ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

IDENTIFICATION AND CHARACTERIZATION OF CONSERVED MICRORNAS IN WHEAT (*Triticum monococcum* ssp. *monococcum*) BY HIGH-THROUGHPUT SEQUENCING AND COMPUTATIONAL ANALYSIS

MASTER OF SCIENCE

SARA BATAW

BOLU, JULY 2016

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APPROVAL OF THE THESIS

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July 29, 2016

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Director, **Graduate School of Natural and Applied Sciences**

To my family

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

SARA BATAW

ABSTRACT

IDENTIFICATION AND CHARACTERIZATION OF CONSERVED MICRORNAS IN EINKORN WHEAT *(TRITICUM MONOCOCCUM* SSP. *MONOCOCCUM)* BY HIGH-THROUGHPUT SEQUENCING AND COMPUTATIONAL ANALYSIS
MSC THESIS
SARA BATAW
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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Wheat is the second most important agricultural crop amongst the top three major cereal crops, with over 615.8 million metric tons of annual production. Drought and salinity are the most adversely influential environmental stress factors that affect yield and quality of wheat cultivars. Plants, on the other hand, have developed their own defense systems to cope with these abiotic stresses. One of these defense mechanisms is the altering of gene expression by microRNAs (miRNAs). MiRNAs are an extensive class of noncoding RNAs, approximately 22 nucleotide lengths, which regulate the expression of genes at posttranscriptional levels by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs. MicroRNA-based genetic modifications, therefore, have the potential to understand the drought and salinity stress response and to enhance the drought and salinity tolerance in wheat. Even though miRNAs, which were implicated under salt and drought stress have widely been reported in numerous plant species and wheat genomes in the last years, studies on einkorn wheat (*Triticum monococcum* spp. *monococcum*) have not been available yet. This study investigated conserved miRNAs under drought and salinity stresses by combining next generation sequencing and computational analysis approaches. Using these approaches, 75,164 unique reads were obtained from 15,139,448 raw reads and 167 putative mature miRNA sequences belonging to 142 distinct miRNA families were identified. For secondary structure prediction, we used available sequences from *Triticum urartu* as template to extract precursor microRNA sequences. 111 of precursor sequences showing 100% homology to *Triticum urartu* genome was analyzed by using Mfold software. Data from this study provides critical information necessary to begin an investigation into a role for these transcripts in the epigenetic regulation of einkorn wheat and provide a basis for further understanding of miRNAs and the biological processes in which they are involved in wheat species.

KEYWORDS:
Computational analysis, Conserved miRNAs, MicroRNAs, Einkorn Wheat, High-throughput Sequencing, *Triticum monococcum* spp. *monococcum.*

ÖZET

SİYEZ (*TRITICUM MONOCOCCUM SSP. MONOCOCCUM*) BUĞDAYINI KORUNMUŞ MİKRO RNALARIN YÜKSEK VERİMLİ DİZİLEME VE SIMULASYON ANALİZLERİYLE TANIMLANARAK KARAKTERİZE EDİLMELERİ
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Buğday; yıllık 615.8 milyon metre küp tonun üzerindeki yıllık hasatıyla, zirvedeki üç ana tahılın en önemli ikinci tarım ürünüdür. Kuraklık ve tuzluluk buğday çeşitlerinin verimini ve kalitesini en fazla etkileyen olumsuz çevresel stres faktörleridir. Bitkiler, öte yandan, bu abiyotik streslerle mücadele etmek için kendi savunma sistemlerini geliştirmişlerdir. Savunma mekanizmalarından biri microRNA'lar (miRNAlar) yoluyla gen açıklanmasının değiştirilmesidir. MiRNAlar spesifik mRNAların 3'-dönüştürülmemiş bölgesine (3'-UTR) bağlanarak post transkripsiyonel seviyelerde gen ifadelerini düzenleyen ortalama 22 nükleotit uzunluğuyla kodlamayan RNAların geniş bir sınıfıdır. MiRNA genetik modifikasyonlarI, bu nedenle kurak ve tuz stresine karşı olan tepkiyi anlama ve buğdayın kurak ve tuz toleransını geliştirme potansiyeline sahiptir. Son yıllarda miRNAların çeşitli bitki türleri ve buğday genomlarında tuz ve kuraklık stresine tabi tutulduğu çalışmalar rapor edilmesine krşın siyez (*Triticum monococcum* ssp. *monococcum*) buğdayında henüz herhangi bir çalışma yapılmamıştır. Bu çalışma; korunmuş miRNAları kurak ve tuz stresi altında, gelecek generasyon dizilemesi ve simulasyon analizinin bir kombinsayonu ile incelemişitr. Bu yaklaşımın kullanılmasıyla 15,139,448 ham okumadan 75,164 özgün okuma elde edilmiş ve 142 belirgin miRNA familyasından 167 olgun miRNA dizisi tanımlanmıştır. Aynı şekilde; *Triticum urartu* tabanı kullanılarak ikincil yapı tahminleri microRNA prekursorları elde edilmiştir. Sonuçta; ve *Triticum urartu* genomunun %100 homologu olan 111 prekursor dizileri Mfold bilgisayar programı ile analiz edilmiştir. Bu çalışmadan elde edilen veriler; siyez buğdayının epigenetik düzenlenmesindeki transkriptlerin rolüne ilişkin bir araştırma başlatılabilmesi için gerekli kritik bilgilerin elde edilmesi ve miRNAlarla bunların dahil oldukları biyolojik süreçlerin daha iyi anlaşılabilmesi için uygun bir zemin sağlayacaktır.

ANAHTAR KELİMELER:
Korunmuş miRNAlar, Mikro RNAlar, Simulasyon analizi, Siyez buğdayı, *Triticum monococcum* ssp. *monococcum,* Yüksek çıktılı dizileme.

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LIST OF ABBREVIATIONS AND SYMBOLS

MiRNA **:** Micro-RNA

UTR **:** Untranslated region

RISC **:** RNA-induced silencing complex

PEG **:** Poly ethylene glycol

RNAi **:** RNA interference

NGS **:** Next Generation Sequencing

CDNA **:** Complementary DNA

MMT **:** Million Metric Tons

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1. INTRODUCTION

1.1 Importance of wheat

On the global scale, wheat is the second most important agricultural crop amongst the major cereals: rice, wheat, maize and, barley, respectively (Rahaie, 2013). According to the Grain and Feed Annual Report (Karabina, 2014), Turkey's wheat production was recorded at 15.8 million metric tons (MMT), compared with rice at 740,000 metric tons (MT), barley at 5.8 MMT and corn at 4.9 MMT.

Wheat occupies a greater land area than any other commercial crop and is the most important staple food source for more than one-third of the world's populace (Goutam et al., 2013), estimated at about 2.5 billion people in developing and underdeveloped countries (Shahzad et al., 2013). The majority of wheat grown around the world is hexaploid bread wheat while tetraploid durum wheat (pasta wheat) represents only 5%. Furthermore, low amounts of wild wheat species such as einkorn, emmer, and spelt are grown in Spain, Turkey, the Balkans, and the Indian subcontinent (Shewry et al., 2009).

1.1.1 History and Geographic Origin of Wheat

The cultivation of wheat reaches far back in the history. Archaeological and botanical investigations indicated that the Fertile Crescent (Figure 1.1), which are stretched from northern Syria through southeast Turkey, northern Iraq, and western Iran, is the birthplace of wheat domestication, where the "founder crops" emmer and einkorn wheat and barley are present in wild (Hopf and Zohary, 2000). More precisely, DNA finger-printing suggested that einkorn wheat was the first cultivated species near Karacadağ in southeast Turkey (Anderson, 1991). Moreover, the wild grass *Aegilops speltoides* and diploid hulled wheat *Triticum monococcum* ssp. *monococcum* was the first hybridization event millions years ago (Patnaik and Khurana et al., 2003).

Figure 1.1. The Fertile Crescent (Hopf and Zohary, 2000)

1.1.2 Classification of Wheat

Wheat (*Triticum* L.) is an annual monocotyledon plant from *Triticum* genus in *Gramineae* (*Poaceae*) family. Throughout the history, the development of wheat classification has passed several phases. Starting from the sixteenth-century, botanists have divided wheat into two groups: *Triticum* corresponding to free-threshing wheat, and *Zea* corresponding to hulled ('spelt') wheat.

The later classification has recognized all domesticated wheat into five species: *T. aestivum*, *T. hybernum*, *T. turgidum*, *T. spelta* and, *T. monococcum*. However, the wild wheat plants were not described until the mid-19th century because of the poor botanical exploration in the Near East, where they have been grown. In modern classifications, wheat is classified into 3 groups according to the number of sets of chromosomes: diploid, tetraploid and hexaploid. The wild diploid wheat plants are *T. urartu* and *T. boeoticum*, they are the wild progenitors of domesticated einkorn, *T. monococcum* (Caligari, 2001).

T. araraticum and T. dicoccoides are the wild tetraploid wheat and the lateral is the ancestor for all domesticated tetraploid wheat except for *T. timopheevi.* All hexaploid wheat are domesticated only because they are the outcome of the hybrid between wild hexaploid wheat and a tetraploid domesticated wheat (Provan and Wolters et al., 2004).

Table 1.1. Classification of wheat (*Triticum*. L) species (Van Slageren, 1994)

Species	**Subspecies**	**Status**	**Chromosome Number**	**Genome**
T. monococcum	*aegilopoides* *monococcum*	Wild Cultivated	2n=14	AA
T. urartu	N/A	Wild	2n=14	AA
T. turgidum	*cartlicum* *dicoccoides* *dicoccum* *durum* *turgidum* *paleocolchicum* *polonicum*	Cultivated Wild Cultivated Cultivated Cultivated Archaeological Cultivated	2n=28	AABB
T. timopheevii	*armeniacum* *timopheevii*	Wild Cultivated	2n=28	AAGG
T. aestivum	*spelta* *macha* *aestivum* *compactum* *sphaerococcum*	Cultivated Cultivated Cultivated Cultivated Cultivated	2n=48	AABBDD

1.1.3 Wheat Production

According to Turkey Statistical Institute's (TÜİK) data, the world's wheat production during (2009-2012) was around 654 million tons and Turkey has produced more than 3% of the world's wheat production at about 20 million tons. Although, Turkey is considered to be one of the most important grain producers in the world, with the yield of 1.95 tons of wheat per hectare, this value is still behind the European Union average yield level, which is 5.66 tons per hectare.

