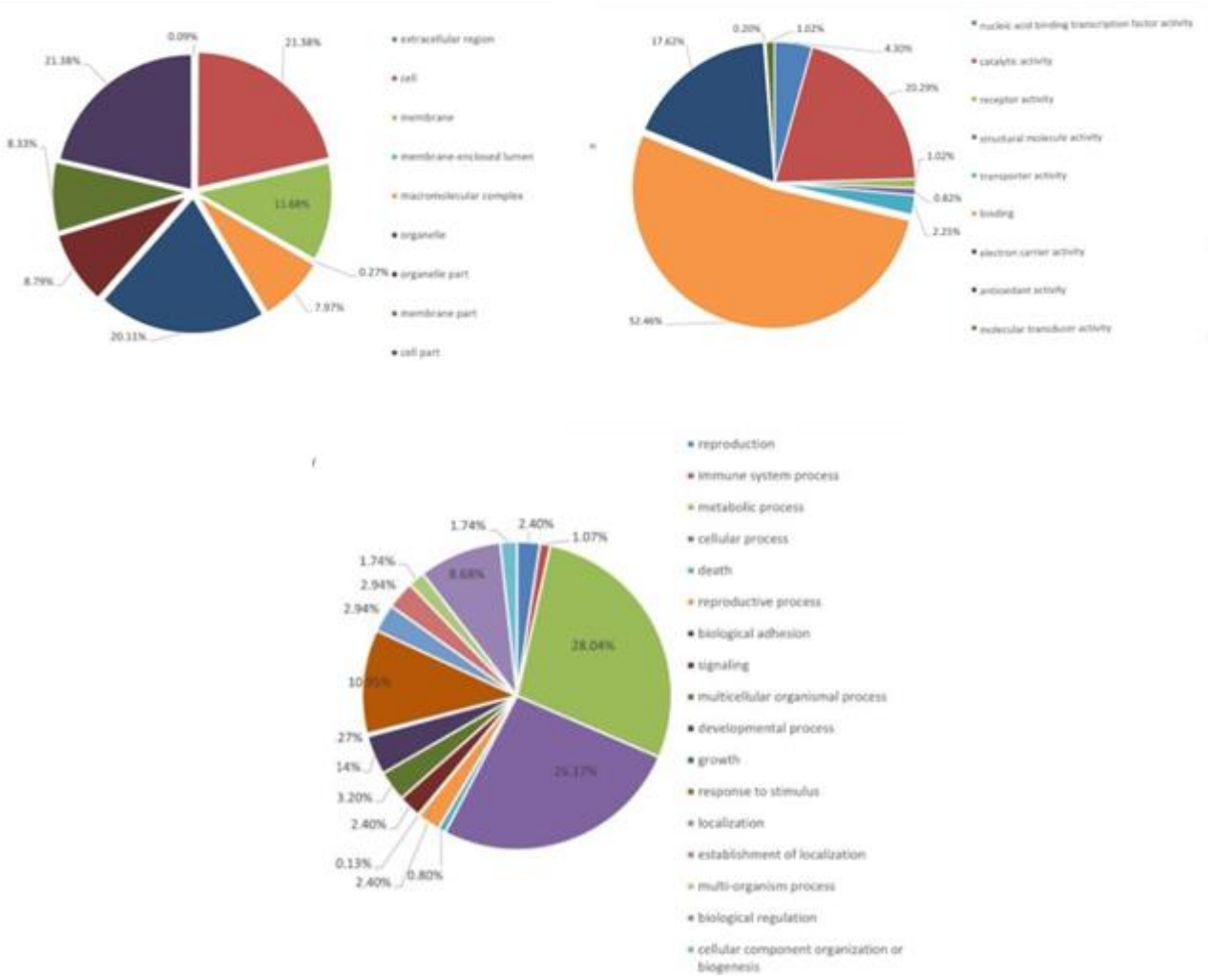

Fig.3.5. The major GO categories of «cellular component», «biological process» and «molecularfunction» forthepredictedgenesof allthe differentially expressed mi RNAs during grain development

In the KEGG database for 13 DE microRNA targets, each may be involved in various pathways, including starch and sucrose metabolism, endoplasmic reticulum protein processing, porphyrin and chlorophyll metabolism, purine metabolism, cysteine and methionine metabolism, histidine metabolism, mRNA surveillance pathway, photosynthesis, biosynthesis of phenylpropanoids, sis, phenylalanine metabolism, RNA transport, ribosomal biogenesis, eukaryotes and spliceosomes

(AppendixesK, L). From the abstract above, it can be indicated that these targets and their corresponding microribonucleic acids may play an important role in the growth and development of wheat.

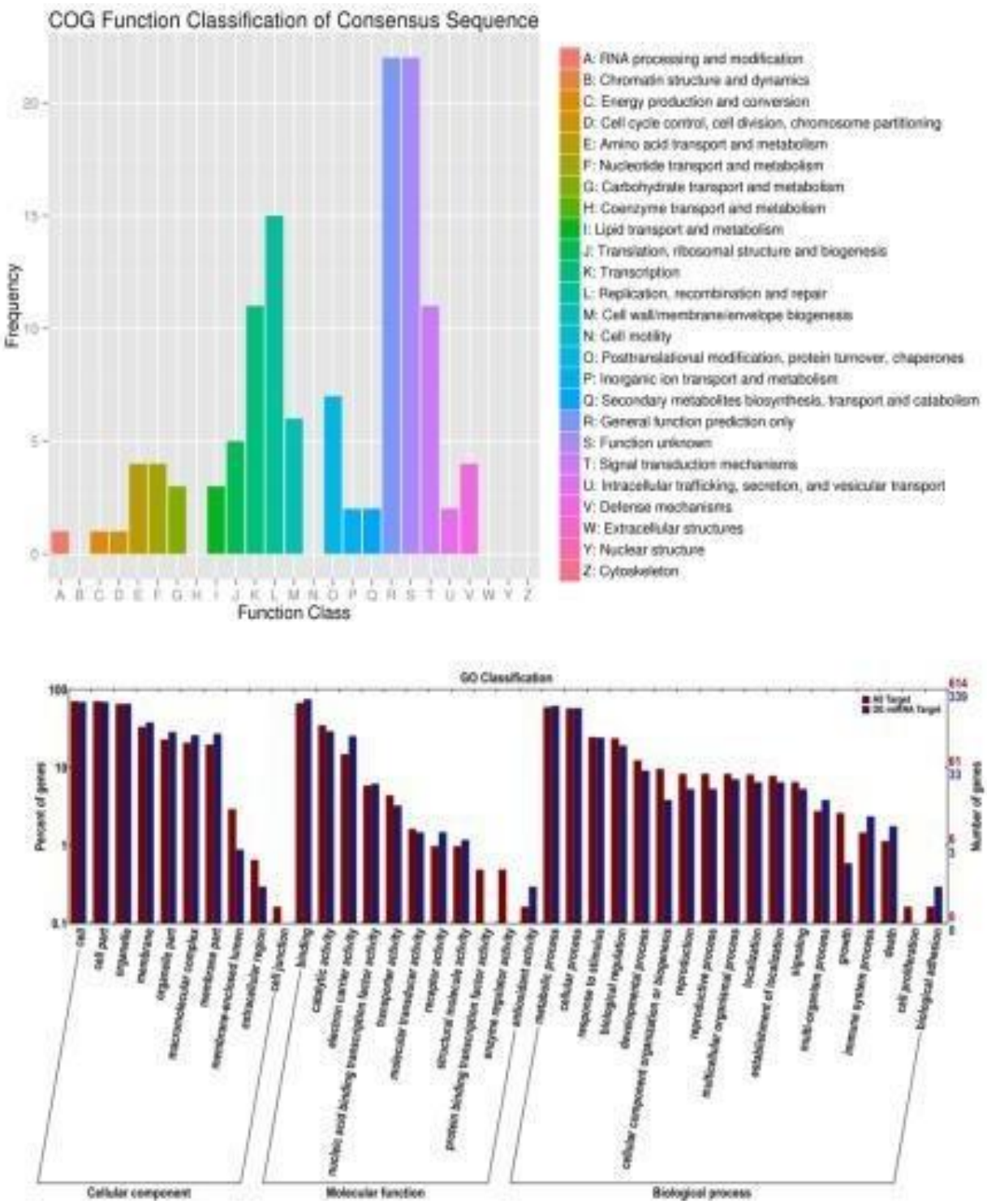

Fig.3.6. The COG function classification of consensus sequence and GOclassificationofall the differentially expressed miRNAs during grain development.

3.4.5. Degradome sequencing and datasummary

After RNA was extracted and poly (A+) RNA was purified from developing wheat grains at 7DAP and 14 DAP, a degradome library was constructed and then subjected to degradome sequencing. A total of 10,620,205 clean reads were obtained, including 4,612,965 unique reads (Table 3.8). A total of 2,776,485 (60.19%) unique reads were mapped to the reference genome. Using BLASTN searches against the GenBank and Rfam databases, structural RNAs (rRNA, tRNA, snRNA, and mRNA) were removed and the remaining reads were used to discover potential miRNA targets.

Degradomic analysis could provide experimental evidence of miRNA-mediated degradation of target transcripts, so miRNA target genes were analyzed by degradomic sequencing. The results show that the 23 predicted targets are cleaved by 7 miRNAs, including 3 known and 4 novel miRNAs (AppendixM).

In addition, most single miRNAs potentially regulate multiple targets, whereas some single miRNAs act on only one target gene. Of the 16 targets, 13 have functional annotations (Table 3.8,AppendixN).

Table 3.8

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

Tae-miR160 targets five genes, including Traes_1AL_147CF243C, Traes_1BL_54CD82AC3, Traes_7AL_E3ADC8C38, Traes_7BL_18D335F08, and Traes_7DL_5. 5ADB3528; tae-miR1119 has three targets, including Traes_6BS_04A3400AF, Traes_6BS_222CE7DA 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-miR0075and tae -miR156 targets Traes_7AS_2084DE83B, Traes_5AL_147EA9565 and Traes_6BS_542961EA4, respectively.

3.4.6. Functions and expression of microRNA targets

To understand the potential functions of miRNAs in the regulatory network of early grain development in wheat, miRNA target genes in small RNA and degradome libraries were analyzed. 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 (AppendixO).

Functional annotations for a set of target genes of differentially expressed miRNAs were performed using BLAST analysis, and the targets were found to include genes encoding mitogen-activated protein kinase, GAMYB transcription factor, WRKY 33 transcription factor, F-box/kelch-repeat protein, factor transcription PCF6, NAC domain-containing protein, ethylene-responsive transcription factor, SPX domain-containing protein, vegetative cell wall protein p1, extensin precursor, cytokinin dehydrogenase 5, calcium-dependent protein kinase and squamous bone promoter-binding protein (SPL), etc.

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 microRNAs), meristem initiation (GO:0010014), carpel development (GO:0048440), cell differentiation (GO:0030154),

cell division (GO:0051301), starch metabolism process (GO:0005982) and regulation of cell division (GO:0051302). This means that these microRNAs may play an important role in regulating grain development.

GO analysis of differentially expressed miRNAs between 7DAP and 14 DAP grains was then performed to classify their target genes according to their cellular components, molecular functions, and biological processes. A total of 10 molecular functions have been identified, of which binding, catalytic activity, and nucleic acid transcription factor activity are the three most common functions. There are 20 categories for biological processes, of which the most common are cellular processes, metabolic processes and single organismal processes. For the cellular component, 11 categories were identified, of which the three most common processes were cellular part, cell, and organelle (Fig. 3.7,Appendix P).

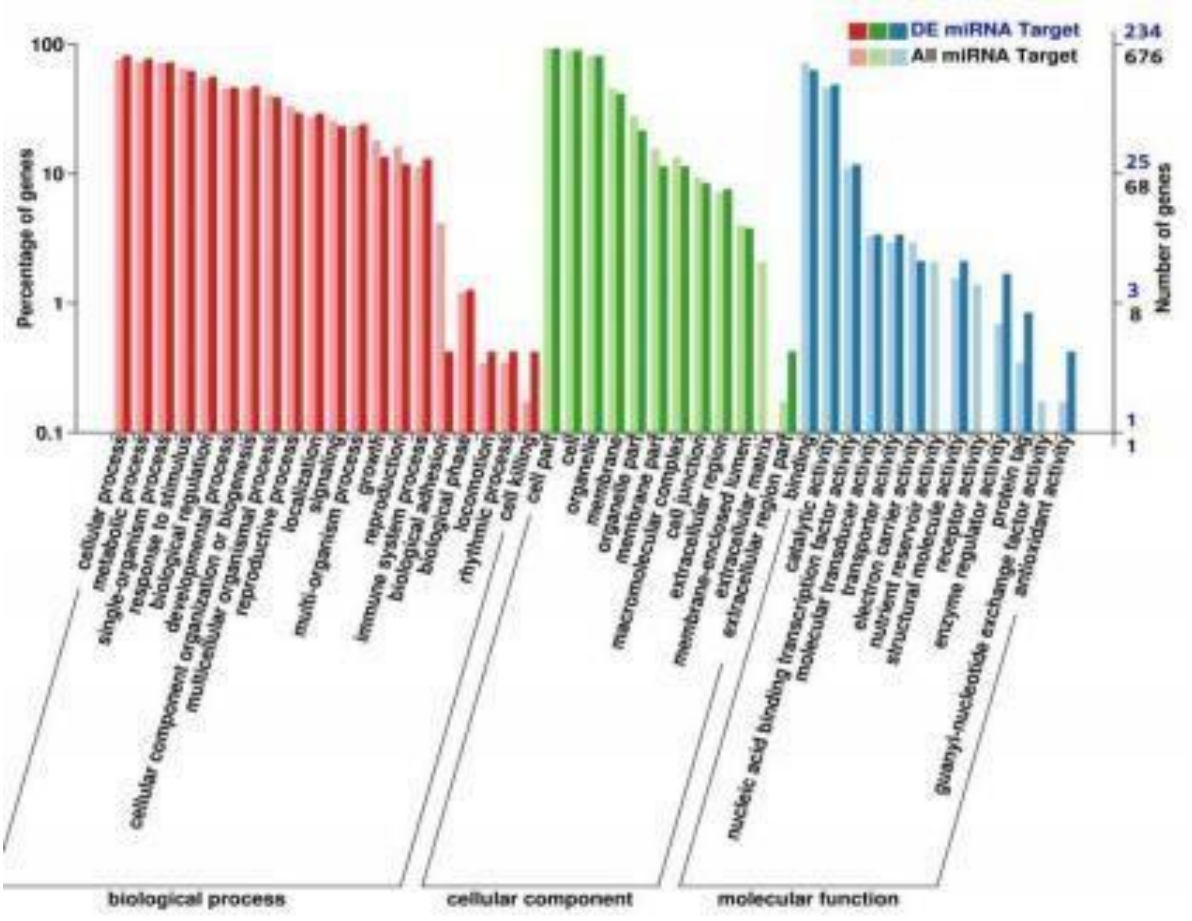

Fig. 3.7. GO analysis of differentially expressed miRNA targets from 7 DAP to 14 grains DAP

In addition, gene-specific qRT-PCR was tested to confirm the expression levels of potential targets. 8 target genes of 8 miRNAs (five known and three new) were randomly selected (Fig. 3.8).