On the other hand, wheat production in 2014 was decreased by 13.8% in many regions of Turkey compared to the previous year especially in Central Anatolia, Çukurova, the Black Sea, and Eastern Anatolia. The reason of this was mostly due to the lower usage production input, unpredictable climatic conditions inducing drought, cold, and salt stresses (Serttas and Paulson, 2014).

Wheat production in most of the Çukurova and Central Anatolian regions has dramatically declined in 2014 due to the dry weather conditions. Wheat germination and harvest delay were the reasons for low yields in those regions. Conversely, the southeastern Turkey was luckier for weather conditions than other regions (Serttas and Paulson, 2014). On the other hand, wheat production in 2015 increased slightly compared with 2013/2014 due to the excellent climatic conditions in most of the wheat-producing regions in Turkey (Karabina and Jess et al., 2015).

1.1.3 Nutrition, Health and Industrial Benefits of Wheat

Wheat is considered to be the dominant crop in many countries for human food and livestock feed, and wheat based foods are essential components in daily human diet; a major source of energy, protein, vitamins, and minerals (Ranhotra et al., 1994). Wheat nutritional values can be fractionated by their contents into starch, gluten, oil, and straw (Bergthaller, 1997).

There are many reports about wheat, and particularly wheat proteins, for medical conditions, which suggest that, whole wheat can treat numerous illness issues due to its high content of catalytic elements, mineral salts, calcium, magnesium, potassium, sulfur, chlorine, silicon, manganese, zinc, iodide, copper, vitamin B, and

vitamin E, and also because of wheat minimal fat contents. Moreover, daily intake of whole wheat can decrease the hazards of heart diseases and regulate blood glucose levels in diabetic patients (John, 2014). Lysine is the least frequent amino acid in wheat when protein content is considered. Wheat has low fat and unsaturated fatty acids (Ranhotra, 1994). Wheat is also an attractive source for biofuel because of high starch content, which is readily converted into sugars (saccharification) and then fermented into ethanol (Murphy, 2008).

1.2 Environmental Stress Factors

Wheat and other crop plants are constantly exposed to the stress of living and nonliving factors such as fungi, weeds, drought, and salinity (Table 1.2) that greatly affect plant growth, development, and productivity worldwide (Mostek et al., 2015). Among the factors mentioned, drought and salinity are major constraints to crop production and responsible for more than 50% of agricultural yield loss, compared to about 10 to 20% crop losses caused by pathogens (Kreps et al., 2002). This might be due to the recurrence of dry intervals in many regions of the world and the problems associated with high salinity in irrigated areas. Moreover, the climate change models have predicted that the frequency of such conditions would increase in future, leading to a decrease in agricultural production by about 70% or more (Cramer et al., 2011).

Plants can tolerate adverse stress conditions and evolve a variety of responses to cope with them since plants are sessile organisms. Plants have evolved many mechanisms to recognize the abnormal conditions and send signals to stimulate stress responsive genes which eventually result in physiological, biochemical and molecular changes (Gao et al., 2007).

Table 1.2. Environmental stress factors

Biotic stresses	**Abiotic stresses**
Pathogens	Cold
Weeds	Heat
Insects	Drought
Herbivores	Salinity
Rodents	Excess Water
Anthropogenic Factors	Radiations
	Chemicals and Pollutants
	Nutrients deficiency in soil

1.2.1 Drought Stress and Its Impacts on Plants

Drought is the most adversely influential abiotic stress on plant growth and yield. The severity of this stress has increased due to the global warming which resulted in unexpected climate conditions, increased the frequency of dry periods around the world and caused about 27% decrement in the average yield of major crops when compared with other stress factors (Blum et al., 1986). Drought can lead to many different plant responses at physiological, morphological and molecular levels, ranging from reprogramming gene expression and cellular metabolism that affect growth and productivity.

The loss of turgor is the first morphological effect of drought stress, which decrease the rate of cell expansion and ultimate cell size and, consequently result in decreasing growth rate, stem elongation, leaf expansion, and stomatal aperture (Bartels and Sunkar et al,. 2005).

On physiological level, drought has a direct impact on photosynthetic activity, by disrupting all major components of photosynthesis system and stomatal closure which is one of the first responses to drought stress and result in declined rate of photosynthesis in plants (Allen and Ort et al., 2001).

Over the evolution, plants have improved their defense mechanisms against drought conditions. They tend to delay the most sensitive stages of development to occur when the stress is less effective. Furthermore, keeping the tissue water potential at high level and decreasing water loss through transpiration are considered to be an effective strategy against drought stress (Shinozaki and Yamaguchi-Shinozaki et al., 2007).

1.2.3 Salt Stress and Its Effects on Plants

Due to the increased use of poor quality salinized water for irrigation and soil salinization in many areas around the world, salinity has today become a major abiotic stress which inhibits plant growth and productivity. It is generally a problem in arid and semi-arid areas where evapotranspiration exceeds annual precipitation (Manchanda and Crag et al., 2008).

Soil salinity causes a significant reduction in agricultural production (Pitman, 2002). Salts have affected more than 6% of the world's arable land (Munns et al., 2005). However, the percentage of cultivated lands affected by high salinity was even greater, with 23% of the cultivated lands being saline and 20% of the irrigated land suffering from secondary salinization (Vardar and Çiftçi et al., 2014). On global scale, salinization is predicted to become a larger problem in the coming decades, increasing every year leading to a devastating impact estimated by 30% land loss within the next 25 years and up to 50% by the middle of the 21^{th} century (Huang et al., 2008; Radi et al., 2013).

In general, salt can adversely affect plants by disturbing the roots water capacity which is disturbed due to high soil salinity or high salt concentrations inside plant cells. These effects can result in the inhibition of many biochemical, molecular, and physiological processes (Monteiro et al., 2011).

The first reaction of the plant against salinity involves the reduction in the rate of leaf surface expansion, considerable decrease in the fresh and dry weights of leaves, stems, and roots, formation of necrotic leaves and wilting (Khan et al., 1999). Furthermore, salt stress may cause water and oxidative stress, alteration of metabolic processes and nutritional disorders, or even genotoxicity and ion toxicity (Zhu, 2007).

Variation of plant tolerance to salinity conditions exist at genetic levels and differ in plant species and even in varieties within the same species (Munns, 2008). In the Triticeae, sodium exclusion is one of the most important adaptation techniques to salinity stress. For instance, bread wheat (*Triticum aestivum*) has a low rate of Na^+ transport to the shoot and maintains a high ratio of K^+ to Na^+ in leaves, compared with durum wheat (*Triticum turgidum* L. subsp. *durum*) which is more salt sensitive than bread wheat (Francois et al., 1986; Gorham and Jones et al., 1990).

1.3 MicroRNAs

MicroRNAs (miRNAs), which are a single strand of non-coding RNA molecules about 22 nucleotide (nt) in size can regulate gene expression at post-transcriptional level in a wide range of organisms (Lee et al., 1993). Early discovery of miRNAs has been involved in the regulation of developmental processes. Since then, much has been discovered about their role in gene regulation in plants under biotic and abiotic stresses (Koyro, 2012). MicroRNAs down-regulate plant gene expression at the post-transcriptional level mainly by mediating mRNA degradation or translational repression in a sequence specific manner (Tomari and Zamore, 2005).

1.3.1 Historical Perspective of MicroRNAs

In 1993, during the study of the role of lin-14 gene in *Caenorhabditis elegans* development, Ambros and his colleagues have discovered microRNAs when they noticed that a short RNA molecule encoded by gene lin-4 had regulated LIN-14 protein abundance. Seven years later, a second microRNA was recognized in *C. elegans*, it was called let-7, short RNA with size of 21-nucleotide that repressed the expression of lin-41 and lin-57 during developmental stage transitions in the worm (Lee et al., 1993; Wightman et al., 1993).

The word microRNA (miRNA) was not used until 2001. In the year 2002, cloning approach in *Arabidopsis thaliana* was used to identify the first plant microRNA (Llave et al., 2002). Since then, many studies were concerned with the identification and characterization of miRNAs and their mRNA targets in many other plants (Park et al., 2002).

At first, plant miRNAs was reported to be involved in the regulation of plant development only. Then, many reports have confirmed that these miRNAs also played an important role in response to biotic and abiotic stresses. Sunkar and Zhu (2004) were the first to identify plant microRNAs under abiotic stress. Since then, many abiotic stress related miRNAs were identified in various plant species (Sunkar and Zhu et al., 2004).

1.3.2 Biogenesis of MicroRNAs

In plants, microRNAs are endogenously produced from their own genes by a chain of reactions, with the help of enzymes (Kurihara and Watanabe et al., 2004). Even though miRNAs are small molecules about 19-24 (nt) they are encoded by much longer genes about 10-1000 (nt). MicroRNA genes are first transcribed to primary miRNAs (pri-miRNAs) by the Pol II or Pol III enzymes (Lin Liu et al., 2012). Pri-miRNAs fold into secondary stem-loop structures, which are processed in a two-step manner by a dicer-like enzyme (DCL1) (Bartel et al., 2004). The primary miRNAs are then cleaved by DCL1 to a characteristic precursor miRNAs (pre-miRNAs) with a stem-loop structure; and the mature miRNA sequence is located at one arm of the hairpin structure. Subsequently, the pre-miRNA is further cleaved by DCL1 into

mature miRNA (single-stranded), which is then incorporated into a ribonucleoprotein complex known as (RISC) RNA-induced silencing complex and responsible for identifying the target mRNA by perfect complementarity (Sunkar et al., 2007).