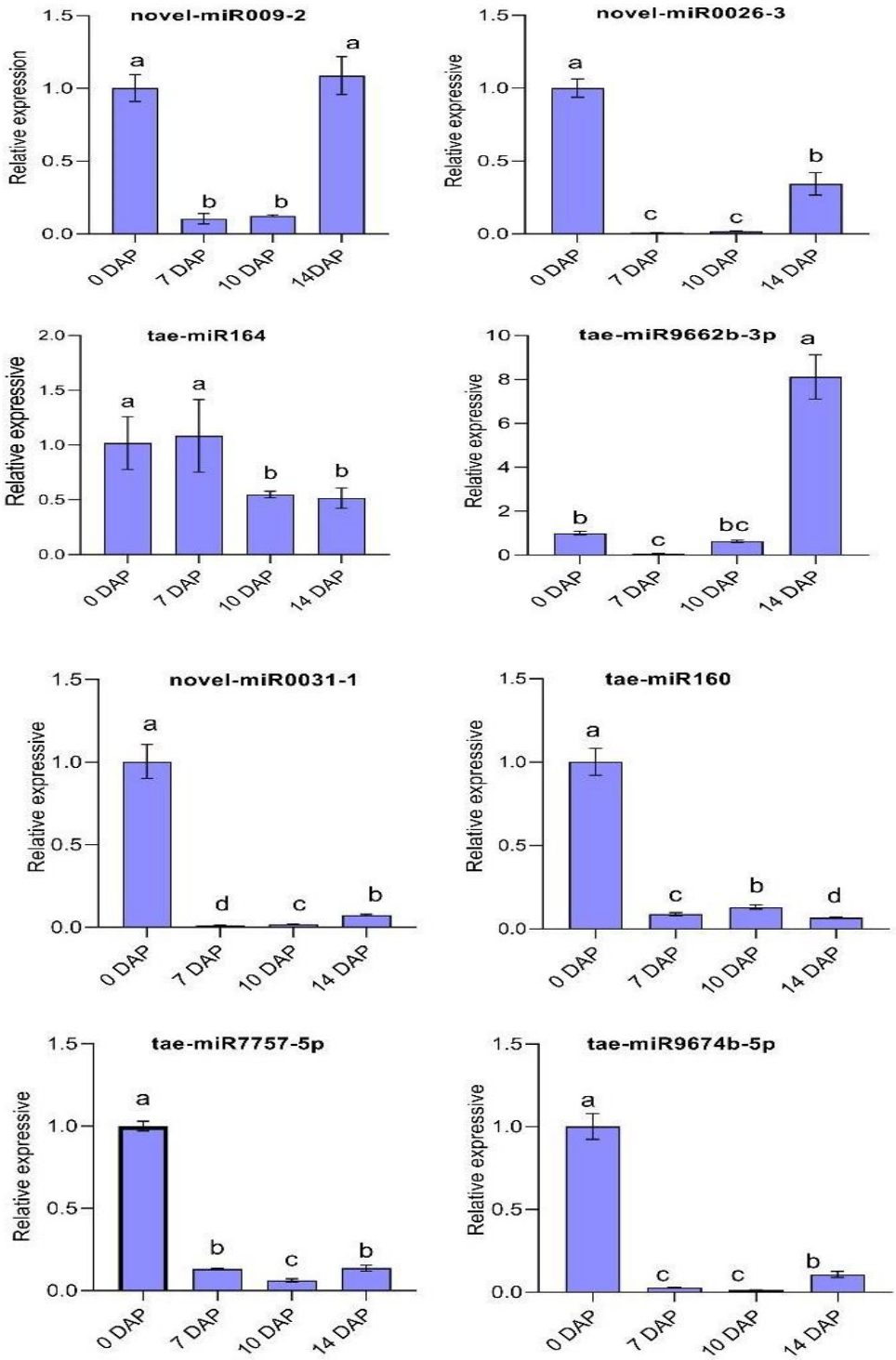

Fig. 3.8. Confirmation of the expression patterns ofpartial miRNA targetsby qRT-PCR

Combined with the results presented in Figures 3.4, the expression levels can be compared between the 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 is negatively correlated with the expression of their target genes, positively correlated for tae-miR9662b-3p and tae-miR9674b-5p and their targets, clear correlation for tae-miR7757-5p and its target are missing. There is likely a complex temporal and spatial regulation between microRNAs and their goals.

Note: MiRNA expression data were normalized to wheat U6 small nuclearRNAgene [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: stages of grain development; Vertical axis: expression levels of randomly selected miRNAs (2-ΔΔct values).

Conclusions to Chapter 3

Thus, the use of RNA and degradome libraries from 7DAP and 14DAP grains during early grain development allowed us to determine changes in microRNA content and compare microRNA expression. The results show that compared with 14 DAP, 7DAP has a broader regulation of mRNA-mediated gene expression. In miRBase/Triticumaestivum, 89 known wheat microRNAs were detected in the two RNAi libraries, of which 46 were expressed in the 7 DAP library and 87 in the 14 DAP library. A total of 16 known microRNAs from other plant species were identified in the existing two RNAi libraries, including 13 expressed in the 7 DAP library and 16 expressed in the 14 DAP library. Degradome analysis can provide experimental evidence for microRNA-mediated degradation of target transcripts, so microRNA target genes were analysed by degradome sequencing. Based on the results of degradome analysis, 23 predicted targets are cleaved by 7 microRNAs, including 3

known and 4 novel microRNAs. Understanding the functions of novel microRNAs and their targets, as well as microRNA-mediated regulatory networks involved in wheat grain development, will help us to elucidate the molecular mechanisms underlying wheat grain development and realise ingenious molecular improvements in wheat breeding.

CONCLUSIONS

1. The results of the variance analysis showed that the parental forms differed at very significant levels according to the two phenotypic characteristics of the spikelet: the number of grains per spikelet and the mass of one thousand grains, which corresponded to the principle of parental selection when creating a population for QTL mapping.

2. The results of the population normality analysis showed that the two traits were mostly normally distributed and there was bidirectional super-parental segregation, indicating that both are heritable quantitative traits.

3. According to the results of the correlation analysis, a highly significant positive correlation with a correlation coefficient of 0.953 was established for the number of spikelets and the weight of a thousand grains.

4. 143 pairs of polymorphic markers with distinct differences and distinct bands were screened for polymorphism using 300 SSR primer pairs for an F1 cross between the Mexican Large Spike and Bainong 4199 parents and the two parents.

5. 143 pairs of polymorphic markers were additionally genotyped and genetic maps were constructed for 145 individual plants of the F2 population.

6. To construct a genetic linkage map with a total length of 3128.17 cM, the average distance between markers was 25.23 cM. The average distance between markers was 25.23 cM, the maximum was 113.85 cM, and the minimum was 3.57 cM, and the genetic density between some markers was greater than 50 cM, which was mainly due to the low density of molecular markers, and more markers should be added in the next study to increase the density of the map.

7. The distribution of 145 polymorphic marker loci in groups of chromosomes A, B and D was uneven and amounted to: 77 marker loci - in the group of chromosomes B (54.22%); 41 - in the group of chromosomes A (28.87%), and only 24 marker loci - in the group of chromosomes D (16.9%).

8. 10 additional QTLs associated with number of grains per ear and 1000-grain weight were found, which could explain 4.922%~21.1044% of the phenotypic variation.

9. According to the number of grains in the ear, nine additional QTL loci were found, 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 - on chromosome 6B , and one associated locus - on chromosomes 1B, 2B and 2D.

10. At the three loci QGNS~1B, QGNS~2B, and QGNS~2D, Bainong 419 increased the number of grains per ear, and Mexican Large Spike decreased the number of grains per ear. Conversely, at the nine loci QGNS~3B1, QGNS~3B2, QGNS~3B3, QGNS~3B4, QGNS~6B1, and QGNS~6B2, Mexican Large Spike increased the number of grains per spike and Bainong 419 decreased the number of grains per spike.

11. QGNS~1B and QGNS~3B2 had large genetic effects and were major loci, explaining 21.1044% and 15.8886% of the phenotypic variation.

12. For 1000-grain weight, one additional QTL locus controlling 1000-grain weight was found on QTGW~3B, which could explain 11.4727% of the phenotypic variation, the QTGW~3B Bainong 419 locus increased 1000-grain weight, and Mexican Large Spike decreased the weight of a thousand grains.

13. Epistatic QTL loci were identified and analyzed for the number of grains in an ear and the weight of one thousand grains. As a result, nine epistatic QTL loci associated with the number of grains in an ear and weight of one thousand grains were identified. Among them, six epistatic QTL loci associated with number of grains per ear were found in chromosomes 2B, 2D, 3B and 6B, the effect values in QGNS~2B, QGNS~2D and QGNS~3B1 loci were less than zero. Therefore, the genetic effect of the epistatic QTL loci belonging to the recombinant type was greater than the effect of the epistatic QTL loci belonging to the parental type, which could explain 41.91% of the total phenotypic variation.

14. Three epistatic QTL loci associated only with 1000-grain weight were found in chromosomes 3B and 5A, among which the genetic effect values of the QTGW~3B1 and QTGW~3B2 loci were greater than zero, which belonged to the parental type of epistatic.

15. Genetic analysis showed that epistasis has a great influence on phenotypic variations in the weight of one thousand grains.

16. Factors influencing the results of QTL localization are mainly the selection of the dividing population, population size, type and number of molecular markers, environmental conditions, statistical methods and density of genetic linkage mapping markers. The above factors can be considered together to more accurately and precisely identify QTL loci associated with target traits.

17. MiRNA identification and expression analysis were performed. This resulted in clean reads after low-quality reads and removal of adapter sequences. Clean reads (tags) of 18–30 nt length were mapped to Rfam, GenBank, and RepBasedatabases sequences to distinguish rRNA, scRNA, snoRNA, snRNA, and tRNA from clean reads.

18. Targeted microRNA annotation was carried out as a result of miRNA identification and their comparison with wheat sequence databases.

19. 20–21 nucleotide sequences were collected for further analysis and obtaining degradome sequencing data, unique reads that perfectly matched the wheat Expressed Sequence Tag (EST) database were saved.

20. Wheat grain development evaluation showed that growth in grain weight and volume was relatively slow at the beginning (up to 11 DAP), increased sharply between 11 and 14 DAP, and continued to increase until about 26 DAP.

21. To identify the role of miRNAs in the early development of wheat grain after pollination, 7DAP and 14 DAP grains of the variety "Bainong 4199" were used to create two small RNA libraries, which were then sequenced.

22. The length of the pure RNA ranged from 18 to 30 nt in both libraries, with the majority between 21 and 24 nt in length.

23. The length distribution of clean mRNA reads in the two libraries was found to be 61% and 73.89% between 20~24 nt, respectively, and the percentage of mRNA with a 24 nt sequence (7 DAP was 26.79%, 14 DAP was 40.42 %) and was higher than in other sequences.

24. 89 known miRNAs from wheat were identified in miRBase/Triticum aestivum in two RNA libraries, of which 46 were expressed in the 7 DAP library and 87 were expressed in the 14 DAP library.

25. Known miRNAs including ata-miR9863a-3p, osa-miR396e-5p, tae-miR9670-3, and tae-miR7757-5p were most highly expressed, while some other known miRNAs such as stae-miR9672b, tae-miR9662a-3p, tae-miR167c-5p ta, e-miR156, tae-miR9777 and tae-miR9669-5p were moderately abundant.

26. Among 88 DEmiRNAs, 477 potential targets of 147 microRNAs were identified.

27. Among the 810 miRNA targets, 174 (21.48%) were annotated in the COG database, 605 (74.69%) in the GO database, and 176 (21.73%) in the KEGG database. However, of the 477 DE miRNA targets, only 84 were functionally annotated by a COG.

28. Both of these targets were found to include global function prediction only and global function prediction only (17.46%), replication, recombination and repair (11.9%), transcription and signal transduction mechanisms (8.73%), post-translational modification, protein turnover, chaperones (5.56%), cell wall/membrane/envelope biogenesis (4.76%), translation, structure and biogenesis of the ribosome (3.97%), defense mechanisms, transport and metabolism of amino acids and transport and metabolism of nucleotides (3.17%).

29. GO analysis revealed that some 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), development embryo (GO:0009790) (4 targets). for 2 miRNAs), meristem initiation (GO:0010014), carpel development (GO:0048440), cell differentiation (GO:0030154), cell division

(GO:0051301), starch metabolism process (GO:0005982) and regulation of cell division (GO:0051302). This means that these miRNAs may play an important role in the regulation of grain development.

PROPOSALS

1. The goal of this project is to clone the major genes that control the number of grains per spikelet in the multigrain type «Mexican Big Spike», and on this basis to study the molecular regulation pathways of the major genes that cause the number of grains per spikelet, which will contribute to further studies of the relevant mechanisms and regulatory networks , and will also provide theoretical guidance for future innovations in wheat germplasm for multigrain spikes and high-yielding wheat breeding.

2. After cloning the target gene and testing its function, combined with the vernalization chamber, artificial climate chamber, intelligent daylight greenhouse, large cold greenhouse and other supporting facilities, it can achieve the breeding of at least three generations of wheat every year (12 months), which ensures the receipt of appropriate experimental materials and the rapid creation of almost isogenic lines.

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APPENDEXER

Appendix A

Certificate

Certificate

The two parental offspring of Bainong 419 and Mexico large spike hybridized .After many years ,many new varieties of wheat were selected from their offsprings, these new wheat strains were named Xinyang 8,Xinyang 10,Xinyang 26,etc..These new wheat strains are participating in wheat approval experiment in Henan province.It is expected that by 2025.these strains will be approved by Henan province.Then they can be promoted and used to the production, they can increase income by more than 15 million yuan for farmers.The company hereby promise the above proof that the achievements were completed by the host of Chen Qiaoyan.

Puyang Xinyang Seed Industry Limited Company

June 20, 2022

Appendix B
The act of implementation

ЗАТВЕРДЖУЮ
Проректор з науково-педагогічної
та навчальної роботи
Сумського НАУ
_____Ігор КОВАЛЕНКО
«__» _грудня_____ 2023 р.

АКТ

про впровадження результатів дисертаційного дослідження
аспірантки кафедри селекції та насінництва імені професора М.Д. Гончарова
Сумського національного аграрного університету
Цяоянь Чень (Qiaoyan Chen) на тему
«Селекційно-генетичні основи ознак колосу озимої пшениці» («Breeding and genetic bases of winter wheat ear traits»)

Даним актом стверджується, що результати дисертаційного дослідження Цяоянь Чень (Qiaoyan Chen) на тему «Селекційно-генетичні основи ознак колосу озимої пшениці» («Breeding and genetic bases of winter wheat ear traits»), що представлена на здобуття наукового ступеня доктора філософії з галузі знань 20 – Аграрні науки та продовольство за спеціальністю 201 «Агрономія», впроваджено у навчальну програму викладання дисциплін «Генетика», «Селекція та насінництво польових культур», «Селекція окремих культур та сортознавство».

Результати дисертаційної роботи Цяоянь Чень (Qiaoyan Chen) щодо генетичних детермінант ознак колосу пшениці і пов'язаною з ними насіннєвою продуктивністю, молекулярної діагностики відповідних маркерів у хромосомах і відповідних можливостей і перспектив селекції даної культури використовуються співробітниками кафедри селекції та насінництва імені професора М.Д. Гончарова Сумського національного аграрного університету при підготовці і викладанні курсів лекцій, проведенні лабораторних занять, наукових досліджень для підготовки фахівців СВО «Бакалавр» та «Магістр» у галузі знань Аграрні науки та продовольство за спеціальністю 201 Агрономія.

Впровадження отриманих за дисертаційною роботою Цяоянь Чень (Qiaoyan Chen) результатів досліджень в навчальний процес підвищує якість підготовки студентів за спеціальністю 201 Агрономія.

Розглянуто і схвалено на засіданні кафедри селекції та насінництва імені професора М.Д. Гончарова Сумського національного аграрного університету (протокол №12 від 11 грудня 2023 року).

Завідувач кафедри селекції та насінництва
імені професора М. Д. Гончарова
кандидат с.-г. наук, доцент Віктор ОНИЧКО

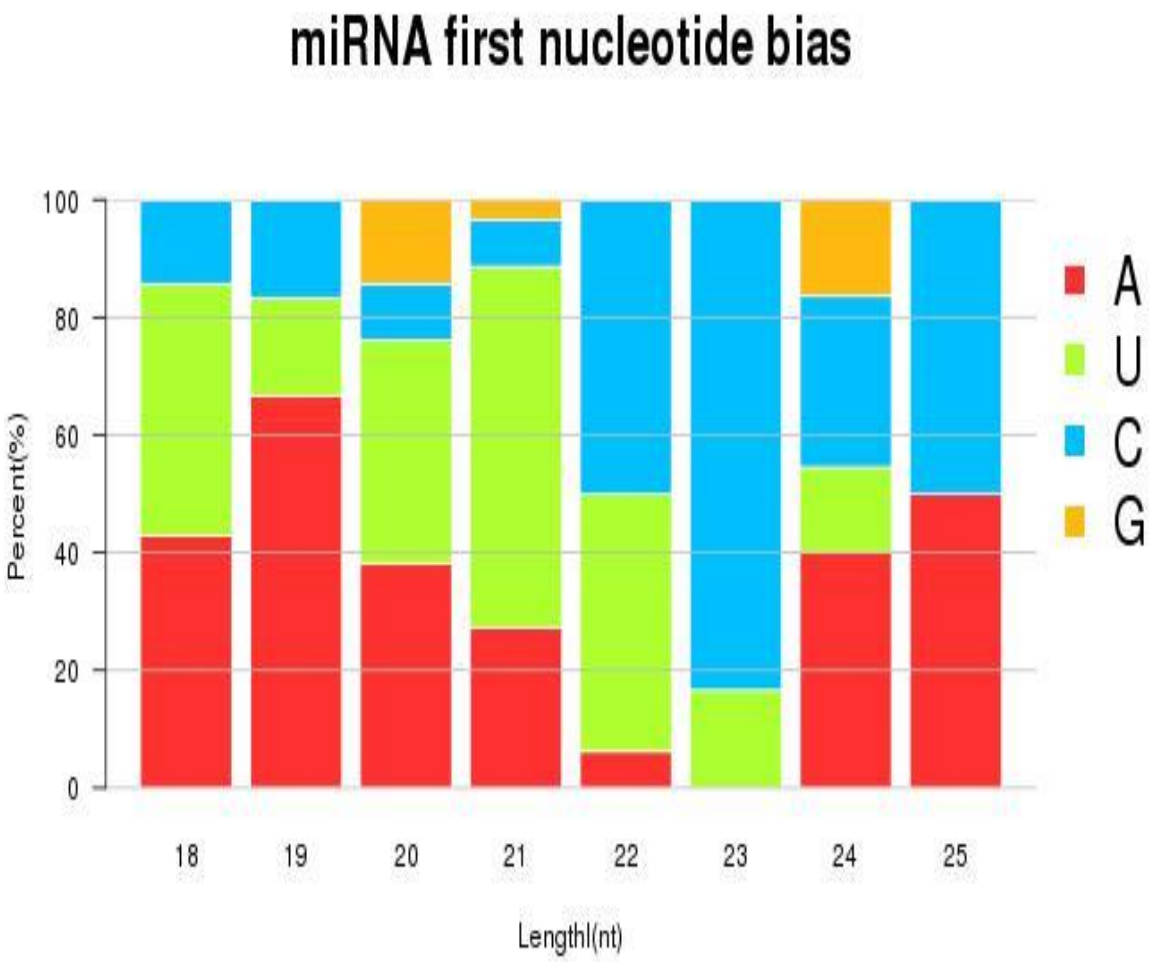
Apendix C

Primer sequences used in this study for qRT-PCR

target	miRNAname	primer	sequence
Traes_1BL_54CD82AC3	tae-miR160	L	GCGTCTCTTGCTTGTTGACAAGTCATCTGCTGCTCC
		R	GTAA
Traes_3AS_6CB1DBD28	tae-miR9674b-5p	L	GTTGCATTAGTGAGGCGAGGCATCAGCCTCAAGAC
		R	CAAGC
Traes_6BS_7F1846171	tae-miR9662b-3p	L	CAGCAATGAGAAGATCGCCGCTGTATGTGAGCAAT
		R	GCCGG
Traes_3B_7FFFF7BCF	tae-miR7757-5p	L	GCATGTGTGGCTCGTACTTAGGCCAATTTCTCGTTT
		R	GCAC
Traes_5AS_E5FD82E36	tae-miR164	L	ATAACAGTGCCAGCAACAGCCTTGTCACAGTTCAT
		R	GGCGG
Traes_1AL_D7A903FD2	novel-miR009-2	L	GTAGCTGGTGGTGGAGGATTGCCATCTCCATCCTC
		R	ATCGA
Traes_2BL_621DAFE84	novel-miR0026-3	L	TTTCTAGCCTCCGGTCGTTT
		R	CGGCAAGGAAGACACAACAA
Traes_3B_325EA6658	novel-miR0031-1	L	ATTCTTTCCCTCCGCTACCCGGGCTTGTCTGAACTC
		R	AACC

Apendix D

Most miRNA sequences showed a strong bias for auridine at position 1, and most 24-bp miRNAs showed a clear preference for 5-adenosine



Apendix E

Known miRNAs from two smallRNA libraries of 7DAP and14DAP grains

miRNA	mature_sequence	7 DAP	14 DAP	miRNA	mature_sequence	7DAP	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					

Apendix F

Detailed information of novel miRNAs from two small RNA libraries of 7DAP and14DAP grains

ovel miRNA name	D	Pre_seq	Strand	Start	End	Mfe_sco	Total score	Star_score	miRNA sequence	Star_s equence	Geno me ID	Hairpin_structur e	Hairpin_en ergy	7DAP	14DAP
ovel-miR 0001-1 ovel-miR0 002-1	ta_i wgsc_2alv16404 501_5978	cuagccgcagcuugugaaguauggaaggcgaucaaauucgag uucgagccgguagauacuauugauuaacuagauaaaacuaaag au aaagaagguauaaauaaaaaagaaacgaaauaaaaagagaaaac aaucaguuaugaaaugcaguaauucuucuuuauucuucuaauu ga uugcaauuaaacucggcuuaaucuuuuuuagaaaaaaaagau ugagccgaaucuuuacuugccgaagcacuucu	-	68 0	92 9	.1	.4	1.3	aaaaagauuga gccgaau	uc ggcuuaaucuuuuu uag	a_iwgsc_ 2alv1640 4501	...((..(.((..((((((........((((((((.((((....)))).((((((...))))))......(((......)))(((((((((((((..............(((......................)))..............)))))))).))))....)))))))). .......((((((((((((((((....)))))))))))))))))..))))))..)) .)..))......	53.1	1 717.84	5984.82
	ta_iw gsc_4asv2593027 6_20488	gugcgagcccauugccguccggcccuuucucacaucuccauu ucucgguggaggggucaagaagucuaucccggaggaggaauc c agagaagcgaggcguuguauauaucaccgcugcacauucgaga ggagaggcagaggaaggaggaggaaggaggaagaagaggguuu u gcuacguggcauggaauucauggcauaguugguucuuucuu cuuccucuuccuaccccugccucuugcucuuccuucu	-	18 0	42 9	.9	9.1	.9	aggaggaggaa ggaggaaga	uuu cuucuuccucuucc uacc	a_iwgsc_ 4asv2593 0276	..((((.....))))..(((((...(((((.((.((((((((....)))))))).)).)))))......)))))((((((.......((((.(.(((((.((.......)).))))).).).))).(((((((((((((.((.((((((((((((((((((((.........(((..(((.((((.....)))).))))))...)))))))))))))))))))).)).))))))))).)))))))))).	98.6	2 14.73	44.02
ovel-miR0 003-2	ta_i wgsc_1dsv1191 1306_4982	gccaaugguggaaguggcggagaaguggaaccagaaguggag gucaacauauuacaggugaugccugcguguaauauguugaccu c	+	95 9	20 8	.7	.1	1.3	aguggaaccag aaguggagg	ucc acuucugguuccac uucu	a_iwgsc_ 1dsv1191	....(((((((.((((((((((((((((((((((((((((((((((((((((((.(((.....))).))) ...)))))))...	117.9	(.00	0.37

		cacuucugguuccacuucuccgccgcugccccaccgucca ggcuccagccccacucccccgucacucccuccuacgcuccucug cag gugcucccccaucacucccccuucuccuccauggcuacug caggcuucucuccucucugaccgcugcuccauccauca									1306	((..((((...........((((.............(((((((((((.........))).........((.....)).....)))))))..............)))).))))..)).....)			
ovel-miR0004-2	ta_iwgsc_7asv14191171_37829	ccaaauacauacgaccuccuagaauuuugagccuaacuuugac uaacaaaauaugaguuauaugcuguaaaaaguauacuauuggaa u ucuuauucgaaagagguuuccaauaguauaagucuuauaacau acaacucauauuuuauuaguuaaauuuaaaaucgaaguuagcu c aaaacuugagggggcucucuuuauuucgacagagauaguacc uucugugagguauuauaaguuuguggguuuugcuc	-	545	794		.3	1.3	auuuugagccuaacuuugacu	uc gaaguuagcucaaaacuug	a_iwgsc_7asv14191171	.((((..((((...((((((.((.((((((((.(((((((((((.((((((((((((.((((.(((((....(((((((((((..((...((....))))..))....)))))..)))).)))))))))).))))..........))))))))))))).)).))))))(((((.((.....)).))))(((((((((....))))))))).....))))..))))...	83	.00	22.01
ovel-miR0005	ta_iwgsc_6blv12921472_32514	agaaauuuuuaaagguaaaaauaugaauuuugaauuuugcaa augggggaccuucaugaagccaggccuccaaaagcaauuuccgag a uugguuucuuuauucagauuccuuggauccguagacauacg auauguacugcaccacgaagaagaacauauacuuccucuguccg g aaauaaaauacuugucggagaaaugguccgacaaguauuuccg gacggagggagcauaugcacuccgcuacuacgcu	-	34	83	.8	.1	1.3	caaguauuuccggacggagg	ucu uccggaaauaaaauacu ugucg	a_iwgsc_6blv12921472	....(((((((....)))))))(.(((((((.(..(((...(((((. (((..........))).))))))...))))..).)))))).))((((((.(((.(((((...))))).(((((((((...))))).))))).....))))))))((((((.((((((((((((((((.....((((((((((.....)))))))))))))))))))))))))).))))))....	81.7	3006.23	438.33
ovel-miR0006	ta_iwgsc_4dlv314357443_22780	augaugccacacaauuugauccacacgucgagaaacaagaaug acaaauucugcucugguaguagcuaauucaaauucagaggugaa aauaguuuugugcauuauuaauauuaacagcagaauuucucau uuuuguuucuucaugugugaucuauuuuguuucuuguaacuu uguggcaucguaguugaaccuugugaacauaaauuuucuuaug	+	464	713	.8	.3	.9	cacgucgagaaacaagaaug	uuu uuguuucuucaugugu	a_iwgsc_4dlv314357443	((((((((((((...(((.(((((((.((((((((((((((.(((((((((((....(((((..(((((.....((((((.((....)).)))))).....)))).)))))..)))))))))).)))))))))))))..)))))))....))))....))))))))).....((((.(((((.....)))))(((((.((((.....)))).))))).....))))..	71	.00	0.18

		accuuuuuuuccuuugaaaugggguucuuaucacuu													
ovel-miR0 007-1	ta_iw gsc_5bsv1226086 4_28356	ccaucccccugcauacggcucggaggugggagguuguuggcug guggcugaaaugaaaauagaggagggaggggcgguggagcug g uugauggacaugcggcgguggcggcaaaggggaccaaaguc uuuauauuuuaggacuuuugucccuucgccgccgccgccacc uc ccauccaucccucaacuccgccgccucucucucuucaauuu cagcccccagccaacaaccuccuaguucccaaccgugug	+	43 1	68 0	.9	8.8	.9	ggugggagguu guuggcugg	agc caacaaccuccuagu ucc	a_iwgsc_ 5bsv1226 0864	..........((((((...((((.(((((((((((((((((.(((((((((.......(((((((((((((((((((((.(((..((((((...(.((((((((((((..(((((((.((((((((. ....))))))).))))))).))))))))))).)......)))))) ..))).))))))))))))))))))))).))))))))).))))))))))))))))).)))).. .)))))))	166.4	.00	43.11
ovel-miR 0008	ta_i wgsc_3alv14448 119_13495	acccgcguucgugcacauugcuauaugagacaguuaguaaua auuugguuugaauuugcacugcuaggggcggcacugccgcac u gcccggucaguguaagcucauuacacuguccgggaagugcgg ccguacugcaagcaguguaaacucaaaucauaucauuacuauu u guuuuauuuuuuauuauuugccacacuaugcaaauuucauga guacuaucuacaauauuuuuuugacauccuucacaug	-	40	89	.3	.6	1.3	uaugagacaguu aguaauaauu	uca uuacuauuuguuuua uuuuu	a_iwgsc_ 3alv1444 8119	.......((((((...........(((((((((.((((((((.(((.((((((((.(((((((((((....((((.(((.((((((((.(((((.((((((((.....)))))))).))))).)))))))).)).)))).)))))))))))).))))))).)).))))))).)))))))))........((((((........))))))..))))))..................	93.5	.00	0.55
ovel-miR0 009-4	ta_i wgsc_2asv15291 774_7109	ucuuguggguuaggguccugugaucaggagagaugacaccg acgccgaucggaugggucggcuuaaccaggccaugcauccguc u ggccggaguuggugucaccucgccugaacauagggcucacua ucccugucaaagcaccagcuucagaggagagcacugcacaggu g auaaugcaacugcagcugagcaccgccuccucuuccuccucg cguuuccucuugcaggucaggucagcuggagaugcgg	-	11 88	14 37	.7		1.3	ucaggagagau gacaccga	gg ugucaccucgccug aaca	a_iwgsc_ 2asv1529 1774	..(((.(((.((((((((((((.(((((.(((.((((((((((.(((.((((((((((((((((....)))))..).))))))))))).))).))))))))))).))).))))).)))))))).).))) .)))...))).(((((((((..(((((((.((..(((.(((.((. ....)).))).)))..))))))))).((((((.(((........))))))..)))..))))))...))))..	114.4	.00	0.73
el-miR0 010-6	ta_i wgsc_7blv167 47143_39914 ta_i wgsc_5dlv144	aagcaguagguuuucaagugaaaaacacucaguagguuuucu uuuugugagauauuuagcaugucaggguucaaguuuggcuga	-	90	15	.2	.5	1.3	a uuguuggucaaa	ca aguuuggcugaca	a_iwgsc_	....(((.(..((((....))))..))))...............(((((((((((..((((..((.(.((((.(((((	103.8	.00	27.50

	ca auuagauuaauaaaauguuguuuauuugacaaagaaaaugaua ucauuggguuuguacugaaaagcuuuuuccaaugguauuaucu c uuugucauauaacccacauuuuauugaucuaauuguugguca aacuugaaucuugugaugcaugaugucuugcauaa		2	1				cuugaaucuug	auu	7blv1674 7143	((((..((((((((((((((...((........))....))))))))))))).))))))))).)))).).)))))))))))))))))))))))))))))))))..))))..)))))))))))...			
	gucuuacugauauguagcugggucugucugcacgggcuuau caggagaggcaaccaacauggucucuaauuggaagcauuuuu g cucauguguagaguuuuaguuccaacaauauuacacucauaa ucagaucuuacaaaccacuuuugcaaacuuaguccuggcacua ua aauuuaaaaaugugcaugugaaaauaugcucauuauaaauuug uaguguucggauugaguuuauaauagucguucuaa	+	69 9	94 8	.3	.6	1.3	a uuuguaguguuc ggauugaguuu	acu uaguccuggcacuau aaauuu	a_iwgsc_ 5dlv1449 5350	......((((((((.(((((((((((((....)))))))...))).(((((..((((...))))))))..((((((....((((((......))))))......))))))...... ....))))).)))))). .......(((.(((.(((.((((((((((.((((((((((((((. .((((.((((((....)))))).)))).))))))))))))))).))))))))))).))).))).))))....	77.1	2 14.73	44.94
	uaauuucuuguacuaauugaggucccagcaagguacauggu acaugcuggcugcauccccuucaacagguguccguccuccac cg aguucaccugauauuccuucuggauccuuuuaugcuucuucu gcaacuuuuguuagcaugcaucaucauuuuguugguugcuuu g cugauuuguggcagcaaccagugagaugaugaugcaugcuaa caaaagauguaggaggagcaaaaugggauccaaaaggu	-	58 3	83 2	.8	.1	1.3	(augcuaacaaaa gauguaggagg	uuc ugcaacuuuuguuag caugca	a_iwgsc_ 7dsv1384 317	...............((((((..((((((..((((...)))).)))))).........))))))((((((.(((........)).)..))))))).....((((.((((((((.((.((((((((((((.((((((((((((((((((((((((((((((((((((((......)))).))))))))))))))))))))))))))))))))))).))))))))))).)).)))))))).)))).	120	(.00	41.28
	ucuguccucagacuaguaguuuccaacuccgacaacauuacua agggcaugugauuauugauguggcuauuaaucaagagaaagca a ugccagagagcuuuagcaacggaaccugauuaucauauuuacc ugcuguugaagcucgucuggcauugcuuucucugaauaguag	+	29	78	.9	5.5	.9	(caacuccgacaa cauuacuaagg	uua gcaaugcuguuggag uugg	a_iwgsc_ 7alv1448 1774	...((.((((((((.((.((((((((((((((((.((((.(((((((((((((((((.((((..((((((((.(((.(((((((((((((((((((((((((((((.(((.. ....))).))))))))))).))))))))))))))))))))).))))))))..)))).)))).))))))))))))).)))).))))))))))))))))))..))))))).))((((	136.3	(.00	82.56

		u cuuguuauuaaugcacaugcccuuagcaaugcuguuggagu uggaagcaccgggucugagcaccgguucagcugaag									...))))...			
ovel-miR00 14-1 ovel-miR00	ta_iwgsc_7blv1 6749817_39957 ta_iwgsc_3alv1 4448716_13502	cuugaggaggaaggcgaggaucugcuugggccggugcuccgucu ccuacaccuggcgccacaucuccgccacgcucuccucggaggcug ucugcucccuccucagccggaccaucccccaucaccagcaucgugc ugcugcucuccccaucauccuccuccuuguucuuaccguccuug ccgccgcgcuugccaacgcucuggcgccggacgaugcggggcag gaggugcaucuggcuguggaggaagaag	18	06 7	.8	.3	1.3	cggacg augcgg ggcagg aggug	ccuc cuccuuguucuuaccg uccuugc	ta_i wgsc_7blv16749 817	.((((((((..((((.((((.((...(.(((((((...........)))).))).))).)))).))))....)))))))).(((.....))).(((((.((((((((..........((.(((((...))))).))..........((.((((((((((((......(((((.(((((..(((........)))..)))))..)))))..)))))).))))))))..)))))))).)))))......	97. 7	29.46	0.55
15-1 ovel-miR00 16-1	ta_iwgsc_4alv2 6019133_1885 8 ta_iwgsc_5bsv1 2254293_28306	ugggcugaaaaccaucggcccccgugugggcgacugcuaucagaau cacuuuagggaggauugcagccgugacucacauuaugacu acaacuacuuaugcaaaaguucauggacgucaugagaaccgcauuu gggaggcguacaucguccaugaacuguucccaugaguaga uguauucaucauacgagucaucgguggaauccucguagauguggu ucucauagcagucgcccaugcgggggccgaggc	51	20 0	.9	.2	1.3	cgugu ggcgac ugcuau cagaau	ucu cauagcagucgccca ugcggg	ta_ iwgsc_3alv1444 8716	..........((.(((((((((((((.(((((((((((((.((((((((.((.(.((((((((.((.(((((((((..((.((((.((((.((((((((((....((((((((((((..(((....((.... ..))....)))....)))))))))))))......))))))))).)))).)))).))..)))))).))))).))))))).).)).))))))))).))))))))))))))))).))))))))))).	13 4.6	.00	00.91
ovel-miR00 17-1 ovel-miR00 18-3 ovel-miR0 019	ta_iwgsc3bv11 0775495_16427 ta_iwgsc_6alv1 5834455_31604 ta_iwgsc_2asv1 5241552_6842	uacuuucccgcaaacuuuuucccuucauuuuauuucccgccauuu ucuuugccgccauuuucuuucccuccauucucuuccgcc acacuaaggagugcuacaucgacggaggaugacgaugaugccuucau guguagugugaaacauugugugagagaagucauaagugu gaaacacucuacgaugaugaugacgaagcugcaaacacuuaugacu ucucuuagacaguguuucacacuccaccagcagc	77	02 6	.9	9	.9	uuaug acuuc ucuua gacag	gu gugagagaagucau aagug	ta_ iwgsc_4alv2601 9133	....((((((.((((((((.((... .(((((.(((..........))))))))........)).)))))))).))((((.....))))(((.(((((((((((((((.((((((((((((((((((((....((..((.((..........)).)).).))...)))))))))))))))))))).)))))))))))))))).))))..)))).	72. 8	14.73	04.57