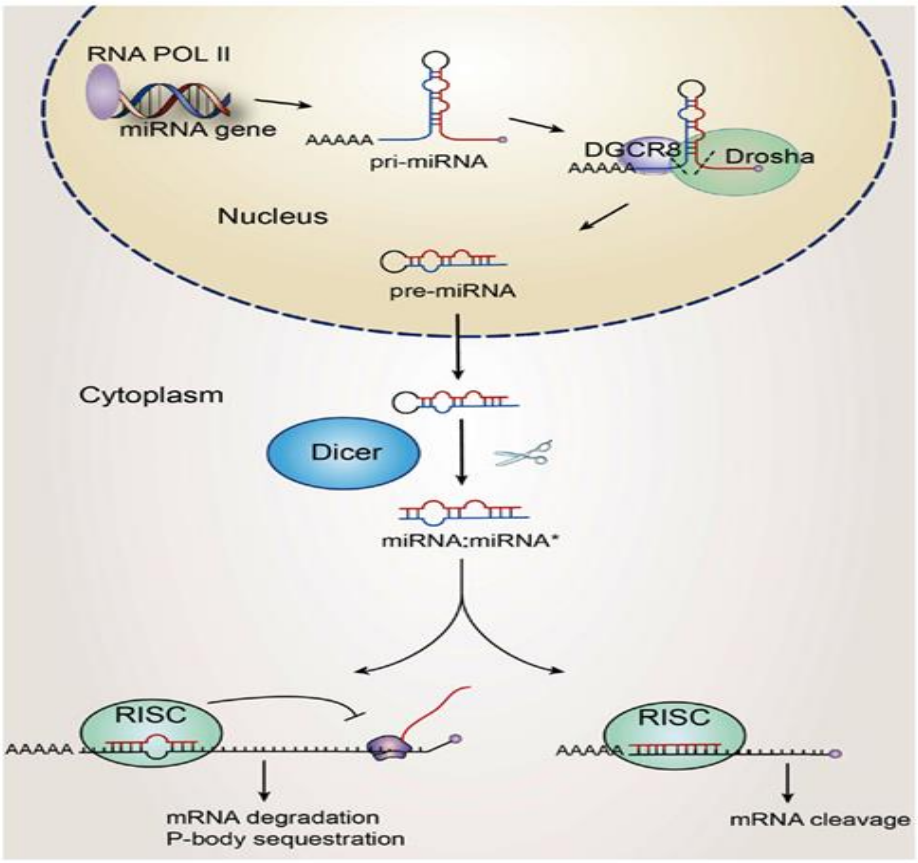


Figure 1.2. Biogenesis of microRNAs (Guo et al., 2010)

1.3.3 Molecular Mechanisms of Plant Gene Regulation by MicroRNAs

After RISC complex is formed, gene expression is inhibited by attaching miRNAs to its complementary target messenger RNA (mRNA) (Bartel, 2004). In plants, this procedure is called post-transcriptional gene silencing (PTGS) (Baulcombe, 2004). However, the majority of miRNAs can find and cleave their targets only by recognizing one single complementary site (Bartel, 2004). In plant, the complementary sites of miRNA targets, can exist anywhere along the target mRNA. Whereas, in animals it can only exist at the 3-UTR.

Recently, another mechanism was identified in plants. MicroRNAs can regulate gene expression by repressing gene translation through inhibition of ribosome movement. For example, miR172 which is perfectly complement to its target mRNAs, it can regulate gene expression by repressing gene translation (Aukerman and Sakai, 2003). Also, it was proved that miR172 can regulate gene expression through the direct cleavage of its target transcripts (Schwab et al., 2005). This suggests that miRNAs may be involved in more complicated mechanisms to control gene expression in plants.

2. AIM AND SCOPE OF THE STUDY

Wheat is one of the most important cereal crops both in Turkey and in the World. Climatic conditions due to the global warming have, however, become increasingly unpredictable and increased the severity of abiotic stresses including drought and salinity which are the most adversely influential environmental stress factors on wheat growth and yield, which consequently affect food security. Therefore, more concentrated efforts are required to overcome these challenges. In this study, we aimed to analyze leaves and roots of einkorn wheat (*Triticum monococcum* ssp. *monococcum)* by using next generation sequencing (NGS) and computational analysis to identify conserved miRNAs under drought and salinity stresses.

In this study, drought and salt stresses were applied to the grown seeds of einkorn wheat by using PEG 600 and sodium chloride solutions. High-throughput sequencing technology and computational analysis were carried out to identify and characterize the conserved microRNAs in leaves and roots of einkorn wheat.

Results are expected to provide much deeper understanding of miRNAs regulations and serve as a point of departure to investigate the mechanism of stress responsive miRNAs and for possible future use of miRNA mediated gene regulation to enhance plant stress tolerance.

3. MATERIALS AND METHODS

3.1 Plant Material, Growth and Treatment Conditions

In this study, seeds of six different einkorn wheat populations were obtained from Bolu Kalite Yem A.Ş, of which from different locations in Bolu and Kastamonu provinces (Table 3.1). The seeds were surface-sterilized in 70% ethanol and 30% sodium hypochlorite (NaClO), respectively, for 15 minutes, then rinsed twice in distilled water. After sterilizing, seed were germinated on two wet filter papers with adding 5 ml of half strength MS solution. Petri dishes was then kept for 10 days in a growth chamber under controlled conditions at 24 ± 2 °C with 16-h light and 8-h dark photoperiod. All stress treatments were initiated after 10 days of normal plant growth. Stress treatments were applied using -0.3 MPa (PEG-600) and 100 mM of sodium chloride (NaCl) for drought and salinity, respectively. PH was adjusted to 5.9 ±1 for all solutions. Both treated (stress) and non-treated (control) plants were kept under the same growth conditions in the growth chamber. Leaf and root samples from treated and non-treated control plants were harvested after 0, 3, 9, 12, and 24h of stress application and immediately frozen in liquid nitrogen. Time point zero (0 h) was used as a control.

Table 3.1. Seeds collection locations

Population No.	**Collection Location**
Population-6	Bolu, Seben-Kavaklıyazı region
Population-9	Kastamonu-İhsangazi-Çatalyazı Köyü.
Population-10	Kastamonu-İhsangazi-Uzunoğlu Mah
Population-11	Kastamonu-İhsangazi-Çay Mah
Population-14 and 15	Kastamonu- İhsangazi

3.2 Total RNA Isolation and Small RNA Sequencing

Wheat leaf and root frozen samples were submitted to Source BioScience Plc (Nottingham, UK) for RNA Isolation, small RNA library preparation, and sequencing using the Illumina MiSeq next generation sequencing platform.

3.2.1 RNA Extraction and Characterization

Total RNA was extracted from stress-treated and non-treated (control) wheat samples via mirVana miRNA Isolation Kit (Figure 3.1) according to the manufacturer's instructions (Life Technologies). Firstly, leaf and root tissues (approximately 100 mg) were ground to a fine powder in liquid nitrogen in an ice-cold mortar by grinding with a pestle. Using a prechilled metal spatula, the powdered tissue was scraped into the Lysis/Binding Buffer with mixing rapidly. Tissue lysate mix was then transferred by spatula into a vessel and 1/10 volume of miRNA Homogenate Additive was added for homogenization by using a motorized rotor-stator homogenizer.

The collected samples that were homogenized were kept on ice for 10 min and then extracted once by adding a volume of Acid-Phenol: Chloroform that is equal to the lysate volume before addition of the miRNA Homogenate Additive. The mixture was centrifuged for 5 min at maximum speed (10,000 x g) at room temperature to separate the aqueous and organic phases. The aqueous (upper) phase was removed without disturbing the lower phase which transferred into a fresh tube. At the end of this procedure, the Elution Solution (nuclease-free 0.1 mM EDTA) was preheated to 95°C and used for eluting of the RNA from the filter. The extract was further purified over a glass-fiber filter by Adding 1.25 volumes of 100% ethanol to the aqueous Phase at room temperature.

After that, the lysate/ethanol mixture was put into a filter cartridge and placed in centrifugation for about 15 seconds at 10,000 (rpm) to pass the mixture through the filter. The filter was then washed by applying 700 µL of miRNA Wash Solution (1) with centrifuging for approximatly (5 - 10) seconds, and washed again twice with 500 µL miRNA Wash Solution (⅔) followed by centrifuging for 1 minute to remove residual fluid from the filter.

Finally, the filter cartridge was transferred into a fresh collection tube and 100 µL of pre-heated (95°C) Elution Solution was applied to the center of the filter with Spinning for about (20 - 30) seconds at maximum speed to recover the RNA. The eluate (which contains the RNA) was collected and stored at (-20°C) for purification.

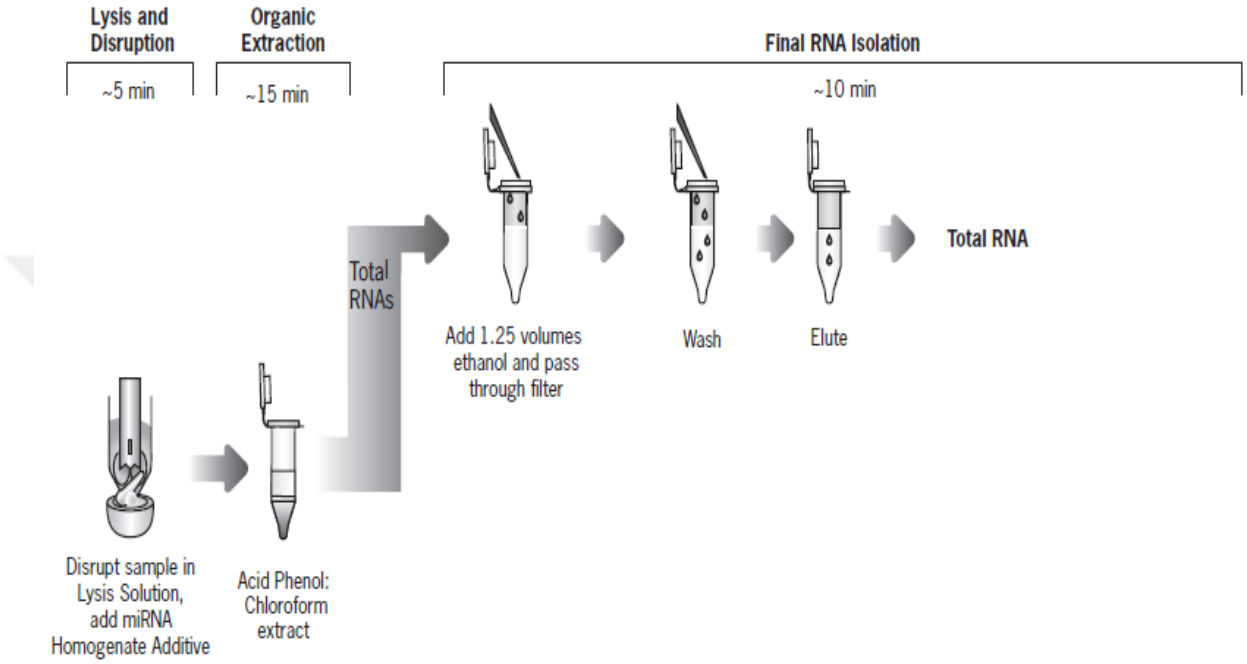


Figure 3.1. Overview of mirVana miRNA isolation kit procedure (Life Technologies, 2011)

Isolated nucleic acids were then quantified by using the Agilent 2100 Bioanalyzer system which provides automated sizing and quantitation information in a digital format to ensure that the quantity and quality of the material meets the specified criteria before progressing through the library preparation. After setting up the assay equipment and the bioanalyzer, Gel-dye mix, destaining solution, denaturing solution, and samples were prepared according to the Agilent protocol. The sample loaded chip was put into bioanalyzer and chip run was started and results were analyzed with 2100 expert software.