ovel-miR0020-1												
	ucagcuagagaacaagaguucagauggugcucaguuuaauuucacugcucaaggaccauggacauauuuuauauuaugucccaaauuuuuuguguccaccuugagcagugaaacuaaacugcgcaacauuugaagcuuguucucuaccugaaauuccugacgauguuuugucaagagcuccuuauuguuggucuuaucuaccagugucaucgagugugacacuugcuuucgguacaccaaaaaugacaaa	473	722		.4	1.3	auggugcucaguuuaauuucacu	ugaaacuaaacugcgcaacauuug	ta_iwgsc_5bsv12254293	((((.((((((((((..(((((((.(((.((((((.((((((((((((((....((((((((........................))))))))))))))))))))))).)))))))).))).))))))).))))))))))).)))).(((.((((((....)))))).)))....(((((.(((((..((((..(.((((((((.....)))))))).).....)))).))))).))))))....	100.1	.00	00.91
	cggcggccgucggugcagaaguucagggacgagcucgauggcggcgauuuggugcggcuccgcucgauuugaugcuccggauggacgaaguugacgguggguuguccuccuggucgcggucucagggcuuggggaggccgguggccgcgucuguggaggcggcauggcgacggugucgcuuggguggggcgagcagaggaggucgcagcauaggggagaggaaggauaggc	382	631	.2	.7	1.3	cggccgucggugcagaa	gagcucgauggcggcga	ta_iwgsc3bv110775495	..((.((((((((.((....(((....)))..))))))))).))....((.((((((((((((((.((((.(....((.((..((((..((((.(.(((.(((.((((((((.(((((((.(..(((((....))))).).))))))).))).))))))))))).)))))..))))..)).)))).))))))))...))).)))))).))....................	94.4	576.77	.00
	aagagcagaagcacgcaguaggagucgacgacgucccgcaugucgcguagccggaucgccaugcuccugccgcuccggacgcggcgccagagcgugcuccgguggguaggagucgacgaggccggccgcgucgacgagcgucugcaccacggggccucccagcuccgagcagagcagcacggcgccgcagcccgucggccacuugagcagcgcguggagccugcggcugcaccgcucgagcuuggcgcacu	711	960	.7	.3	1.3	cguggagccugcggcug	gccgcagcccgucggcc	ta_iwgsc_6alv15834455	....(((((...........((((((...))).)))(((.((((((((.(((((.((((...((((((((((..(((((.(((.(((....)))))))))))))))))))))).).)))..))))).))).)))))).))))))))).....(((....))).((.((((((.((((.(((....((((((((((.((((..........)).))))).).))))))))))...))))..)))))).))...	115.6	58.92	0.18
	uugaguuugcauugcccgggcagcucugccgcagcugcccggacaguguuacacuguuugggcagcugcgguagugcugcuagugcauuguaauucagaucacaucauuauuauguaucauauuuuugauaguuuagcauuuuaugcaaaguuuguuuuauuaauacauaaaguuuuguaauauuuuuguccggucagcguuuucguuguuucaaauucaugcaucauauuaagaaggucaauuuuuuga	1	30	.7	1.4	.9	cucugccgcagcugcccggacag	guuugggcagcugcgguagugcug	ta_iwgsc_2asv15241552	.((((((.(((.(((.(.((((((.((((((((((((((((((((((((...)))))))))))))))))))))))).)))))).).))).))))))))(((((...((.(((.((((((((((......)))))))...........(((((.((((((.........((((........))))).................(((((....)))))...))))))..))))))))).))))).))))..........	88.6	.00	41.28

ovel-miR0021	ta_iwgsc_7dlv13377298_41627	gcgucgcuguaagccauccgauccggcacggcuugcagccggaacaaaacugcugaccggccuaacccuaggucacuuucuccccugacccccucucgcguuuagcgcuacugagagcgacgacgacguucaacgguggcccaaggcacaccggccaaggcggcgcagcggcugcacucaccggagcagcgugccguggacuuaguccggcgcgcuccuccgucgagcguggcugcugacgcugcgcgcg	3132	3381	.8	.1	1.3	uccggcgcgcuccuccgucgagc	ucaccggagcagcgugccguggacuu	ta_iwgsc_7dlv13377298	((((((.((((((((.((((...))).).))))))))(((((..((.......)).)))))........(((((..........)))))...((((((.(((((.....))))).)))).))))))))....((((((((...))).)))))((....(((((((((((((((.(((..(((((.((((((((..((((...))))))))))))).)))))..))).)))))))))).))))))..)).	121.4	.00	41.28
ovel-miR0022-3	ta_iwgsc_4blv16981217_21187	ugaucgaggaguugcuuggguugaugaccauauagacaaccucagccuaugcguggucugcccauugggccauguucaccacgcaaauaggacaagugugcaccaacuauggugccaucucuccaagcucaugaagccaggaauaccacugaguacuuccauaagaugaccaguuacccgaugaccagcauggugucggcguaaccggucaucuuguggaaauauucugugggcaccguguguuucucuu	797	046	.4	.7	1.3	caucuuguggaaauauucug	gaguacuuccauaagaugacc	ta_iwgsc_4blv16981217	(((.(..((((...((.((((((............)))))).))((((((((((((.((((...))))...)...))))))))..))))....(((.((((((....)))))))))..)))).).))).((((((((.(....((((.(((((.((((((((((((((.((((((((((.((((...)))))))))).))))).))))))))))))))).))))).)))).....).)).)))))....	105.9	29.46	01.83
ovel-miR0023-2	ta_iwgsc_2dlv19872788_11101	accgaguguuaagcuuaagcacucccucuguacacaaauguaggauguuuuugcaguuuaaauuaaacugcaaaaauguccuacauuuguuuacagagggaguagaugguugaacaauuuaagcaccauuauaacauugcuagcauuggaggucucucucuaagcacucaugcagguguaaauucaggccuaaauucuguguugggagcuucuuugucuguauauuuugcaguacuaaucaaauguaucu	729	978		.9	1.3	cccucuguacacaaauguag	auuuguuuacagagggaguaga	ta_iwgsc_2dlv19872788	......((((.((((.....(((((((((((.((((((((((((((((((((((((((...))))))))))))))))))))))))))))).))))))))))))....)))).)))).....((((...........)))).(((..(((((.(((((....(((((......((((.(......).))))).......))))))))))))))).)))(((((.....)))))...................	90.8	.00	0.55
ovel-miR0024-1	ta_iwgsc_6bsv12999099_34032	aaguguauugguaaguacucccucuguaaacuaauauaagggcauuuagaucacuaaagaucuaaacgcucuuauauuaguuuacggcgggaguagaaauuagugguaggaauagaguaagaaaauggaauaacaauuuguugcuuaaauacuacacaauuccuuugauauugcugguaagucaacaugcacauacuugaaagaacacuuacuucaaacaaagucuggacggaugauaucgucgggga	217	466	.1	99.8	.9	cuguaaacuaauauaagggc	ucuuauauuaguuuacggcg	ta_iwgsc_6bsv12999099	.....(((((((...(((((((.((((((((((((((((((((.((((((((......)))))))).))))))))))))))))))))).))))))))...))))))).((((.....((((((.(((.((......)).))).))))))).....)))).......(((((...(((..((((((((.......((......)).......))))))))))).)))))(((.((((........)))).))).	84	576.77	047.61
ovel-miR0025-1	ta_iwgsc_5dlv14575265_29704	gcuugcucccuguggcggccguugagacgagaucuccccauaccacauagcggcagcucggcuccgugcacgcguauggggagaucucgccucaacagccgcuccgugugagcuuuaccuguacguaugac	101	350		.3	1.3	gagacgagau	auggggagaucucgccucaac	ta_iwgsc_5dlv14575265	(((((.((((.((((((((.((((((.((((((((((((((((......((((.(((...))))))))......))))))))))))))))).)))))).))))))....((((((((((..(((.((....(((((.(((......((((((.((((.......((((((............))))))).......)	100.9	.00	62.38

		gaugcuuuugguccuuuccugcaucgcuuguugguaacgcgcucuccucuacuaguugaucuugcaugagagguagccuuguucaguccagccgagaguucacugcugggauuaaugcu						cucccauac			))).)))))).))).)))))..)).)))...)))))))).)).)))).))).)).			
ovel-miR0026-1	ta_iwgsc_2dlv19907038_11391	acguacgacccuaguuagaguuuggacgaggaugugcagcugcgguaggacucucagcuacgaccggcagcugcacauccacguccaagcgcuagcuaggaucgucaacaacggccggcagcaggacauccauguacguggagguuaaucuugauaggcgacgccgaguucaggaaggagaaccugacgggggguguucgagguggagguggcgcugucgggcgaggucagguaccaggcgcacuccagcg	657	906	.7		1.3	ggacgaggaugugcagcugc	agcugcacauccacguccaag	ta_iwgsc_2dlv19907038	....((((.((((((((.((((((((.((((((((((((((((.(............).))).))))))))))))).)))))))).)))))))).)))).((..((.((((((((..(((.(((((....))))).))).(((((.((....((((((....(((((........)))))....))))))....)).)))))...))))))).))..))..........(((......)))	114.9	.00	01.83
ovel-miR0040	ta_iwgsc_5dlv13183779_28677	auguaugaccaacaacaaccacagcagcagguccauaacgcaguggguagcucuaccacugcgcuauggacccacugcuuuugcuguuguuagagcaauugacugcagaucaauaggucaccgguucgaacccgguugggcccuauuguuuucuuuuguaauuuuauuucuuugucaacuacggucuuggccuuccacacuugcgucacagcuauucacuuucaaguuuuaaccauaucuagcuuuauuc	440	689	.9	2.5	.9	gcagcaggucauaacgcagugg	acugcgcuauggacccacugcuuu	ta_iwgsc_5dlv13183779	..(((((.(.(((((((.(...(((((..((((((((.((((((((((....)))))))))).))))))))..)))))...).))))))).)(((...((((.((((..(((((((((((((((.......)))))..)).))))))).........................(((((........))))).........))))))))..)))...................)))))............	71.9	.00	88.05
ovel-miR0041	ta_iwgsc_4bsv14945417_22357	uucaagcuuuuugcaagaauucagcggaaaaaaacaucagaggagcucucggcaaaaaaaacaaaaucaauguucuaaguuugcgcacuguuugagcacuguuugagcauugauuuuguuuuuuuugugagagcuccucggauguuguuuuuagcugaaacuuugcaaaaaguucaaacauuugaugaagcuuguugucucaagauuucagauuuuucggaccuuguuuuaucacauuuuucgaaguucc	378	627	.2	.1	1.3	gcggaaaaaaacaucagagg	ucggauguuguuuuuagcuga	ta_iwgsc_4bsv14945417	....(((((((((((((..((((((.(((((.((((((.((((((((((((.((((((((((((((((((((((.((...(((.((.....)).)))...)).)))))))))))))))))))))))))))))))))))).)))))).))))).))))))..)))))))))))))........(((((((((..(..((((.((((........)))).)))))..))))))))))..............	103.1	.00	0.73
ovel-miR0042	ta_iwgsc_6alv15785293_31177	acauguuccauucucggacaccaagcucauaauguuauuuagggguacgugauaaccaaaauaccacaugucauuucuauacaucuuccucuuaucaguacuuagcucaaagcuuaugucucuucaauggaauuucagcaaugugcuacucuauuaaugaaugcucccuccguucggaaauacuuguccuagaaauaguuguauuucuaggacaagua	660	909		.3	1.3	ggacaaguauuucggac	guucggaaauacuuguccuag	ta_iwgsc_6alv15785293	....((((((((...(((((..(((((......((((.....(((((.((((((..............(......)..............)))))))))))))))....))))).)))))....))))))))...((((...))))........(((((((((.(((.((((((((((((((((((((((((((....))))))))))))))))))))))))))).))).)))))))))........	83.2	14.73	46.77

		uuuccggacguagguaguauuuauuuuuuauga						gu						
ovel-miR0043	ta_iwgsc_3asv13328771_13886	ugcacaccaaccucucuucuuucuucuuagagaggggcacacacaauucgcagagagagagagaaauugagaaauagagagagagagagugagggagggagggaggggggagagagagcaacaaugccuuggggaagcauccaaagggaucgcauugauccuuccauccauggguguugauggcugucaucaaucaaccggcucgacagaucaugcgauccuuuuggaagcuuccucuagccauuacuccac	064	313	.8	5	.9	ucaugcgauccuuuuggaag	uccaaagggaucgcauugaucc	ta_iwgsc_3asv13328771	......((..((((((((((((.(((((...............((((((.............).)))))...............))))).)))))))))))..)).((((.(((.(((....))))))((((((((.((((((((((((((((.((((..((...((..((((((((((.....))))))).)))))...))..)))))))))))))))))))).))))))))).........))))..	88.8	.00	44.02
ovel-miR0044	ta_iwgsc_7alv1297285_36285	cuaugauuuuuguugcagauggcaaagugagcucuuugccgucugugcuuuuuuugcagacggcaaaguuuauuuugccgucaguuuuucuuugucgucug		01	.5	.3	1.3	aaagugagcucuuugccgucugu	agacggcaaaguuuauuuugcc	ta_iwgsc_7alv1297285	..(((((.......((.((((((((((((((((..(((((((((((.........))))))))))))))))))))))))))))).))........)))))...	43.6	14.73	62.38
ovel-miR0045	ta_iwgsc_7alv14536617_37090	agccucuuuuagcauauuuuaucaagaacuucuauuuuguuguagaguugcaugugacguuacagcgaagugccgaagaucuagcauuuuuauauguuuucuaaaaauaugagagaaacaaguugaaaguuuuacaacauucgugcuggaagagaagcgugugugcauucccaugauauaauuuucauuuuaugaauguagacuacgcaacgagaaaugaacggguuuguuuuucaaauauuacaacauc	539	788	.3	.9	.9	aagaacuucuauuuuguuguaga	acagcgaagugccgaagaucuagc	ta_iwgsc_7alv14536617	...((((((((((((.........(((.(((((((((((((((((((.(((......))).))))))))))))))..)))).)))....(((((.((((((((...)))))))).)))))....((((.........))))....))))))))))))..((((((.((((((((..((((........))))).....))))))).)).))))...((((((((((....))))))))))..............	61.9	.00	0.55
ovel-miR0046	ta_iwgsc_7blv1571312_38763	uuauaagcccugaguguagagacaaugaagggagaggcgcagugcucaccagcauguuucuuaccuucaagccuugaggaaguggcguagacugcacggagcuaugaggguuuuggacaagaacuacacauagcuccauguagucuacacuauuuccucaaggccugaagcuaagcaacgugcaggugagcauugcgccu		04	.9	.2	1.3	aaggccugaagcuaagcaacgug	uguuucuuaccuucaagccuugag	ta_iwgsc_7blv1571312	.......((((...(((....)))....))))..(((((((((((((((.(((((((.((((.(((((.((((((((((((((((.((((((((((.((((((((((..((((((.....))))))...))))))))).))))))))).)))))))))))))).))))).)))).)))))))).)))))))))))))))	124.7	14.73	392.62
		cuguuguccaaccggcuauggacacaaccucugaguccaagaac									.((((((.((.((((((((((.(((((.((((((((((((((((((((.(((((((.((((			

ovel-miR 0047	ta_iwgsc_3asv1 3388517_14007	ucuguuuccuugaacauaguacaccugugguuaagggcgcag aguucuuggacucagagauuguggccauagccggcugcacaaca accccuuuuuguguggucugcuuacgugguuuccgccucgu acuguauaacaaauguuucaucucauuggaaacaucuuuggaag gagacgggcacgcccuuagacugcuacugccaccg	812	06 1	.6	.5	1.3	acaacc ucuga gucca agaac uc	guu cuuggacucagaga uuguggc	ta_ iwgsc_3asv133 88517	(.....))))).))))))).))))))))))))))))))))).))))).))))))))).)).))))))..........(((((((((....((((...((((.(((.(.(((........(((((((.........)))))))...))).).))).))))))))....)))))))).))........	11 3.7	.00	21.10
ovel-miR 0048	ta_iwgsc_7blv1 6691063_39345	uaauuuuccuuccaaguaaacccucuacuuuucauucacaaaau uguaaggccgggggggagggggggaaagcccccucccccugac uacacuaagcuccgccauuagugaguuguuucaauuucaacccc auguguccugcaugcgggagcuuguaguacauguaaaucaua gaaaaguaaaaaauguuguccuuucuauguugcagguuuccuua gccuagacugugguuggaagaccggcgauuacac	201	45 0		.3	1.3	agguu uccuu agccua gacug ug	caug uguccugcaugcggg agcuugua	ta_ iwgsc_7blv166 91063	............(((((.......)))))............(((((.(((((((((((((((.....)))))))))))).((((.((((((.........)))))))))).......((((((((.((... (((..((.((.((((((((((((..........((((((((.((((......))).).))))))))))))))))))))).))))...))))).))))))))..)))))..))))).	82 .6	006.23	432.98
ovel-miR 0049	ta_iwgsc_6asv1 4350518_31819	caucugguggcaaugagaagucauaucauaugacugagcaauga aaugccaucuguaauguuucgggacugccucaccauugccuu ucaacuguggaugauuuugccauuuguacuugugaagaauguuc auacgugcugcaacaaaacagauguuauuugugauguugcgg ucauggauuaaacuacaucauagacaagcauaugucugaauuuu cuuauguggaacuuuguguguuucuuauaagugu	0502	07 51	.4	.7	1.3	auau caua ugac ugag caa	gc cuuucaacugugg augau	ta_ iwgsc_6asv143 50518	((...((((((((.....((((((.((((((((..(.((((((....((..((((........))))...)).....))))))))..))))..)))))))))))))))))))).......((((((.....))))))((((((((((((..((((((...))))))..)))))))))).))))(((........))).((((((......))))))......((((((.(((((.......)))))).))))))))).	64	582.99	642.06
ovel-miR 0050	ta_iwgsc_1alv2 3976442_839	ggcauggcauguaucccacggccauggccaaggccucugaggu cgcgugaccuuuuaggccucgcccauggcuaugguguucaug gucuuauuuguccaucagaucuaucuaaaucaccaaagucuguc uggaucuuucauguaugucggccaugcccagguauuguucu uuuugaucuacucccuccgucccgucccaaauucuugucuagau acaucuauguucagaaaaauuuaagacaagaauuuu	1955	220 4	.6	.9	1.3	augg ccaag gccu cuga ggu	u uaggccucgccc auggc	ta_ iwgsc_1alv239 76442	((((((((((((.((.(((.((((((((.(.((((((..((((((....))))))..)))))).).))))))))).))).)).)))))..........(((((.((((((.................))))))..)))))...............)))))))..((....(.((.....)).)....))........ .......((((((((((((((((...(((......)))...)))).)))))))))))).	79. 4	29.46	66.95
ovel-miR	ta_iwgsc_1dlv1 2289383_4330	ggcauggcauguaucccacggccauggccaaggccucugaggu ucgcgugaccuuuuaggccucgcccauggcuaugguguucau	906	15	.8	.1	1.3	auggc	u uaggccucgccc	ta_ iwgsc_1dlv122	((((((((.(((...(((.((((((((.(.((((((..((((((.....))))))..))))))).).))))))))).))).....((((.(......).)))))..((((((((.....................)	70	294.61	359.50

0051		ggucuuauuugucuaucagaucuaguaucuaucuaaaucaccga agucuggauccuucauguauguugcguugacggccaugccc agguaaauuuuuuuuagaucuauauaagucgcucaauuuuuug cuaguucccuuuuaaagagcaaacucuaacagauccu		5				caagg ccucu gaggu ucg	auggc	89383	))))))....(((((...)))))..)))))))))))................(((((.....(((.((((........................)))).)))).....))))))..	.7		
vel-miR0 027-1	ta_iwgsc_7ds_v 1_3958882_430 31	cugcgcgcggcuaauuaaugggucgccgggagccggcgaac cgcggcgggggaugcgagaucccagcgggagagagcccgcc gga cucgccaaggaaacgccuaccuccaugcggggaaucgccucg agguaagaagcaaagagcacaugcauucaacugaucuaaaucc ua aucccccacuucucaucagcccagaaaugaaugcgugugcu cuuuccuucuuacccucgagccgacccccggagccgg		72 4	973	.7	5.9	.9	u gugcucuuuccu ucuuaccc	u aagaagcaaagag cacaug	ta _iwgsc_7ds_v 1_3958882	.......(((((......(((.((((((((...))))))).))))....(((((..(((((.(((..(((((......))))).))))))))..(((.....))).))))).. .(((((..(((.((((((((((((((.(((((((((((((((((.(((..((.............. ....))..)))...))))))))))))))))).))))))).))))))).))).))))).))))).	11 6.8	.00
ovel-miR 0028-1	ta_iwgsc_2al_v 1_6416280_606 3	ccauggcggcaugguggccccguucgucggacgagcgugc cuaggcaucggccgcgcgcuccuccgucgaacggcaccugc cgu gucgcacgcaugcaguuaggcggcggggaaaggggaugcgu ucgccggugcugcugcggcauaaggaggugacggcaacgcgu uu ggcacgguggccuugcacgucgucguccauggguguggc ggcccggcgacuugaucuuccgcgcgacuucuccauggaga		17 0	419	.8	7.2	.9	u ucgucggacgag cgugccu	c gcgcgcuccucc gucgaac	ta _iwgsc_2al_v1 _6416280	(((((((((((((((((..(((((((.((((.((((((((...(((. ...))))))))))).)))).))))))))..)).))))))))))..(((..........)))(((.(((....((((....(((((((.(((((..(.((((......(.((((((.(((.((..(((... ...)))..)).))).)))))))....)))).)))))))))))))....))))))).))).......))))))...	12 8.4	503.11
ovel-miR 0029	ta_iwgsc_5dl_v 1_4571082_296 89	ucgaccgugcuguccccccgacgguauccuaugggcuau ggcgacgcgcgugccucucucucucucucccuucuuggcug gcu ggggugaucaugcaugcacaugcugccaugcaucucgaccc auccucauauacuagcacacuuguacugcuuacucccucugu ac		65	214	.3	.2	1. 3	u ucuuauauuuug ggacaaag	c uguaccaaaauau aaaaaca	ta _iwgsc_5dl_v 1_4571082	......((((((((......))))))))).....((((....(((...)))..)))).................((((((((...((((....((((((((((.....)).))))))))....)))).......... . .((((.........))))((((((((.(((.(((((((((((.(((.(((((((((...)))))))))).))).))))))))))).))).))))))))))))))))........	76. 5	.00

		caaaauauaaaaacaauuuuaacacaauguuaaaaucguucuu auauuuugggacaaagggaguacuaguuaacuacuacu												
ovel-miR 0030-1	ta_iwgsc_5ds_v 1_2782660_307 71	guguuacuuugaaucuuauagguauuucacaggaaacaguuc aaugucuaccgaacugaaagaguuuuuccugaccauggcuuuu c uagucaauaggaauacaccauguguuggaagacaaacuucuu ugcagcaucuaguagauguugaacuguuuccucugaaauucc ug uaaauuucaugaguaguuucguauuccuuugaaaaaccuaua ugagacuucguaguuuuuucaaaggcucaaaauaug		00 13	0262	.2	53.6	.9	uu gaacuguuuccuc ugaaauucc	ua uuucacaggaaaca guucaaug	ta _iwgsc_5ds_v 1_2782660	...((((((((((..(((((((.((((((.((((((((((((((((((((.((.((((((((((((((((.((((((((..((((((.....))))))....))))).)))))))))...)).))))).)))..))..))))))))))))))))))))).)))))).)))))))..)))).))))))...((((((((((((((((....(((((....)))))..)))))))))))......))))))	92	.00
ovel-miR 0031-1	ta_iwgsc_5ds_v 1_2761097_305 88	uuuguuacuuuuauuugauguuuguauuaucuagaaccuu gcaagauucaaacaaagcuaucaauaauuaugauguuaacuugc au uugauguuucuuaagaaacaucaaauacaaguuaacaucauaa uuauugauaaguuuguuugaaucuuccaagguucuagauaau ac aaauaucaaauacaaguaauaaaacguaugcuuuguaugagag acaaaaugagauacacuuauuggauauacaucu		84 9	098	.8	.2	1. 3	u uguauuaucua gaaccuu	g guucuagauaau acaaau	ta _iwgsc_5ds_v 1_2761097	((((((((((.(((((((((((((((((((((((((((((((.((((((((((((((..((((((((((((((((((((((((((.((((((((((((....)))))))))))) .)))))))))))))))))))))))))..)))))))))))))).))))))))))))))))))))))))))))).))))))))))....((((((.....)))).)).((((((.....))))))............	12 4.8	.00
ovel-miR 0032-2	ta_iwgsc_3al_v 1_4435088_134 19	cauugccauugaggccgccacacucaugacucagcugaaugc cuaucuugccuucauguacgccacgccaagucacucaccucu a ucgccaucgugugauugaggggagggacuuaccauggcguac auagaggugagauggacguuuagcuggguccugagcgugggg c cuccaugguuggucgccauuaaugaauagcuuggagugcauc aaugggucaauacguugcguggauguauagauguuggg		87 7	126	.8	.1	1. 3	u uuagcugggucc ugagcgug	cu caugacucagcug aaugcc	ta _iwgsc_3al_v1 _4435088	....(((((.((((((.((((.((((.(((((((((((((((.((((((((((((.((((((((((.(..(((((.(((.((((.((((((......))))).)))).))).)))))..).))))))))))).)))))))))))).))))))))))))).)))).))))))))))).)))))......(((((.(((.((........)).))).)))))(((.(((((((.....))))))).)))......	12 5.7	.00
	ta_iwgsc_7as_v1_	cuaacaaaauuugugaguguuuaaaaggcaugugucuacaaucug							aa	agg	ta	..........(((((((((((((.(((((((((((..((((((((((((		

ovel-miR0033	4123870_37669	uaaaaaguucaaaucaaaauucgaaacacacaucgagaaacaaaaaauaaaaauuuaguaugaauagugccacaaaaagccaaaagccaaaugguacuauuuacguucagauuuguccuuuuuguuucccuugugcauuuugaaauuuugaguugaacuuuuuacagguugucaacauauaugucuuaugaacacucauaauua		260	509	.5	.8	1.3	aaggcaugugucuacaaucugu	uugucaacauauaugucuuauga	_iwgsc_7as_v1_4123870	((((((((.(((((((((((((..((((..(.((((((((((....((((((....((((((((((((......((.....))......))))))))))).....))))))....)))))))))))..))))..))))).))))))))).))))))))))))))))))))....)))))))))))).)))))))))))...	95.1	.00	
ovel-miR0034	ta_iwgsc_1al_v2_3947972_580	ugcgccagaaacauauuuguaucguccccacgacaggugaacaaggcagugacggcacggucuagcaagguuagaagucccuuggcauguucuuccuuccaaaaaucugauguauuuguguuagucaacacuccaaaaaauuaauggagugcauauacucccuccguuccuaauuauaagucuuuguagagauucaaaucucuacaaagacuuauauuuaggaacggggggaguaguaaaagaugaaguc		253	502	.7		1.3	aagacuuauauuuaggaacgg	guuccuaauuauaagucuuug	ta_iwgsc_1al_v2_3947972	.........((((((..(((((((.......((.(((.(((((.(.(((....((.((..((((((...)))))).)).))))).).))))).)))))........))))))..))))))......((((((.........))))))....((((((((((((((((.((((((((((((((((((....))))))))))))))))))).)))))))))))))))))..............	96.8	.00	
ovel-miR0035	ta_iwgsc_4al_v2_7174831_20292	ccuaagcguuuuaaagauuauuugguuuucgugaugugccuaaugcaccgguagggagggagugguacaugcuuacagcuuagggauggaugcuuuuauaauagcaugauggauauggagagcgaaguaguggugcaggggcaccauggugcccuuuauuuugaaaaaaaauaaaacauauuuagaaguucuaaaaaauucaaaaaaauuauguggaaacuuguauguguucaccauggauucguaaaau		526	775	.3	.6	1.3	aauuauguggaaacuuguaugugu	auauuuagaaguucuaaaaaauuca	ta_iwgsc_4al_v2_7174831	(((((((((.......(((((((..(((((.....((((.....)))).....))))).)))))))........)).)))))))).....(((((........))))).............(((((...((((((..(((((((....)))))))((((((((.....)))))))(((((((..((.((((((((...((((......))))...))).))))).))))))))))))))...))))).....	62.2	44.19	(
ovel-miR0036	ta_iwgsc_4bs_v1_4729575_21772	gcacgucuuuggcgaccagcaaacggagggcuaaaaccuguagcuuaaagaaaaaagauagguguugcucuguuguuugcuuugagguuuccccuaguuggggagaccgaga		24	73	.6	.5	1.3	acggagggcuaaaaccuguagc	gauagguguugcucuguuguuu	ta_iwgsc_4bs_v1_4729575	...((((...))))...(((((((((((((....((((((..(((........))))))))...))))).)))))))))....(((((((((.....)))))))))....	43.1	717.84	1
ovel-miR0037	ta_iwgsc_6dl_v1_2950520_34565	gugagguccgcuccaaauuucaacucauuuggauaucugaacaacucucggcaaaaacgacaaucgggucaaaacaguucgugaacaauaacuuuuuuacagaccccgauuuugucuuuuuacagagagcugcucaaauguccaaacgaguugaaauuugcaccgaaccucacgcaccaaauuaucuaccacacaaaaaaaugggaauuugaugaag		910	159	.3	.7	1.3	acucauuuggauaucugaacaacu	cugcucaaauguccaaacgaguug	ta_iwgsc_6dl_v1_2950520	(((((((.((...((((((((((((.(((((((((.(((.((.(((((.(.(((((.(((((((((((((((((.((((.((.....)))))).))))...)).))))))..))))))))).).))))).)).))).))))))))).))))))))))))...)).))))))).................(((((.....((((((((....))))))))).....))))	76.7	.00	

		uuuucuacuauuuguuuugauuuuguuucgucg										).................		
ovel-miR0038	ta_iwgsc_5al_v1_2757370_24456	acacaauuagucuauaaacucgagaaucauaguagagcaaaacggcaagucaaagaaaaguuuucauaagacugaagacuaaaacaugagaacuaucuucccaaggguuaaaccaaagcagcaaggaguaauucucauguuuuaaucuucaagucuuauggaaacuuucuuugccuugccgcuuugcucgaguaugauucucagguuuauacgcuacuuauguuuuaucguccugcugaucauuucauua		085	334	.3	5.1	.9	agaaucauaguagagcaaaacgg	cgcuuugcucgaguaugauucuca	ta_iwgsc_5al_v1_2757370	(((.((.((((.((((((((.((((((((((.(.(((((((.((((((((.((((((((.((((((((((((((((((.((((((((((((((.(((..(((.....(((.......))).....)))))).))))))))))))).))))).))))))))))))))))))))).))))))).))))))).).))))))))).)))))))).)))).)).)))...((((......)))).........	105.3	.00
ovel-miR0039	ta_iwgsc_5bl_v1_3540269_27988	auuuauuacacucuuguuaaggagauggacuaucacuuugaguguucaccugucucgagcagagaacauucaaagugauaggccaucuucuuaaugguggaugccgguguuuuuuguuguuguuuuauuucaugcuuggcaacaac		3	12	.2	.5	1.3	agauggacuaucacuuugaguguu	cauucaaagugauaggccaucuuc	ta_iwgsc_5bl_v1_3540269	.......(((((...((((((((((((.((((((((((((((((((..((((.....)))).))))))))))))))))))).)))))))))))))(((....)))))))).....(((((((((.............)))))))))	59.8	.00
ovel-miR0053	ta_iwgsc_2bs_v1_5198356_9507	aaugcuucucuugcuauucacuguugagaccucuuggugugguuguaaacuuguaaugaaacgaaucgcaaagguggccggcaaccauuagaggaagguuuaggguucgaaugagcuuaaauaaauuaaacccucuguucuuaaauauaaaacguuuugauaguuuaaauacucccuccgucccauaauauaagaacguguuaaaaacguucuuauauuuugggacaaagggaauauuuugaaacaaguaac	+	571	820	.7	0.5	.9	ccgucccauaauauaagaacg	uucuuauauuuugggacaaag	ta_iwgsc_2bs_v1_5198356	..((((((((..((........)).))))((((((((((.((((((...((((....((((......)))...))))...))))))))))..))))).(((((((((((((....)))))........))))))).((((((...((((.......)))).))))))...((((.(((((..((((((.((((((((((((((.......)))))))))))))).))))))..))))).))))........))))...	70.5	.00
ovel-miR0054	ta_iwgsc_5bl_v1_10919911_27652	acggcgacggcgguuccauccaccucgagaugaacggcgacggcagucccauccaccucgagguguacggcaauggcguccacauccaccucgagguguacggcgacggccgucccauccaccucgagguguauggcaacggcgucccgguggagaaccuagccgcagcug	+	993	242	.8	.7	1.3	ccucgagaugaacggcgacggc	cgucccauccaccucgaggug	ta_iwgsc_5bl_v1_10919911	.(((.((((.(((...((((.(((((((((.(((...((.((((((.((((((...((((((((((((...(((......))).....)))))))))))))...))).)))).))))))))...)).))))))))).))))...)).)))))))((((((...((.(((...(.((((((((((...(((..((((..((((((.....))))))......))))..)))))))))))).)..))).))))))))......	123.1	.00