3.2.2 Construction of Small RNA Library and Deep Sequencing

The library was prepared according to the TruSeq Small RNA Sample Preparation Guide (Rev. C, March 2012). The 3` and 5` adapters were ligated to each end of the RNA molecule and a reverse transcription reaction was used to create single stranded cDNA. Then, cDNA fragments having adapter molecules on both ends underwent 11 cycles of PCR to amplify the amount of prepared material. The resulting library was validated diluted prior to sequencing. The library was then loaded at a concentration of 8pM onto an Illumina MiSeq Flow Cell. Samples were then sequenced using 50bp Paired-End runs.

3.3 Computational Sequence Analysis

Small RNA library sequence data was processed using Perl codes developed in functional genomics laboratory at Abant İzzet Baysal University to remove multiple reads, contaminants, and adaptor sequences. Another Perl code was then generated to blast small RNA sequences against a database of 30424 known mature miRNA sequences belonging to 203 different species that were downloaded in FASTA format via (ftp://mirbase.org/pub/mirbase/CURRENT/mature.fa.gz). To reduce the risk of false positives, our code was designed to select short sequences that having more than 90% identity. In order to extract miRNA precursor sequences for secondary structure studies, a Perl code was developed to blast small RNA sequences against the database for known mature microRNA sequences. Since the sequence data for *Triticum monococcum* chromosomes are not currently available, *T. urartu* chromosomal sequence data were downloaded from NCBI and used as a reference. The matching sequence was extracted in addition to 80 nucleotides upstream and downstream of the reference miRNA. Extracted miRNA precursor sequences was uploaded to RNA Folding form application at http://mfold.rna.albany.edu (Zuker et al,. 2003), where the default parameters were used to display secondary structures of the selected sequences.

4. RESULT AND DISCUSSION

4.1 Small RNA Sequencing

After deep sequencing, approximately 15,139,448 raw reads were processed by using a designated Perl codes to remove redundant sequences (the low quality reads, adaptor reads, and reads with length < 16 or length > 30). A total of 75,164 unique sequences were run in encoded Perl blast code against a database file containing all known miRNA sequences obtained from the NCBI database and formatted using Blastdb tool. The analysis has identified 167 putative mature miRNA sequences belonging to 142 miRNA families (detailed data analysis is given in Table S1 and S2). The read length of 18 nucleotides was the most abundant at 24.5% of the total miRNAs identified. This was followed by reads with lengths of 21 nucleotides (18.7%) and 19 nucleotides (16.7%), respectively (Figure 4.1).

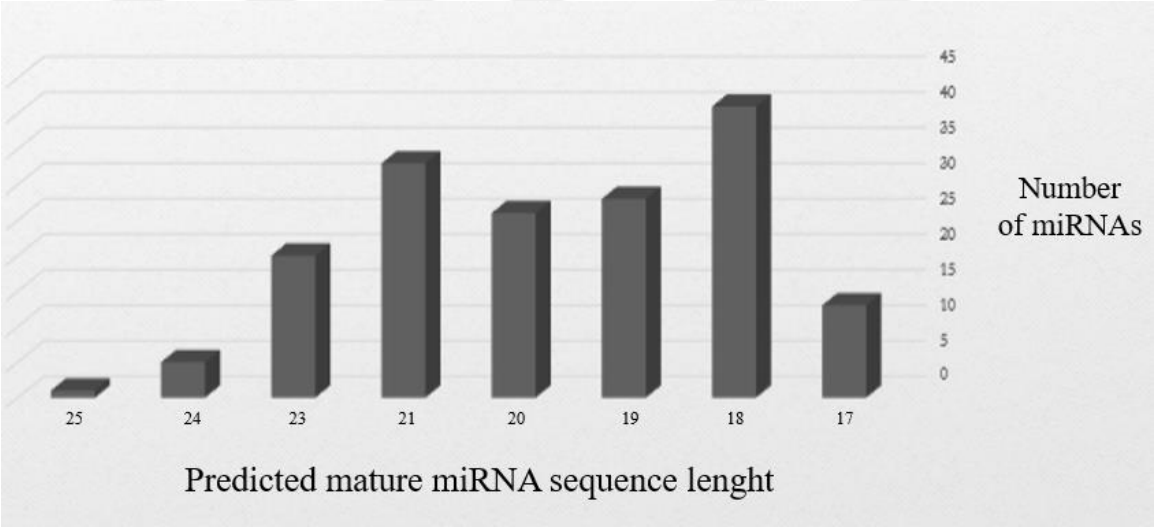


Figure 4.1. Nucleotide lengths of each identified miRNA sequences

Base distribution analysis at each position of the identified miRNA sequences has revealed that a uracil and guanine were the most abundant at the first and second position with 64 and 53 of the transcripts respectively (Figure 4.2).

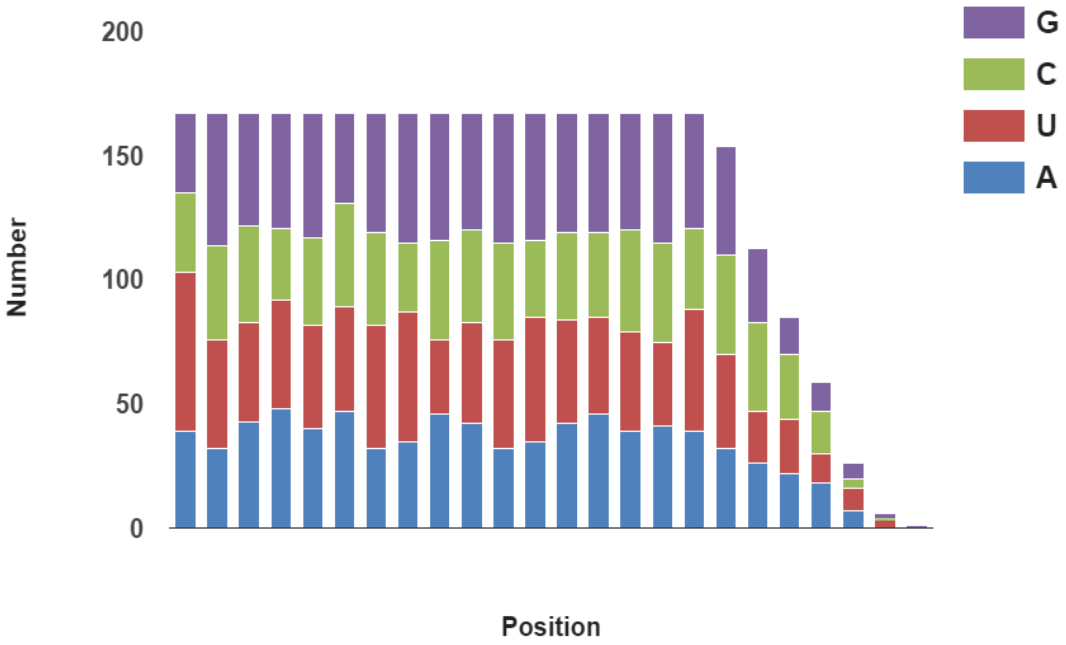


Figure 4.2. Base distribution at each position of the identified miRNA sequences

4.2 Validation of *T. monococcum* miRNAs by Secondary Structure Prediction

For secondary structure studies, a Perl code was developed to blast small RNA sequences against the database for known mature microRNA sequences. Since there is no available sequence data for *T. monococcum* chromosomes, *T. urartu* chromosomal scaffold sequence data was downloaded from NCBI and used as reference to extract precursor miRNA sequences. 1,455,436 scaffold sequences, with sequence lengths ranging from 50 to 82,078 nucleotides, were searched for 100% positive matches. In addition to the matching region, the sequence from the region ~80 nucleotides upstream of the start of the mature miRNA and ~80 nucleotides downstream of the miRNA was selected.

111 pre-miRNAs were analyzed using Mfold software, where the default parameters were used to display secondary structures of the selected sequences. In total, upstream and downstream nucleotide sequence information was obtained for 86 of the 111 *T. urartu* homologs. The failure in prediction of the secondary structure for the remaining 25 *T. monococcum* miRNAs was likely due to the incomplete and fragmented nature of the *T. urartu* scaffold sequences used for analysis.

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences

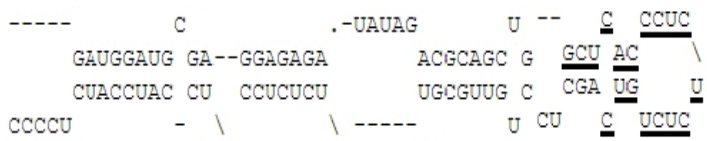
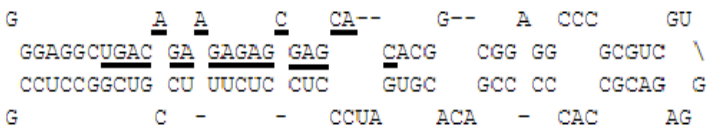
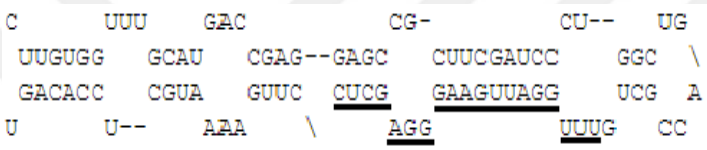
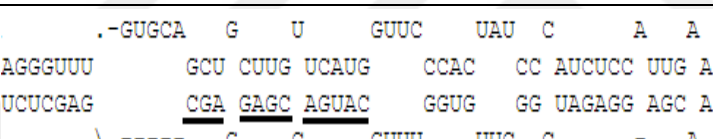
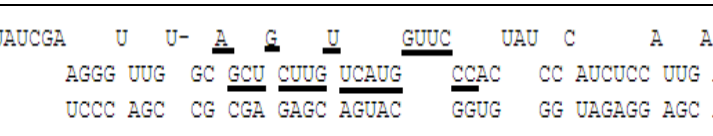
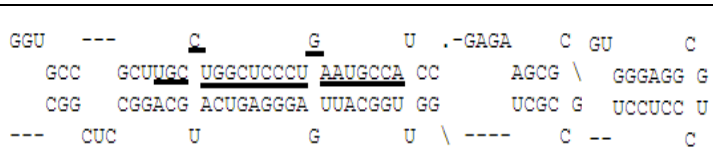
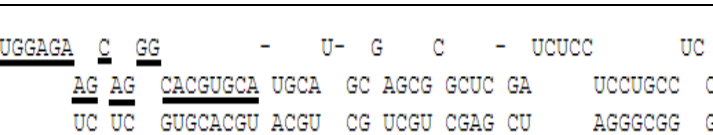
	Predicted Secondary Structure	Predicted miRNA Name
1		tmo-mir-156-3p
2		tmo-mir-156-5p
3		tmo-mir-159
4		tmo-mir-159-3p
5		tmo-mir-159-5p
6		tmo-mir-160
7		tmo-mir-164