		gucgccggcgcaccuccgcugcacgccccacgugccccc cacggaucuguggcgaccaccgaggugagcucugccua ccu											
ovel-miR 0055	ta_i wgsc_2bs_v1_5 197055_9481	ccuuaggcccaguucuuuuggccgauucucccagaauuuu gagaaucgagccucccccagcuucucccagaaucuugaaca uaua uuucuuuuacaauucuagccaguuaugauguaaacagcuagg auugucaaaaaaauauggguucaagauuccgggagaaguuga gug gaagguugauuuuuaagauuccgagagaaucgaccaaaa gaacugggccuaaucacaguggaacacaggucccccgga	-	107	356	.8	.1	1.3	cg auucucccagaa uuuugag	ua agauuccgagag aaucgac	ta_ iwgsc_2bs_v1 _5197055	..((((((((((((((((((.((((((((.(.((((((((((((((.((((((((.((((((((((.((((((((((.(((((((.(((.(((((((((((..(((........))).))))))))))))..)))..)))))))..)))))))))).)))))))))) .).)).)))))))))))))))))).).)))))))).)))))))))))))))))) ....((.((....)).)).....	130. 2	.00
ovel-miR 0056	ta_i wgsc_2bl_v1_8 024098_8304	ugcgcgugacgcuggagcuacgcucgccggaggagcgugc gggacucgguccguugcacguugcuccagcgaggcuagcc accg cgccgccaugggccagcgagccucgaagccgccgcucggu cgccgcuccucuggagagagcgagugaaucgggaguggac ggggga aaugaggcuaggguuugacccaguggcaguuuugcucca cuccggagcgauuggagccaucggcucgauccgauggacg aa	+	451	700	.8		1.3	c ucgccggagga gcgugc	a cguugcuccag cgaggc	ta_ iwgsc_2bl_v1 _8024098	....(((..(((.(..((((..(((((.((((.((((((((((((...)))))..)))))))).))))).)))))..))))..).)))..(((((((((.((((.(((((((...(((((((((..(((((((((((....)).)))))..))))).....)))))).))).....)))))))...))).).))))).))))....(((((((.....)))))))......(((((((.....)))))))))..	121. 5	44.19
ovel-miR 0057	ta_i wgsc_2as_v1_5 241042_6839	ggcagauucaugcacccuagcagauaagauagauaguaaua augugauuugacuuugcauugcccgggcagcucugccgcag uugc ccggacagaacacuguccgggcagcugcgguagugcugcua gugcauuguaaauucagaucacaucauuauuacauaucuuau uuu ugauaauuuagcauuuuaugcaaaguuuguuuauuaaauac	-	0376	0625	.5	.9	1.3	gau aagauagauagua auaaugug	cau cauuauuacauau cuuauuuu	ta_ iwgsc_2as_v1 _5241042	((((((....(((.....)))(((((((((..(((((((.(((((((((((.((((((.(((.(.((((((.((((((((((((((((((((....))))))))))))))))))))).)))))).).)))).))))))..)))))))))).)))))))..)))))))))......(((((.((((...)))).)))))..........((((........)))).....)))))).....	109. 3	.00

		auaaaguuuuguaauauuuuuguccggucagcguuuua											
ovel-miR0058	ta_iwgsc_1bs_v1_3442002_3022	ugggcacguaguggacgacugcggggagugaaugccguuuggggaccucaaccaugccgacgcgucaucaugcaagugcaugaccugcagacgacacugcagcuuccaccgcaaaggugaugaugggagaggucgccgccgugcgguagcggcggcgaccucuccaucaucuucuugcggugcaagcuguaguggcaauaguaggccgccccacuuguaugacgaugcgucggcgggguucagaucccccaa	-	2704	2953	.9	.2	1.3	gcccacuuguaugacgaugcg	cgucaucaugcaaggcaugaccu	ta_iwgsc_1bs_v1_3442002	..((((((((((.....))))))).....))))....(((((.((.((((.((((((((((..((.(((((.....(((((((((((.(((((((((.((.((((((((((((((((((.....)))))))))))))))))))).)))))))))).)))))))))).....)))))..))..)))))))))).))))))))))).))))...)).)))))...	162.1	.00
ovel-miR0059	ta_iwgsc_1bs_v1_3428948_2970	uggcggauccauugguguacgccgccuuugccugcguuuccaggggguggucguacuacagucugccaacguacgccggcgauguacuaggccuaggcguggugugcaagugcaugucaauuuuggucguccccguacgacuuugccauggcggauccacuggccgccuggaacgccaccgacgggacgccgugagggcgggguucucggugcgacggaaguuugacgcccgcuggugcagcgagugggagg	-	927	176	.3		1.3	gcgacggaaguugacgc	gucaauuuuggucgucc	ta_iwgsc_1bs_v1_3428948	(((((((((....((..((((((((((((.....)))))))))))..)))))))))).(((.((((((((...(((((.((....)).)))))((((....)))((((((((((.(((((.(((...(((((((((((((((..(((.(((..((..(((.....))).)).)))))))))))...)))))))))..))).)))))).))))).)))))))..)))))))..)))......	105.4	29.46
ovel-miR0060	ta_iwgsc_7dl_v1_3341310_41122	aaaaaugcaaguaaaaaaguaaagugcaaccuugugaaaaaaaaacguaaaguugcacuuuucauuagauugaaguugcacuuuccugaaucgaucgggaaaaucacaguccacuagacauacaucgaucaugaaaagcuggugguaagggaagcuacgaauggcuucguuuuugucaugucgacuagcagauucgugaagauugccaccucacggguauguagcucagaugguagagcgcucgcuuagcaugcga	-	286	535	.6	.9	1.3	gguauguagcucagauggu	caugucgacuagcagauucgu	ta_iwgsc_7dl_v1_3341310	....(((((((.........(((((((((.(((((((...((....))).....))))))).)).....)))))))((((((.....)))))))).....(((((((.....(((.....))).....(((((((....)))))))((((((((((.(.(((((.((.(((((((((.((....)).)))))))).)))))).)))).)))))....)))).))))....	68.3	1165.99

ovel-miR0061	ta_iwgsc_3b_v1_10686187_15724	aagagggagccaguuauccgguuuaauuuguccauagcauccgcgaucccgccauucagcugugauagcugcugcccuauugcacguugcggaagauagcuggugcuauggauaaauucaacccgaugguugugcaugcccgaaugaaagaagaauccugucuugaagcucgacugagccaacagucuguuugaagcagggaucucggucaucugcuugaauuuuucuguauuauuaucauacaaccugc	+	85	34	.5	5.2	.9	gugcuauggauaaauucaacc	uuaauuuguccauagcauccg	ta_iwgsc_3b_v1_10686187	....(((.((((((((((.((((.((((((((((((((((.((.(((((((.....((((((((((........))))))))).))))))))..))).)).)))))))))))))))).)))).))))))))).))...)))..((((..((.((.(((((.(((.((((..((((((......))))).)))).)))))))).)))))..)))).............(((((.......)))))......	86.6	.00
ovel-miR0062	ta_iwgsc_2al_v1_6345017_5515	agcacgggaggugacugcucacaggggggauccaaauccaaaucccgcaccacaucacuuauugcgguggggaagauugcaacuauaccuggccaucugucgggccgggccuaccaaagcccgaugcaaaaaacccaggcccgagcccgacccagcccagccgucgagccuaaaaaucaagaccgagcccagcccauagcgcuaaaagccuguugggccuugggccgggccucuucguuaacucaugaaua	+	292	541	.5	.4	1.3	guugggccuugggccggg	cgagcccagcccauag	ta_iwgsc_2al_v1_6345017	...(((((((((((.(((....(((((((....))))...(((((.((((.((........)).))))))))((((.((...........)).))))(((((((((((((......((.....))........))))))).)))))))....)))))).)))...............(((.((((((((((((.(((...)))))).))))).)))))))))))))).)))).)............	95	29.46
ovel-miR0063	ta_iwgsc_4bl_v1_7036829_21561	uaaauccaaccggaugguugcgacauacccgguugaaggaagcuuugcucccgcgcgcauccaggccggugggugcagagugcccugguggcggccccagccgguccaucgucgaccgcggggagccaauccucugguuugacuuguccauagcaucauccauccugccauucugcugagguagcugcugcccuuuugcacgauggguagaauggcugguguauggaugaauucgaccagauggcuagc	+	148	397	.8	4.7	.9	uagaauggcuggugcuaugga	auagcaucauccauccugcca	ta_iwgsc_4bl_v1_7036829	......((((((((((.......)))..))))))).(((..(((((((.(((((..((......))..))))).))))))))..)))..((((..((((.((.((((.......)))))))))).)))))..(((((((((.((.((((((((((((((.((((.(((((..(((((.((((..((....))))))..))).))..))))).)))).))))))))))))))).)).)))))))).)).....	120.6	14.73
	ta_i	agcgugggucuuccuugcacucuauuuggaggauaugcaag	-						uau	cau	ta_	.(((.(((((.(((.(((.((((((((((((((((((.((((		

ovel-miR 0064	wgsc_4al_v2_71 70245_20131	augaguaugauuuaucaucuguauucaucguguauauucuuc aag uggagagcauggaugacugauguauuuuuuuggagcuauac ucggauacccgagaguggaucucgguaaacgugaaguugccg aga guagcccugggguaaagauaaggccaccaucaaaauuuag ucccaccucgccuauagacaagcaacacgaccuguuuaa		622	871	.2	1.9	.9	uuggaggauaugc aagaugag	cguguauauucu ucaagugga	iwgsc_4al_v2_ 7170245	((((((((.....))).))))))))).))))))))))))))))))).))).))).))).(((..(((((..(((.(((.((. ((...(((((.((.((...((((((((.(.....))))))))))...)).)))))))..)))).)))..)))..)))))...)))...)).)))... ((((((.(........).)))))).	92.2	.00
ovel-miR 0065	ta_i wgsc_2al_v1_6 399394_5948	uguucaagcuuuuugcaagaguucagcggaaaaaaacaucag aggagcucucggcaaaaaaaacaaaaucaauguucuaaguuu gcg cacuguuugagcauugauuuuguuuuuuuuugugagagcuc cucggauguuguuuuuagcugaaacuuugcaaaaaguucaaa cau uuaaugaagcuuguugucucaagauuucagauuuuucgg accuuguuuuaucacauuuuucgaaguuuugcaaacuc	+	165	414	.9	.8	1.3	uc agcggaaaaaaa caucaga	gg auguuguuuuua gcugaaa	ta_ iwgsc_2al_v1_ 6399394	((((..(((((((((((((..((((((.(((((.((((((.((((((((((((..((((((((((((((((((((((((..(((......)))....))))))))))))))))))))))..)))))))))))).))))))).))))).)))))))..)))))))))))) ..))))......((((((((((......)))).)))))....(((((((...(((.... ..)))...)))))))..........	99.7	.00
ovel-miR 0066	ta_i wgsc_5dl_v1_4 507581_29026	uauuuggcgcggacaaggcuccucugacguacugcagggu gcaguuggcucacuaagagccaacugcacccuguaguacgu caga ggagcugagucuggcgcagaacaaggguagacagaaaucga aaaugcugcuucgcgcgcgagcguaucguuuccuuacugga agaa auaguagugggaggcagaggaauagaauucgguggagaaa gugaagagggggaggggaauaaauaagcggaaacguuuu	+	019	268	.7	.6	1.3	uc ugacguacugca gggugca	ca cccuguaguacg ucagagg	ta_ iwgsc_5dl_v1 _4507581	......((((((((..(((((((((((((((((((((((((((((((((((((.....)))))))))))))))))))))))))))))))))))))))..)))).)))))....((.......((.(((.((..... ..(((((((.(((((((.....))))((((.....)))). ....))).))))))).......)).))).))........)).............. ......(((((....))))).	117. 9	.00
ovel-m iR0067	ta_i wgsc_5al_v1 _2791901_2 4713	caaaaagguucacauagcuucuugaaccuuggg gaaaagccgcauaacaccauuaacuugaaaacca uguauuuccuaucccaaaac ugcuccuagcacaagggguaaaagaugguuuuu	-	122	371	.8	0. 1	.9	ug aaccuugggg aaaagccgca u	g cggauuuu ucaccaaga	ta _iwgsc_5al _v1_27919 01	..((((((((((.((((((.((((((.(((((.(((((((((((((...((((((((((.(((((((((.(......((((((... ..((((...))))...))))))..).)))))))))))))).))))).)))))).)))))).))))).)))))).)))))).)))))))))).(((	88.9	29.46

		aguuguguggucaugcggauuuuucaccaagauucaagugguuaucuggaucuuuugacuuugaaaucuauacgugcuagugcacauuggauguaagugugucccuggugcaugugcucacugaagugca										((......((((.(((((((.(((((((.......)))))))..)))))))).)))))......)))))...		
ovel-miR0068	ta_iwgsc_5bl_v1_10924917_27843	ggcauuucgacguuggggaagaugaaguagagcagagaccucacgcucaagaagcggugaggugccugaccuacuucaucuucaccaacgaagagaacggcgaaugcaacauugacggcugcuugcgcugcagaaaacuaucaaggagcucaacgagaccacaauaaaaaagaauacauauaaggauauguucguauaauuaugucauuuuaaacuuauuuggcauaugcaguguagaugugacaac	-	0652	0901	.8	.1	1.3	ugaaguagagcagagaccuca	aggugccugaccuacuucauc	ta_iwgsc_5bl_v1_10924917	.((.((((..((((((.(((((((((((((..(((..((((((((((((.....))).)))))))..)))..))))))))))))).))))))..))))...))((((((((((((((..((((..(((.(((.(.((......))..).))).)))..)).))....... ........((((((....))))))........(((((((............)))))))..))))))...))))))).....	81.6	29.46
ovel-miR0069	ta_iwgsc_1al_v2_3916116_359	uuaaaaagucuacauaaccucuugaaucuugggaaaaagcugcauaacuccaauaaauuggaaaccucauuuucuaucccaaaauugcuugcaaacacaauuucuggguaaaauauaugguuugaaguuaugagguuaugcagauuuuucaccaauauucaagugguuaucuagauuuuuuuagaaacuauggaacauauaucauugaagguacucuucgaccaaucuuacuuuauuuaggacaauuua	-	634	883		.9	1.3	ugaaucuugggaaaaagcug	gauuuuucaccaauauucaag	ta_iwgsc_1al_v2_3916116	..((((((((((.((((((.(((((((.(((((((((((.((((((((((.((...((((..(((((..((.((.((((((((((((((..........)))))).))))))).)).))..)))))..)))).)).))))))))))).)))))).)))).))))))).)))))).))))))))))).........(((...........((((((.....))))))))).(((((.......))))).......	73.2	.00
ovel-miR0070	ta_iwgsc_2dl_v1_9769407_10602	auaucaaacuauauguccugcaagguagaagaccucagucuuaaaaaucucaagaacucuucgaugaaguauugcgcguccgagaguacauuagacguugguuacuugggagcgcaaaaa	-	248	497	.8	.1	1.3	ugagaucuucaagacugaga	ucagucuuaaaaaucucaag	ta_iwgsc_2dl_v1_9769407	.......(((((((.(..((((.....(((((.((((((((((.(((.(((((((.(((((((.((((((((.(((((.(((.((((.....((((((((((((((((.....((.........((((((((((.........))))))))))))))))))))))))))))	97.8	.00

		cuuggguucuaauacauugguggaaccuagcag uaaccaacgucuaacaucauc uuagacauaugcaagauuucauccaagagcuu guugagaucuucaagacugagaucuuucgug caaaagauauagu									.....)))).)))...))))).))))))).))))).)).)))))).)).)))))))).)))))..))))..).)))))))		
ovel-miR0071	ta_iwgsc_5ds_v1_2782660_30778	uucuuguacugaaagcaagagugucacacucga ugacacuaguagauaagaccaacaauaaggagcu uuugacaaaacagcgucagaa auuucagguagagaacacggcuucagauguugu gcaguuuaguuucacuacucaagguggacgcaaa aaauuugagaaaugguauaa aauauguccaugguccuugaguagugaaauu aaacugagcaccaucugaacucuuguucucua gcugaagcuucu	-	2751	3000	.9	227.5	.9	uuaaacugagcaccaucugaacuc	cuucagauguugugcaguuuaguu	ta_iwgsc_5ds_v1_2782660	.((((((.(((........((((((((....).))))))) ..))))))))).........((((((((((((........)))))))..(((((.(((((((((.((.((((((((.(((.(((((((((((((((((((((((((((((................... ....))))))....)))))))))))))))))))).))).)))))))).)).))))))))).))))))))))	99.3	14.73
ovel-miR0072	ta_iwgsc_3dl_v1_2881909_16753	ggagcuguggggugaacaguauaaguguccauu ucuucagaaucuucagacuuuaggagcucgcca agauugggcccugacagggac gauggcuggugcuacggauaaaauucaacccgau uggugugcaugcccgacugaaagaagaagccuu gcugagaguuaagugagccg ggaaucaguuugaaacagggagccaguuauc cgguuuaauuuguccauagcauccgcgauccc gccauucagcuguga	+	43	92	.3	.1	.9	uuaauuuguccauagcauccg	ggugcuacggauaaaauucaac	ta_iwgsc_3dl_v1_2881909	(.((((((..((((............(((((..((((..((. .((((........)))).))...)))).)))))(((....)))..(((.((. (((((((.(((((((((.((((.(((.(.((.((...(((......(((..((..(((.(((.(........).)))).)))..))..))).......))).)))).).))).)))).))))))))).))))))).)).))).))))...))))).)..	76.5	.00
ovel-miR0073	ta_iwgsc_2bl_v1_8046566_8407	caauaucuaugagauaugagauacaaauuugucu cauaauuaaagagaucuucuaggaucuuuauauc uaaauuuguucuugccauu aguuuucauauaacuuuccaacgcaguuucuuu	+	5313	5562	.6	.9	1.3	uuaggaacaugugugcucucuugu	aagagagcacacgucuuccuaaac	ta_iwgsc_2bl_v1_8046566	(((((.....((((((((((((......))))))))). ....((((((((....))))))))...............((((....((((... ....))))........))))))))...)))))........((((((((((((((((((((((((((((((((((((	95.7	14.73

		uguugaacgcaaaaaaucaucauuuaugaugaua caagagagcacacgucuucc uaaacaagagaucauaaacuaacaucacuugu uuaggaacaugugugcucucuuguaucaucau aaaugaugauuu										.((((((((((((.(((..........))).))))))))))).)))))))))))))))))))))))))))))))))))))		
ovel-m iR0074	ta_i wgsc_5ds_v1 _2732637_30 405	ccuacgugagcaacgaugacgauuaugaagaga guacaacugaaggaggcagacucauggacauuuc cucugaagaagacagcuagu uuugaccaccauguuaucuuaaauuuucaacauc ggucuagacgauaaacagaaauuuaagauaacaug guggucgaaacuaguugu cuucuucagaggaaaugucaaugacuccgc cuccuuuaguuguacucucuucauaaucgu caucguugcacacgua	-	3139	338 8	.9	.3	1.3	uu augaagagag uacaacugaag g	u uuaguugua cucucuuc	ta _iwgsc_5ds _v1_27326 37	..((((((.((((((((((((((((((((((((((((((((((((((((.((.((((.(((((((((((((((((((((((((((((((((((((((((((((((((((((((((((....((((.......)))). ....)))))))))))))).)))).)).))))))))))))))))))))))))))))))))))))))))).))))))	187	.00
ovel-m iR0075	ta_i wgsc_5as_v1 _1552033_25 658	gauccuagaugagcuaggaaaugauuaaaaaauc cuauaguuuccaugcauaauaugguguuacuuug aaucuuacagguguuucac aggaaacaguucaaugucuaacgaacugaaaguu uuuuuuuccugaccauggcauuucuagucaaua ggaauacgcuauguguugg aagacaaacuucuuuccagcaucuaguagaug uugaacuguuuccucugacauuccuguaaauu ucaugaguaguuu	-	960	209	.2	.5	1.3	uu gaacuguuuc cucugacauu c	g uuucacagga aacaguucaa ug	ta _iwgsc_5as _v1_15520 33	..((((((.....))))))...((((....))))........ ..(((((.....)))))..((((((((((..(((((((.((.(((.((((((((((((((((((((.....(((((((.(((.((((((. ((((((((((((((((.....))))))))..)))))) .)))))))))).)))...)))).)))........)))))))))))))))))))).))).)).)))))))..)))).))))))...	84.3	29.46
ovel-m iR0076	ta_i wgsc_5bl_v1 _10799490_2 6116	ggcuugcucccuguggcggccguugagacga gaucuccccauaggcccauaccacauagcggc agcucggcuccgugcacgcgua uggggagaucucgccucaacagccgcuccgug	+	3156	340 5	.8	.1	1.3	u ugagacgag aucuccccau a	u ggggagauc ucgccucaac a	ta _iwgsc_5bl _v1_10799 490	((((.(((((.((.((((((.((((((.(((((((((((((((((.((............((((.(((...)))))))....)).))))))))))))))))).)))))).))))).)).).)))).......((((..(((((((((((....((......)).))))))).	97.8	.00

		ugagcuuuaccuuuacguacgaugaugcuuu ugguccuuuccugcaucgcuu guugguaacgcgcucuccucuacuaguugau cuugcaugagagguagccuucuucaguccagc cgagaguucacugcuggg									))))..))))........(((((.(..((.......)).)))))).)))).....((((((.(........).))))))		
ovel-miR0077	ta_iwgsc_2ds_v1_1133030_11630	guggacgaagagggagcgaugagagccaaacau agcguuggaauacgagcaggagacgacggcgua ggagaagcggcgcgggucgc gggggcgggcgcgcucccccuuguacucuuaca accagaaguccucgguucuuagucuaaaucau cccauccucaacauucuuuca cagccauggccaagcuacauauauugugaaag uauauugaggaugggaugauuuagacaaaaag cucuggguaauacau	-	004	253	.9	.2	1.3	uu gaggauggga ugauuuaga	u aaaucaucc cauccucaa ca	ta _iwgsc_2ds _v1_11330 30	(((.........(((((..(((((((.......((.(((. ....))))).((.(((..(((.(((((((..(((((....)))))(((((((....)))))))......)))))))..)).)..)))))..)))))))(((((((((((((((((((((.((.(((((((((...((((...)))).....)))))))).)).))))))))))))))))))))).....)))))........)))	99	.00
ovel-miR0078	ta_iwgsc_4ds_v1_2274320_23450	ugcgcuguuggggccggggucuguuggggccuu gggccgggugcgcuguuggggucggggucugc ugggcccuaggcuggcuccuggccugccuag cccucgcccgcuuggggcuggcuggggguggac cgggucgcggcucuuggggccuccuggguguc cuaggcccagucacccuacgagguggauaug gccucgaggggauuuccccugugccccccuucc acucccgcagccgccaccgcuuccuccgcgca c	+	344	593	.8	.3	1.3	u ugggccuug ggccgggu	c uggccugc cuagcc	ta _iwgsc_4ds _v1_22743 20	(((((.(..((((.((((...(((((((...(((((((.(((((...(((((.((.....)).)))))..))))..)).))))))))))))))))))))))((...(((.((((((((((((..(((. (((((....(((((((...((....)).)))))))(((.((((.((((((.......))))))))))))....)))))..)))..))))))))...)))))))...)).....).)))))).	130.2	58.92
ovel-miR0079	ta_iwgsc_1as_v1_2129014_1024	ccagucgcugcggggcuuuccuuugugcuuga ucuaaccauguggguggaacgauggaaacggaac augguucugucaagcaccgc ggaaagcaccgugcucuccugcagcauggcc	+	3	72	.6	.9	1.3	uu gugcuugauc uaaccaugu	a ugguucugu caagcaccgc g	ta _iwgsc_1as _v1_21290 14	...((((((((((((((((((...(((((((((..(((((((((..((.....)).)).)))))))..)))))))))...))))))).))(((((((...((((..((((..((((..((....)).)))) (((....))).....)))))...	69.6	288.38

		cgccaccgccgccaccaccgcuggacaccucu ccucugcucuggagcaccgcag cccaccaccugccagacccacc ucuccc									))))))))))))).....)))....		

Apendix G

7DAP and 14DAP differential expressed target gene known mi RNAs

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

Apendix G (Continued)

7DAP and 14 DAP differential expressed target gene nove lmiRNAs

miRNA name	ID	7 DAP	14 DAP	log2FC	P Value	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

Apendix H

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
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

Apendix H (Continued)

Target ID	Gene ID	Gene sequence	Target ID	Gene ID	Gene sequence
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

Apendix I

7 DAP and 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_525339E9F6	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	0.00733092	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	0.00291577	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_29B4E	0	up	Traes_1DS_F957	8.66E-05	up	Traes_2DL_0A7F75	0.003304	down	Traes_3B_FAA7	0.005795	up

633D			D1B2C			7AB	355		BF6EB	692	
Traes_1BL_30CF86D20	0.00330435	down	Traes_1DS_FD33F6FB81	0.006881614	down	Traes_2DL_0D2359A4B	0.006752 72	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	0.006752 72	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	0.006752 72	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	0.006752 72	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	0.006752 72	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

Apendix I (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_24902825	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_847006BB7B	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	0.00733092	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	0.00579 5692	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	0.00733 0092	up	Traes_6BL_066E7881C	7.39E-06	up	Traes_6DS_93C7DA2261	0	up
Traes_4DL_F064084A9	0	up	Traes_5DL_51EE2478D	0.00579 5692	up	Traes_6BL_5320E59A6	0.00675272	down	Traes_6DS_9A9B26465	0.00675272	down
Traes_4DS_261E93270	0	up	Traes_5DL_5E1EF029C	0.00291 5577	up	Traes_6BS_0159CBF63	0.0025484	up	Traes_6DS_9DF272FBB	0.00688 1614	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

Apendix J

7 DAP VS 14 DAP differential expressed target gene list

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_87E0FB8854	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	0.00291557	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

Apendix K

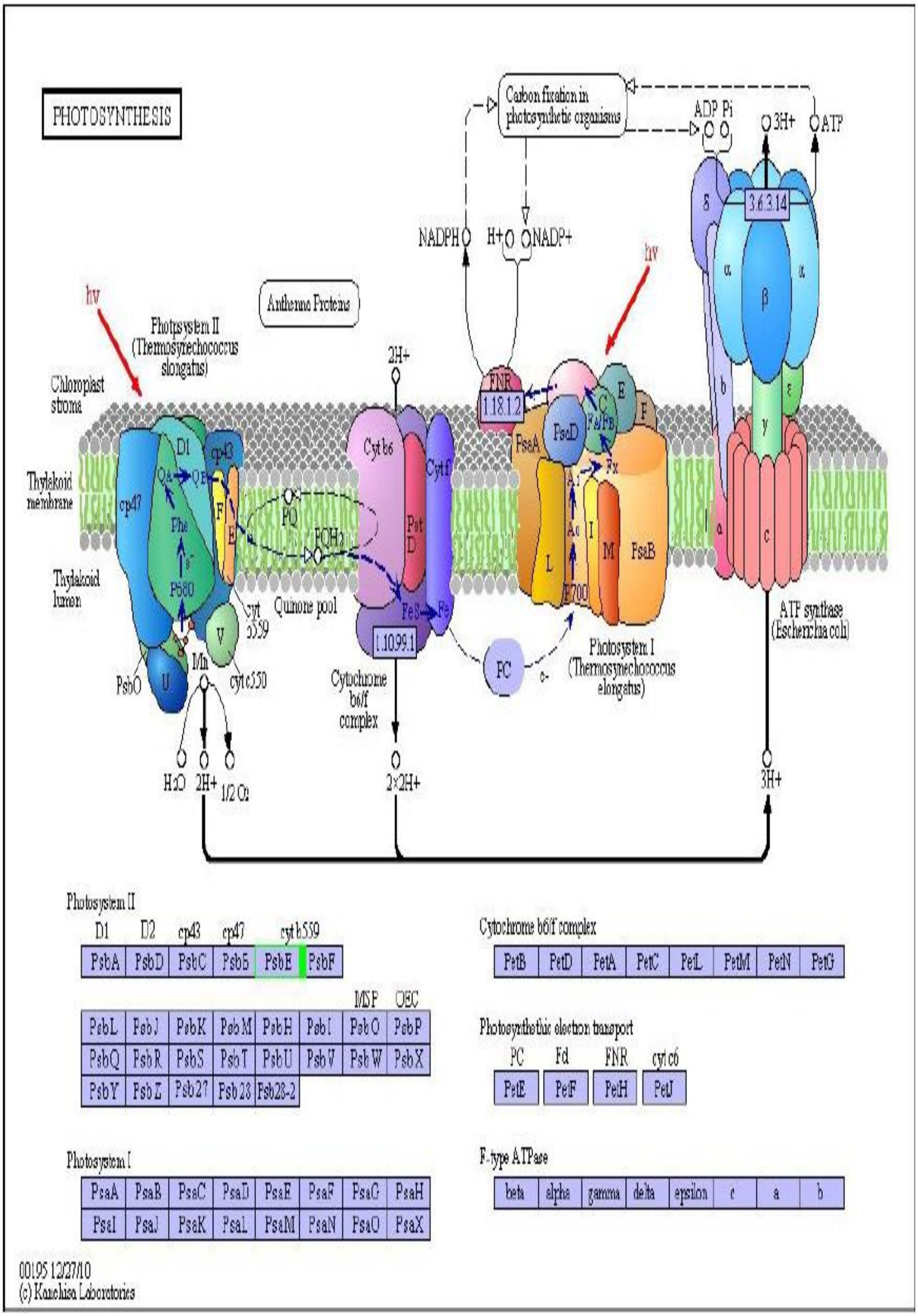
Summary ofKEEG pathway of differentially expressedmiRNA targets.

№ Pathway	ko_ID	Unigene	gene	Unigene_ all	gene_ all		
Starchand 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+K 00510+K00510
Purine metabolism	ko00230	4	9	101	145	Traes_1AL_8475A65E8;Traes_1BL_1E61EBE23; Traes_1DL_7AC3AE1EB;Traes_1DL_DE87A275 A	K01952+K01952+K01952+K 01952
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;T	K02707+K02707+K02707+K 02707+K02707 +K02707+K02707+K02707+K 02707+K02707+K02707+K02 707+K02707+K02707+K0270 7+K02707+K02707+K02707+ K02707+K02707+K02707+K0 2707+K02707+K02707+K027

						raes_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_5320E	07+K02707

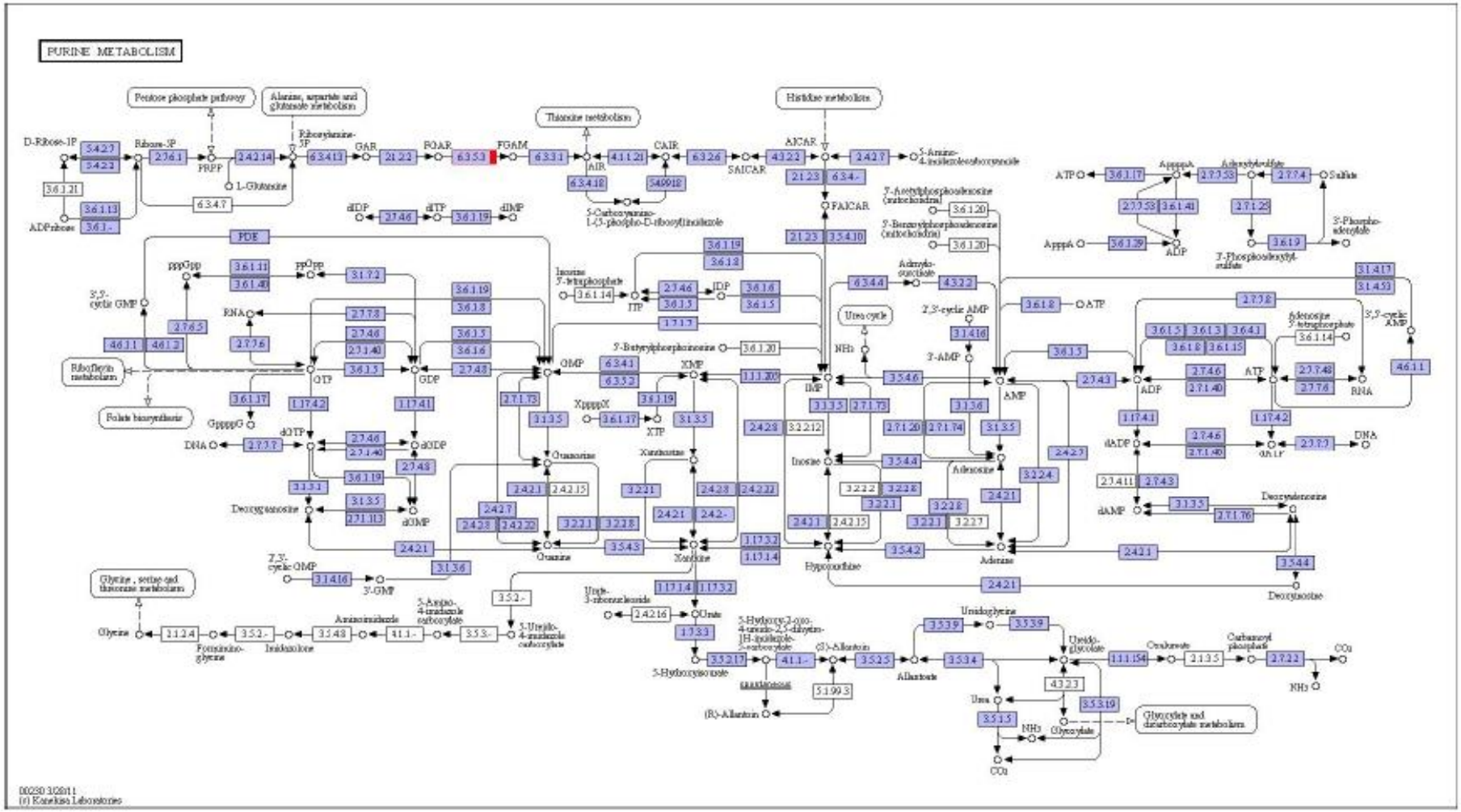
						59A6;Traes_7BS_5320E59A6;Traes_7DL_5320E59A6;Traes_7DL_5320E59A61;Traes_7DS_88B703F52	
Phenylpropanoid biosynthesis	ko00940	1	2	101	145	Traes_1BS_BA046E212	K00430
Phenylalanine metabolism	ko00360	1	2	101	145	Traes_1BS_BA046E212	K00430
RNAtransport	ko03013	3	6	101	145	Traes_2AS_EC7E6EAF0;Traes_2BS_80474BB5B;Traes_2DS_1A728B7C8	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