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

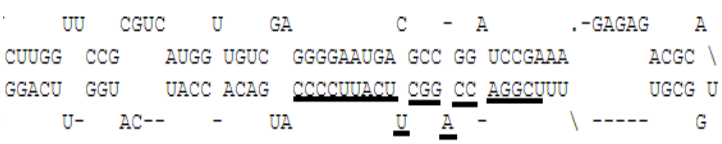
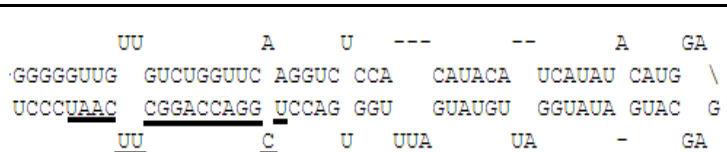
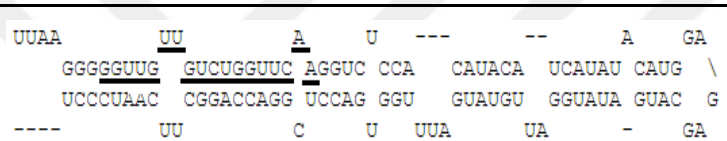
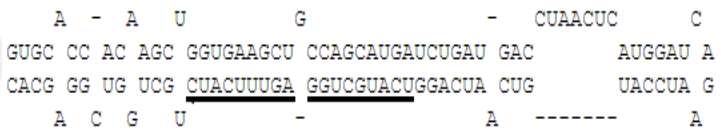
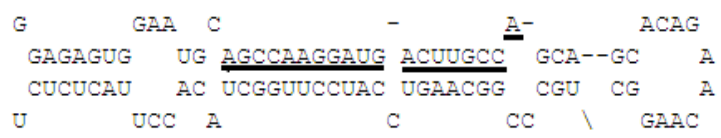
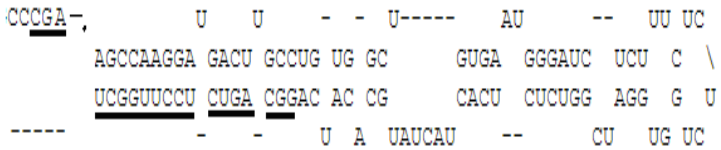
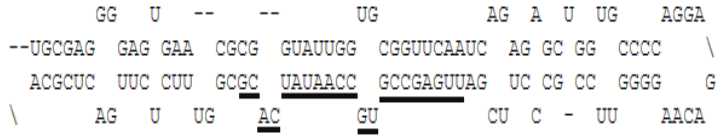
	Predicted Secondary Structure	Predicted miRNA Name
8		tmo-mir-166
9		tmo-mir-166-3p
10		tmo-mir-166-5p
11		tmo-mir-167-3p
12		tmo-mir-169
13		tmo-mir-169-3p
14		tmo-mir-171

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

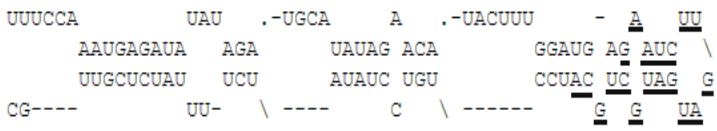
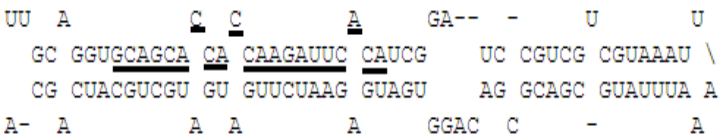
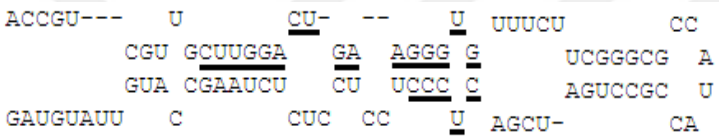
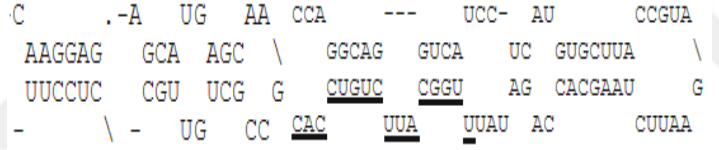
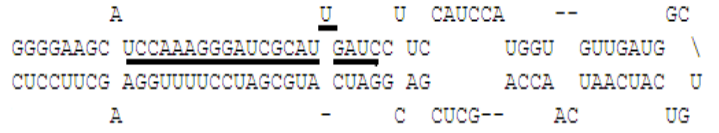
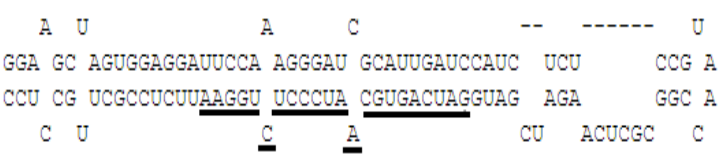
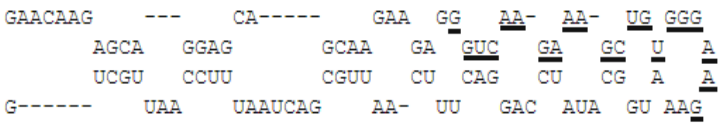
	Predicted Secondary Structure	Predicted miRNA Name
15		tmo-mir-172
16		tmo-mir-172-5p
17		tmo-mir-319-3p
18		tmo-mir-384-5p
19		tmo-mir-393
20		tmo-mir-393-3p
21		tmo-396-3p

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

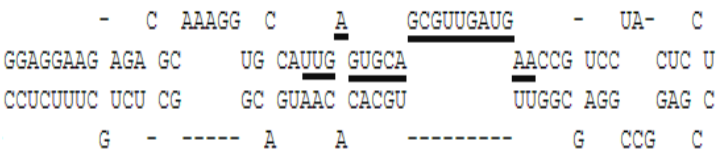
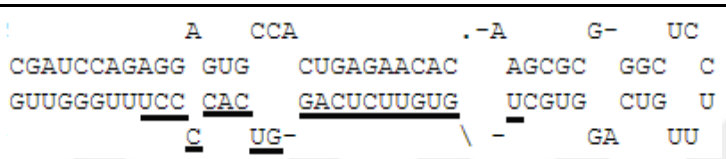
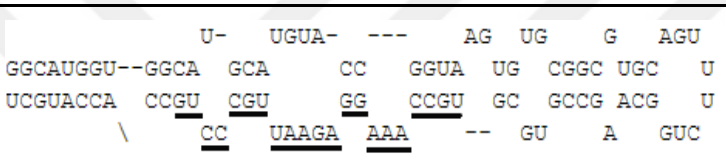
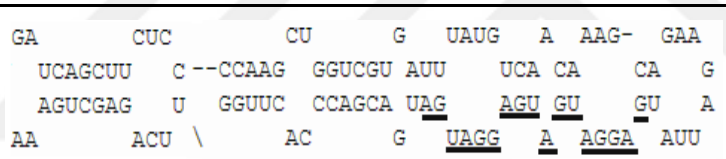
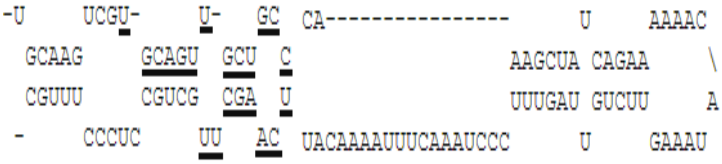
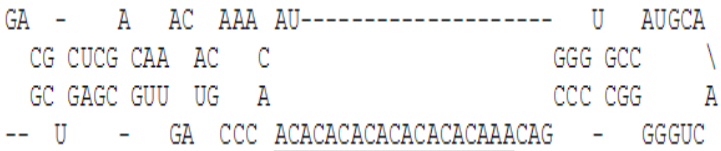
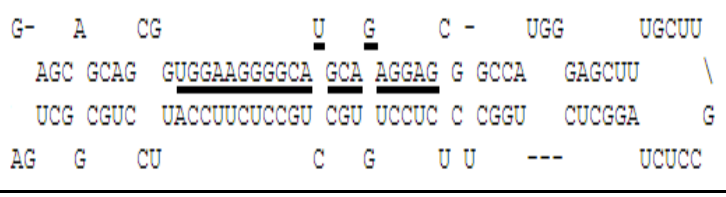
	Predicted Secondary Structure	Predicted miRNA Name
22		tmo-mir-397
23		tmo-mir-398
24		tmo-mir-399
25		tmo-mir-414
26		tmo-mir-444-3p
27		tmo-mir-466-5p
28		tmo-mir-528-5p

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

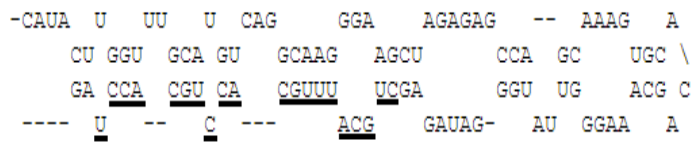
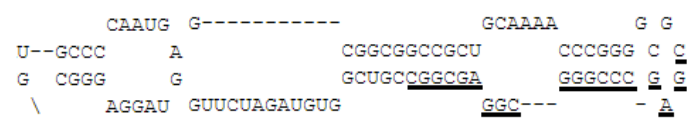
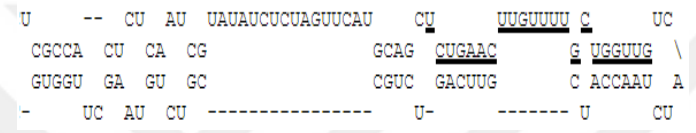
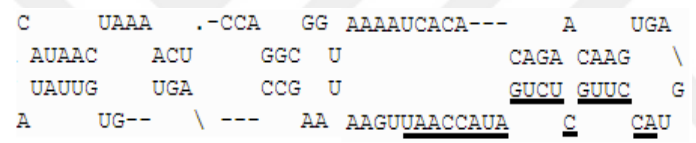
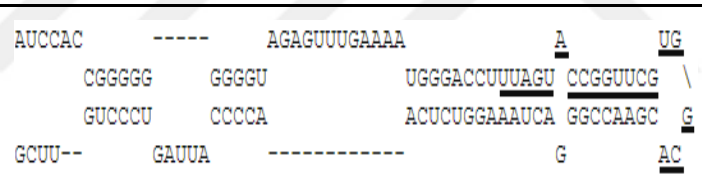
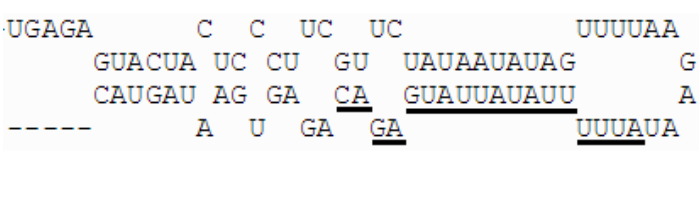
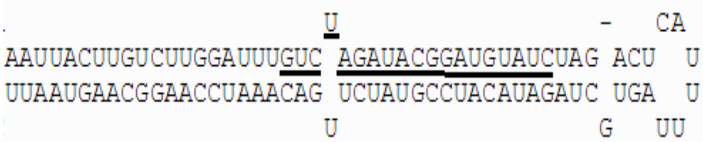
	Predicted Secondary Structure	Predicted miRNA Name
29		tmo-mir-530
30		tmo-mir-716
31		tmo-mir-827-5p
32		tmo-mir-845-5p
33		tmo-mir-1117
34		tmo-mir-1120
35		tmo-mir-1122

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

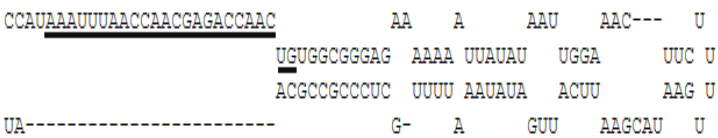
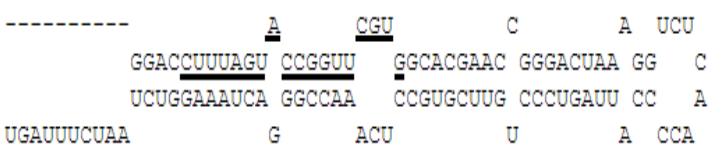
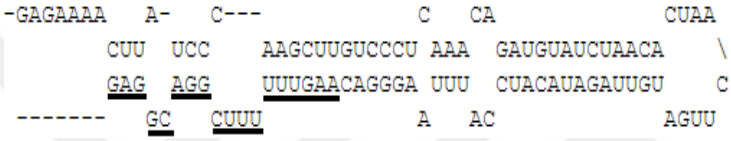
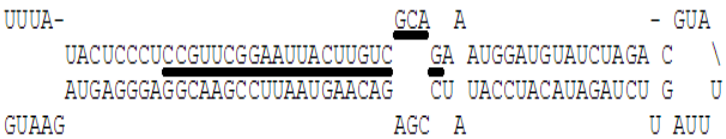
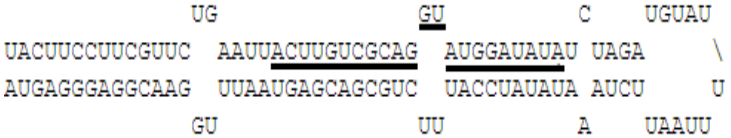
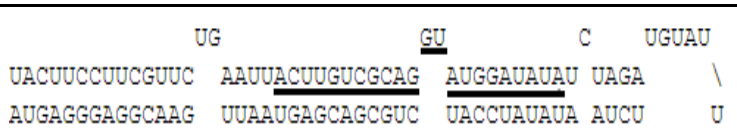
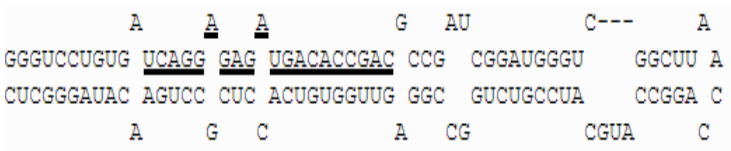
	Predicted Secondary Structure	Predicted miRNA Name
36		tmo-mir-1125
37		tmo-mir-1131
38		tmo-mir-1133
39		tmo-mir-1135
40		tmo-mir-1136
41		tmo-mir-1137
42		tmo-mir-1432-5p

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

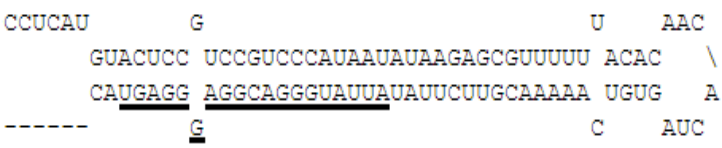
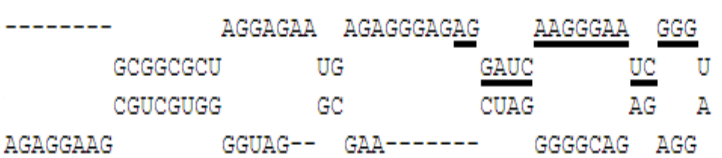
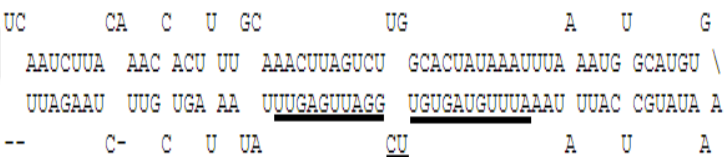
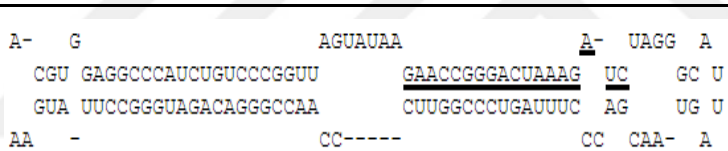
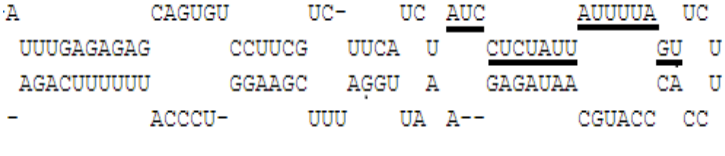
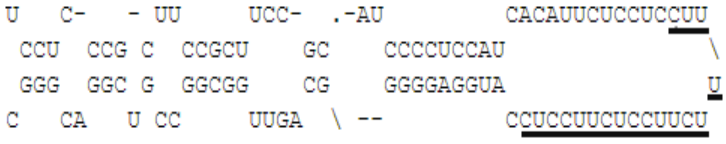
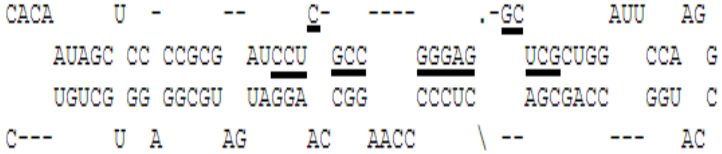
	Predicted Secondary Structure	Predicted miRNA Name
43		tmo-mir-1436
44		tmo-mir-1584
45		tmo-mir-1878-3p
46		tmo-mir-2120
47		tmo-mir-2538-5p
48		tmo-mir-2673
49		tmo-mir-3348

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

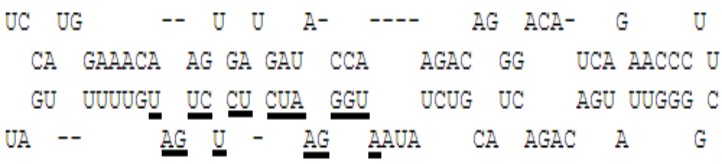
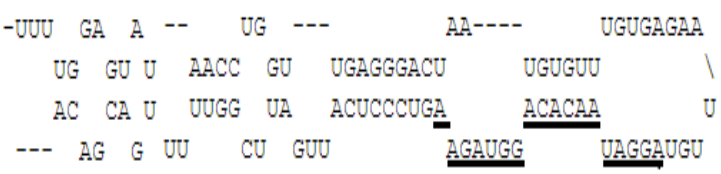
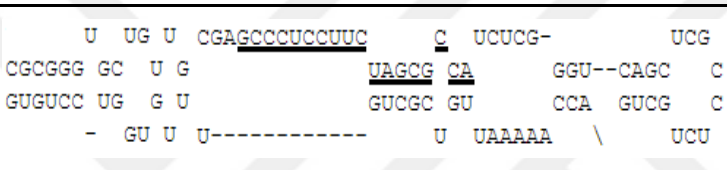
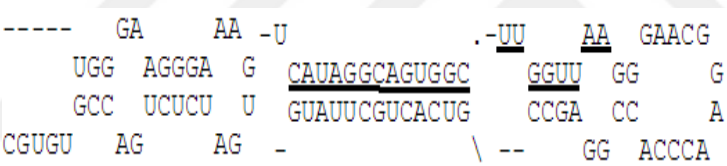
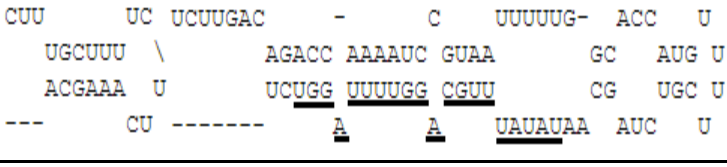
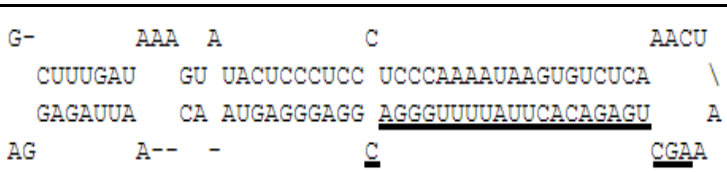
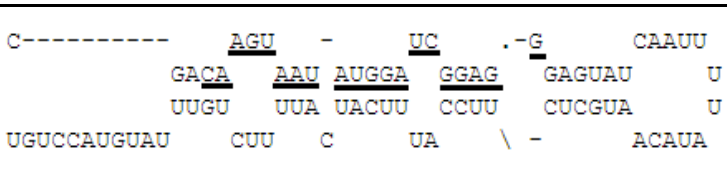
	Predicted Secondary Structure	Predicted miRNA Name
50		tmo-mir-3630-3p
51		tmo-mir-3682-5p
52		tmo-mir-3711
53		tmo-mir-4995
54		tmo-mir-5048
55		tmo-mir-5049
56		tmo-mir-5049-3p