Apendix L

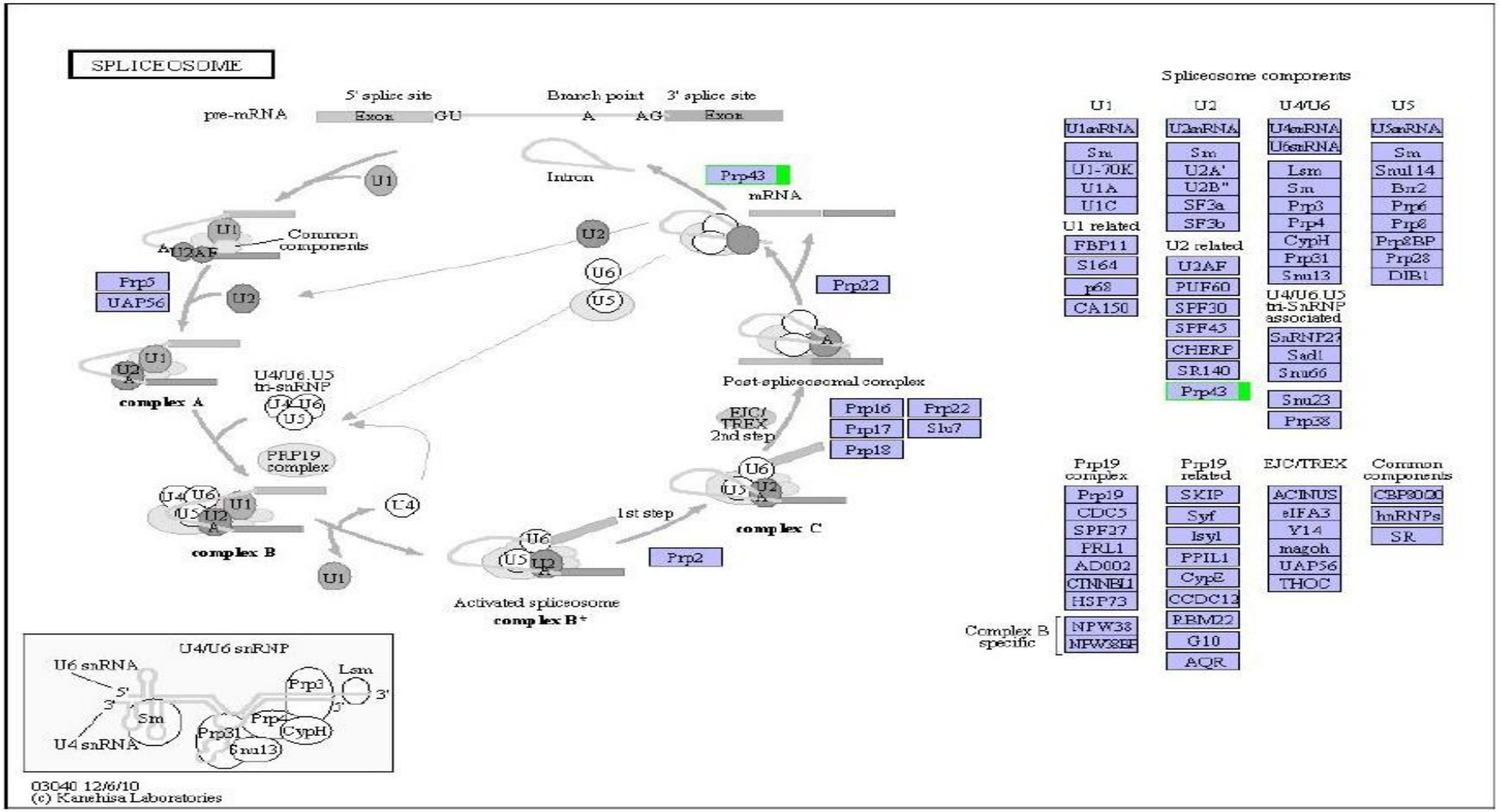


PHOTOSYNTHESIS
Carbon fixation in photosynthetic organisms
ADP Pi
3H+
ATP
3.6.3.14
NADPH
H+
NADP+
hv
Anthenna Proteins
Photpsystem II (Thermosynechococcus elongatus)
Chloroplast stroma
Thylakoid membrane
Thylakoid lumen
2H+
FNR
1.18.1.2
PsaD
PsaA
PsaB
Cyt b6
Cyt f
Pet D
FeS
1.10.99.1
PQ
PQH2
Quinone pool
cp47
cp43
D1
Phe
P680
cyt b559
PsbO
U
V
Mn
cyt c550
H2O
2H+
1/2 O2
Cytochrome b6/f complex
2×2H+
PC
e-
Photosystem I (Thermosynechococcus elongatus)
P700
ATP synthase (Escherichia coli)
3H+
Photosystem II
D1 D2 cp43 cp47 cyt b559
PsbA PsbD PsbC PsbB PsbE PsbF
MSP OEC
PsbL PsbJ PsbK PsbM PsbH PsbI PsbO PsbP
PsbQ PsbR PsbS PsbT PsbU PsbV PsbW PsbX
PsbY PsbZ Psb27 Psb28 Psb28-2
Cytochrome b6/f complex
PetB PetD PetA PetC PetL PetM PetN PetG
Photosynthetic electron transport
PC Fd FNR cyt c6
PetE PetF PetH PetJ
Photosystem I
PsaA PsaB PsaC PsaD PsaE PsaF PsaG PsaH
PsaI PsaJ PsaK PsaL PsaM PsaN PsaO PsaX
F-type ATPase
beta alpha gamma delta epsilon c a b
00195 12/27/10
(c) Kanehisa Laboratories

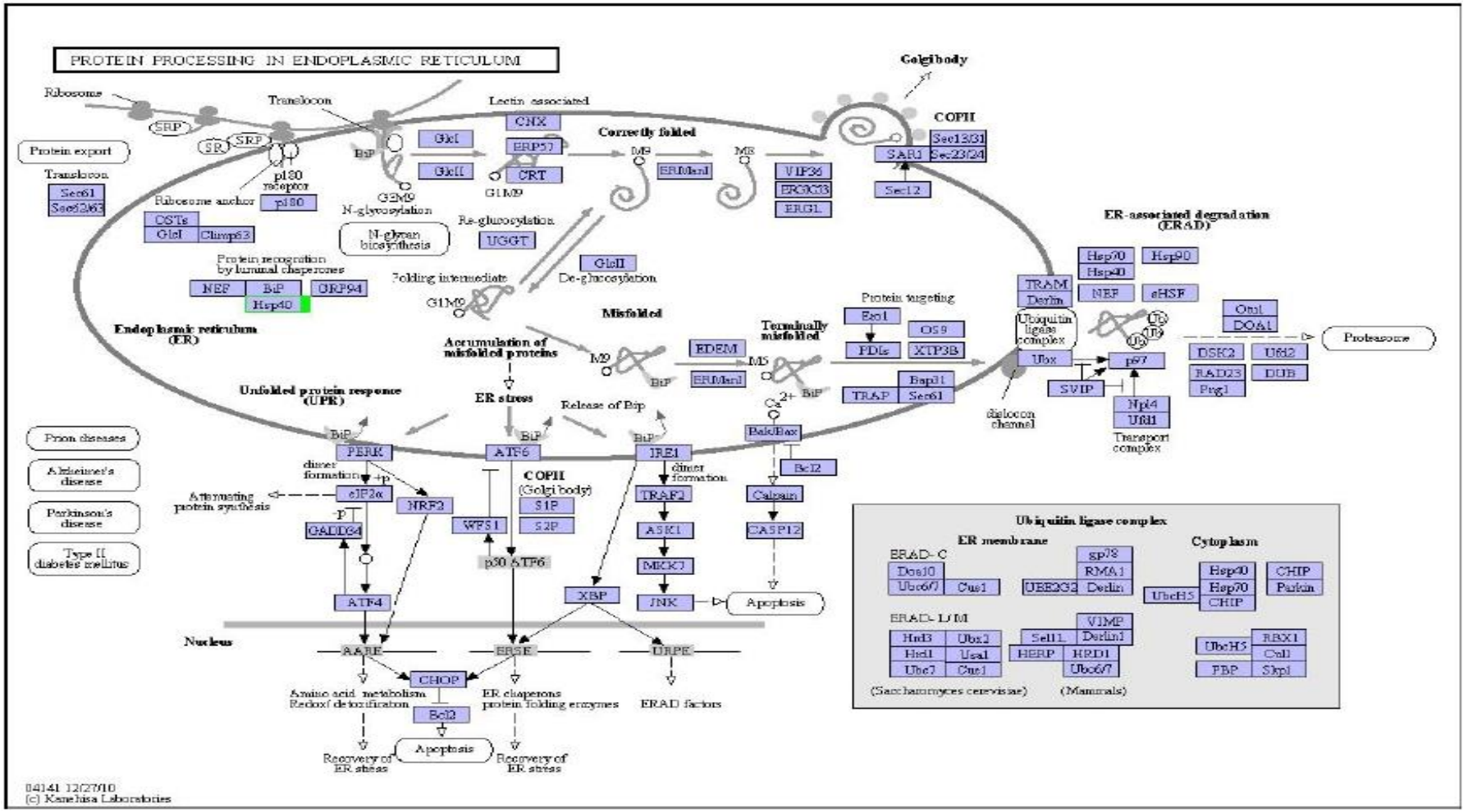
Apendix L (continued)



Apendix L (continued)



Apendix L (continued)



Apendix M

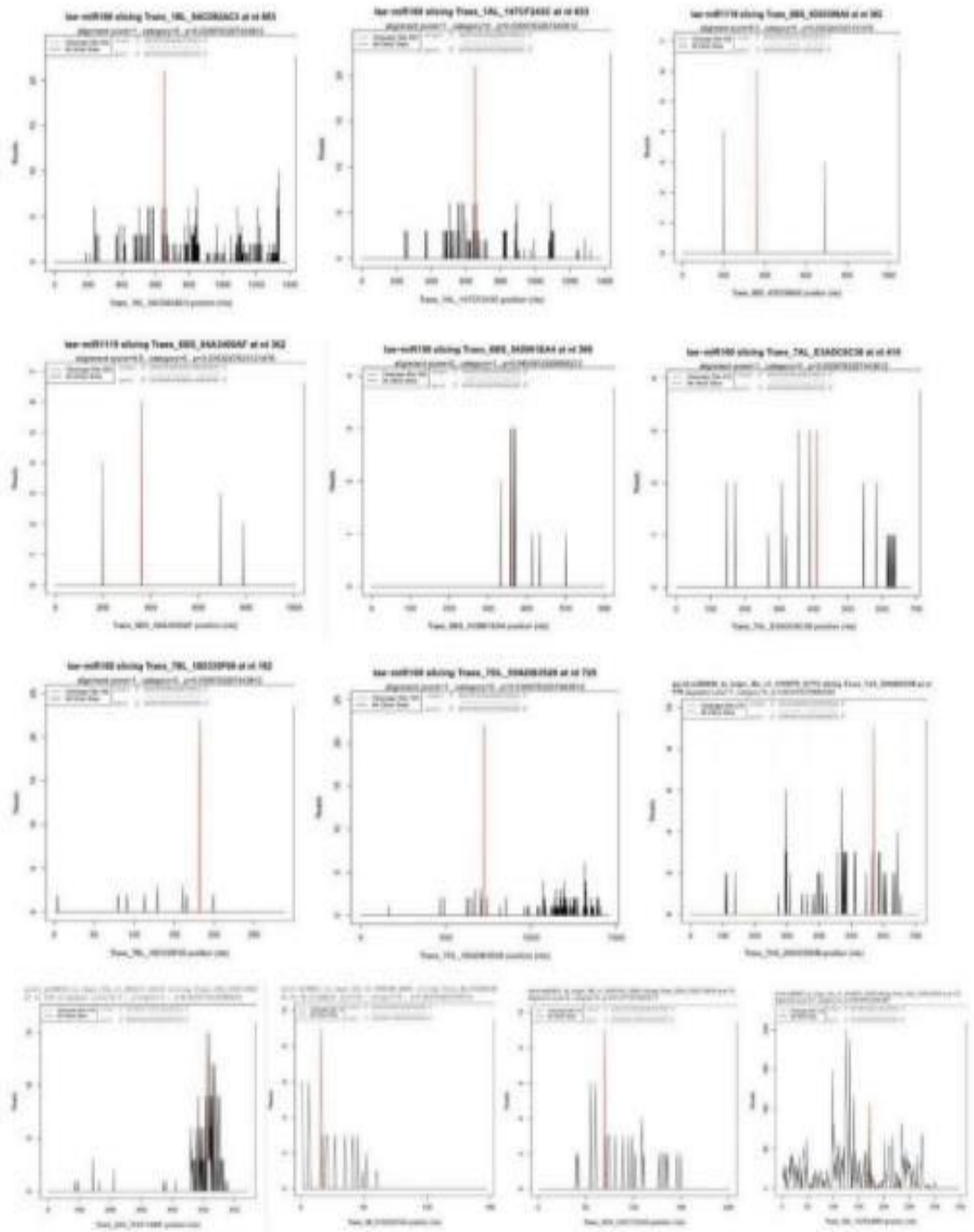
Sequencing data of degradome library fromearlydevelopinggrains

RNA name or ID	RNAname or ID	Target	Alignment Score	Alignment Range	Cleavage Site	Cate-gory	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

Apendix N

Degraded sites of 13 annotated targetgenes predicted by degradome sequencing



Apendix O

miRNA name and Target ID

miRNA name	Target ID	Target ID	Target ID	Target ID
novel-miR009	Traes_1BL_19E9499CD	Traes_4AS_4B81C93C0	Traes_2BS_31C9F9A6F	Traes_1DL_696E3F6A2
novel-miR0034	Traes_3DS_D42FCD586	Traes_6BS_3013D3E79		
novel-miR0079	Traes_1DL_52D511CDD	Traes_1AL_14DCD6020		
novel-miR0059	Traes_2BL_2D4F1DB93	Traes_5AL_619BDF609	Traes_2DL_EE130E7CF	Traes_4AL_96928889D
novel-miR0062	Traes_4DL_1120E8821	Traes_7BS_418111C53	Traes_4AS_53B185E1A	Traes_1AL_3A716350F
novel-miR0056	Traes_5AL_2FA4FA08C	Traes_4BS_DDDD362D5	Traes_7DL_90CD3809F	Traes_2AL_4D3C861F2
novel-miR0053	Traes_6BL_507268FE5	Traes_7DS_5A117F2B1		
novel-miR0070	Traes_3DL_1D73EFA2C	Traes_3AL_755D48EBC	Traes_3B_ECD436CB6	Traes_4DL_85939BE5A
novel-miR0026	Traes_2AL_CF41123D7	Traes_4DS_F6F01D11D	Traes_2BL_621DAFE84	
novel-miR0018	Traes_6DL_FA861D610	Traes_6AL_9A0ED2378	Traes_3B_0BA89B2AC	Traes_2BS_832F28506
novel-miR0072	Traes_1DS_112FD8244	Traes_1DS_810C7F474		
novel-miR002	Traes_4DL_AEF5AB905	Traes_6DL_506278E82	Traes_3AS_D5CE7702B	Traes_4BL_63A544CAE
novel-miR0036	Traes_7AS_2084DE83B			
novel-miR0078	Traes_4BL_399250A77	Traes_4AS_53B185E1A	Traes_3AS_7E128A012	Traes_3AS_396386369
novel-miR	Traes_5AL_147EA			

0075	9565			
novel-miR 0076	Traes_6AS_05309 C728	Traes_6BS_0159C BF63	Traes_6BS_6EE55 88AC	
novel-miR 0068	Traes_4AL_D8CC E0D2E	Traes_7DS_50747 298E	Traes_4BS_5EC01 BC81	
novel-miR 0011	Traes_3DS_C6D17 D438	Traes_3B_E7D2E8 720		
novel-miR 0025	Traes_6BS_0159C BF63			
novel-miR 0031	Traes_3B_325EA6 658	Traes_4DS_F5383 0B2B	Traes_4AL_2A5D EA0BB	
novel-miR 0019	Traes_5DL_D897 DFC04	Traes_5BL_03D63 7203	Traes_4BS_973D1 B453	Traes_2DL_CED2 097FF
novel-miR 0049	Traes_3B_BCBA9 A6CB			
novel-miR 005	Traes_3B_D2DA3 4BB5	Traes_1BL_32F55 19AA	Traes_6BL_066E7 881C	
novel-miR 0014	Traes_4DL_FA07 D8414	Traes_3B_6396F53 A9	Traes_4BL_5091D E58E	Traes_4BL_F6C3 B5069
novel-miR 0012	Traes_3DS_2F5F2 C276	Traes_3B_8824DB B56	Traes_3AS_7EEF1 386F	

Apendix P

Detailed information ofthe differentially expressed known miRNAs between 7DAP and14DAP grains

miRNA name	7 DAP	14 DAP	P Value	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

Apendix Q

Detailed information of the differentially expressed novel miRNAs between 7DAP and 14DAP grains

miRNA name	ID	7 DAP	14 DAP	log2 FC	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