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

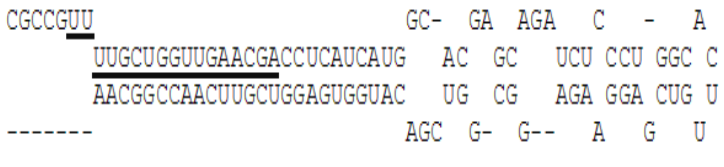
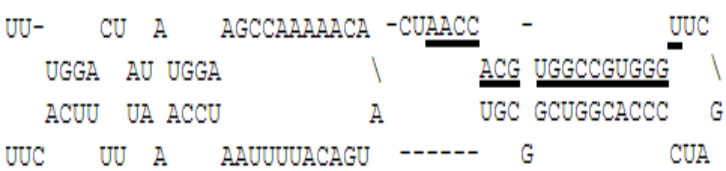
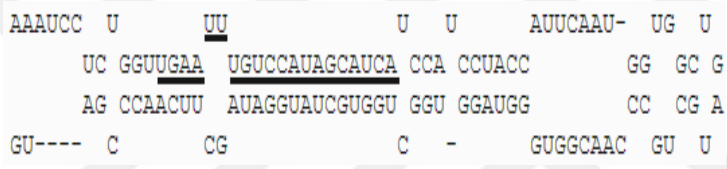
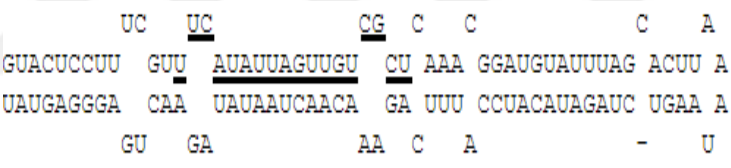
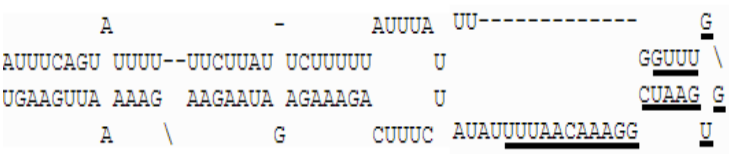
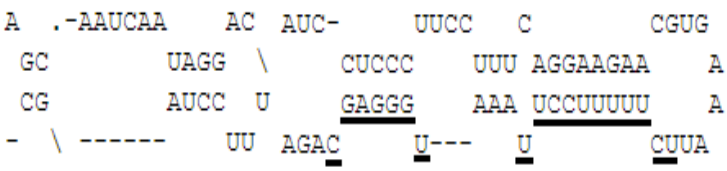
	Predicted Secondary Structure	Predicted miRNA Name
57		tmo-mir-5050
58		tmo-mir-5054
59		tmo-mir-5064
60		tmo-mir-5067
61		tmo-mir-5073
62		tmo-mir-5076

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

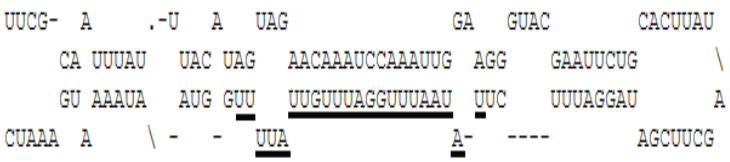
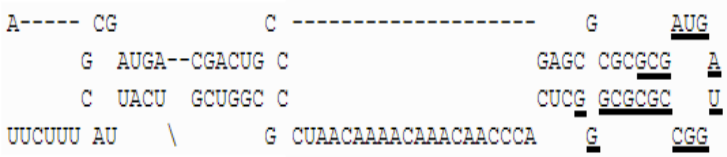
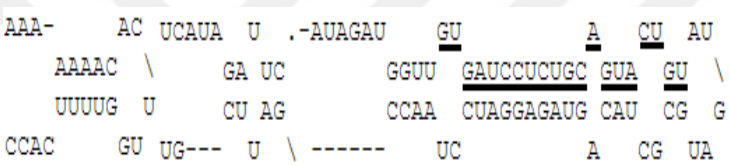
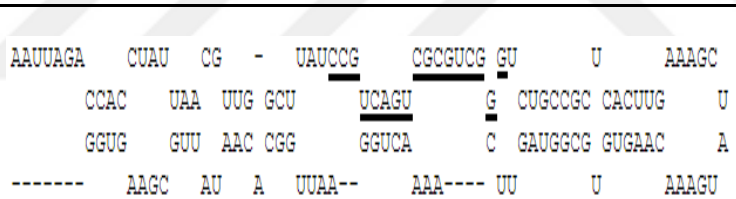
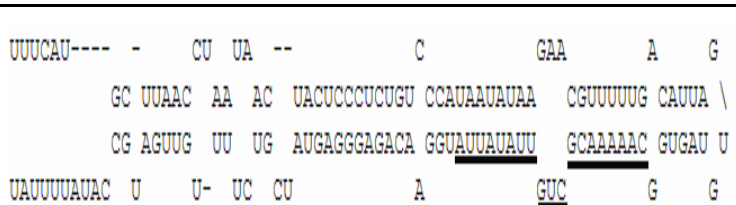
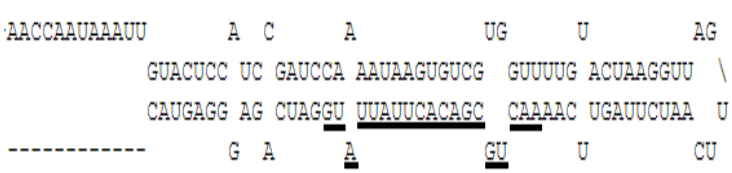
	Predicted Secondary Structure	Predicted miRNA Name
63		tmo-mir-5079
64		tmo-mir-5082
65		tmo-mir-5084
66		tmo-mir-5141
67		tmo-mir-5174-5p
68		tmo-mir-5181

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

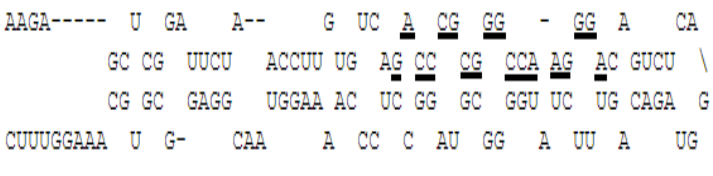
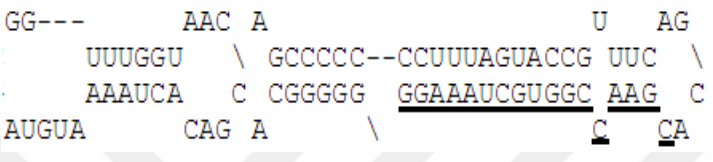
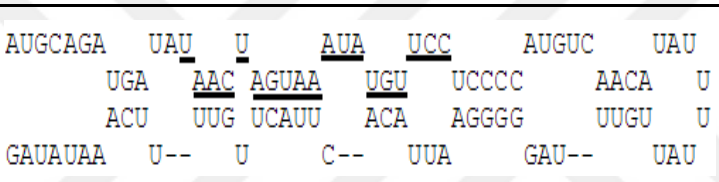
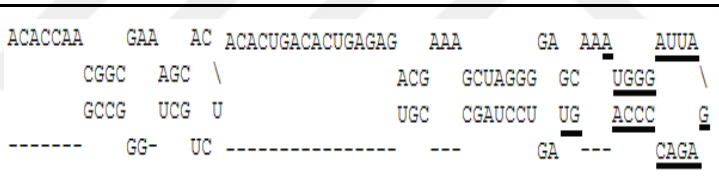
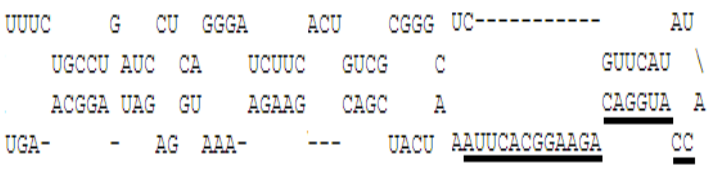
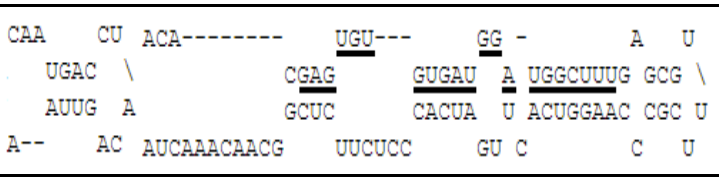
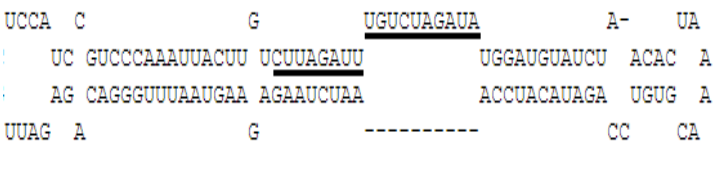
	Predicted Secondary Structure	Predicted miRNA Name
69		tmo-mir-5368
70		tmo-mir-5387
71		tmo-mir-5523
72		tmo-mir-6173
73		tmo-mir-6177
74		tmo-mir-6182
75		tmo-mir-6191

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

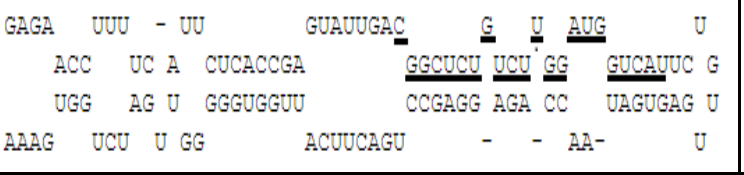
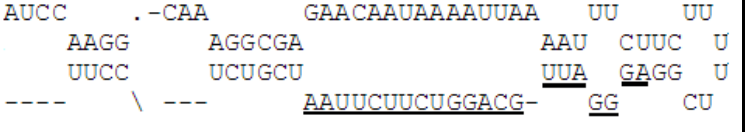
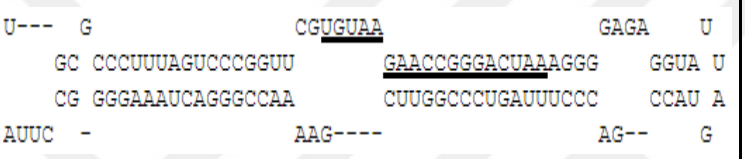
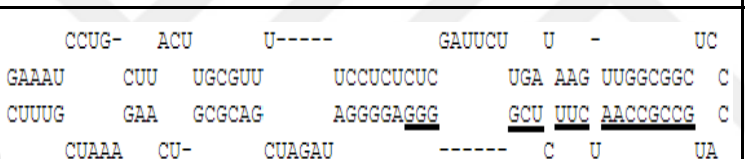
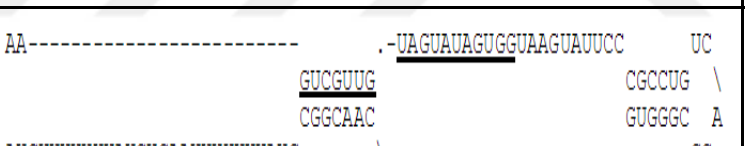
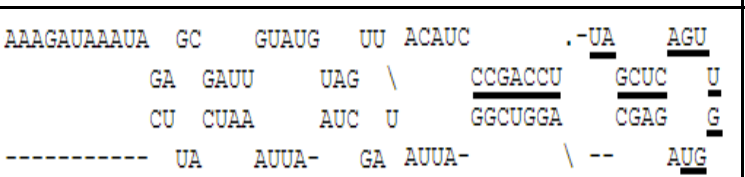
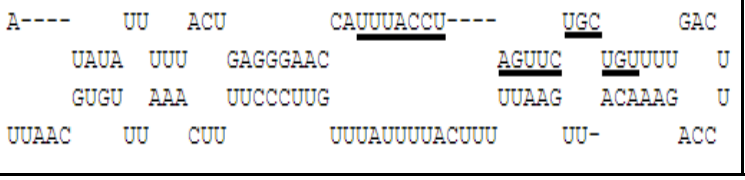
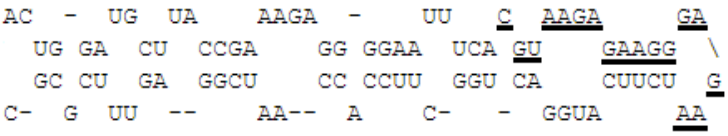
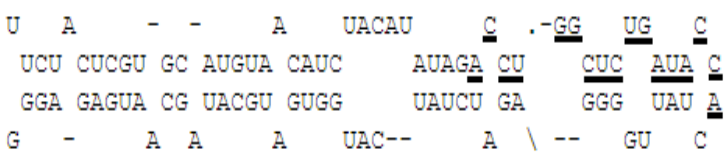
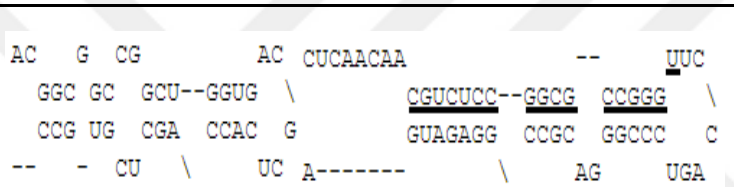
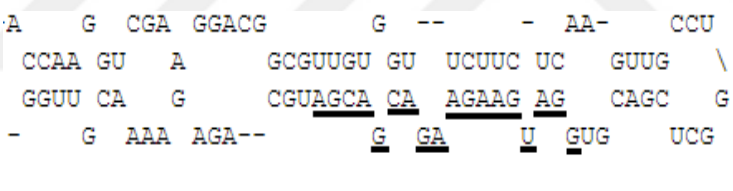
	Predicted Secondary Structure	Predicted miRNA Name
76		tmo-mir-6198
77		tmo-mir-6203
78		tmo-mir-6219-5p
79		tmo-mir-6250
80		tmo-mir-6300
81		tmo-mir-6478
82		tmo-mir-6874-3p

Table 4.1. Predicted secondary structure visualizations for putative *T. monococcum* miRNA sequences (continued)

	Predicted Secondary Structure	Predicted miRNA Name
83		tmo-mir-7398-3p
84		tmo-mir-8155
85		tmo-mir-B6-3p
86		tmo-mir-l5-3p

4.3. MicroRNAs Expression and Regulation Pathway under Salt and Drought Conditions

Recent studies have indicated that the expression of miRNAs, an important class of gene regulators, is altered by abiotic stress treatment (Ding D et al., 2009) in different plant species such as *Arabidopsis* (Sunkar and Zhu et al., 2004), rice (Zhou et al., 2010), cowpea (Barrera-Figueroa et al., 2011), tobacco (Frazier et al., 2011), soya bean (Kulcheski et al., 2011), and common bean (Arenas-Huertero et al., 2009).

According to our result, a number of conserved miRNAs in *T.monococcum* were proved to be expressed differently *in Arabidopsis* under salt and drought stress. For instance, miR156, miR159, miR167, miR169, miR171, miR319, miR393, miR396, and miR397 were up-regulated under salt stress (Liu et al., 2008). On the other hand, miR156, miR159, miR167, miR171, miR172, miR319, miR393, miR396 and miR397 were up-regulated, while miR169, miR171a and miR319c were down-regulated in response to drought stress (Liu et al., 2008, Sunkar and Zhu et al., 2004).

Moreover, another study has reported that, two members of miR169 family in wheat was upregulated during salinity and down-regulated in drought conditions (Stephenson et al., 2007).

Interestingly, other studies has showed that, the same miRNA in the same plant species can be differentially expressed in response to the same stress For instance, members of the miR319 family (expressed in *T. monococcum*) were reported to be up regulated and down regulated in response to drought stress in rice (Zhou et al., 2010). Furthermore, according to Trindade and Capitaoin (2010), it was confirmed that, two members of miR398 family in *Medicago truncatula* was upregulated during drought stress (Trindade et al., 2010), while another study has showed that the same miRNA in *M. truncatula* was down regulated under similar conditions (Wang et al., 2011).

MicroRNAs are also differentially expressed between different tissues or developmental stages under drought stress (Reinhart et al., 2002). This has been the case for miR169 which was identified in *T. monococcum*, it was induced more prominently in the roots of rice compared to its shoots (Reinhart et al., 2002).

Many studies were concerned with the identification and characterization of miRNAs in wheat (*Triticum aestivum*), the close relative to *T. monococcum* by using computational and experimental approaches (Bharati P et al., 2013, Chun S et al., 2014, Han R et al., 2014). In a recent study that has examined two genotypes of wheat (*Triticum aestivum*), a total of 14 upregulated conserved miRNAs and 6 conserved down-regulated miRNAs were identified under drought stress (Xingli et al., 2015).

Furthermore, the expression levels of miR160, miR164, miR166, miR169 and miR444 were analyzed with predicting the possible regulatory mechanism involving differentially expressed miRNAs and their target genes in two wheat genotypes under drought conditions (Figure 3.3) (Xingli et al., 2015). Because these miRNAs were also extracted in our study, this result may provide better understanding of miRNAs function in *T. monococcum* under drought and salt conditions.

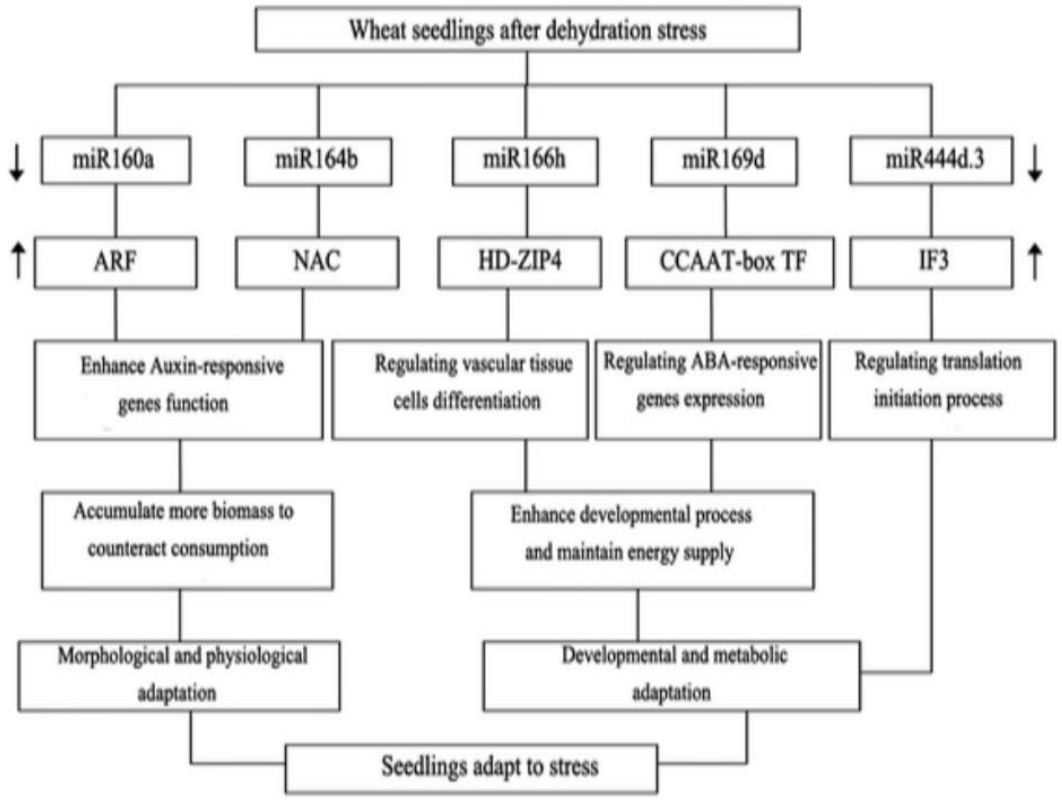


Figure 4.3. Regulatory mechanism of differentially expressed miRNAs in *Triticum aestivum* under drought stress (Xingli et al., 2015)

According to Xingli (2015), mir160a (member of mir160 family) is down-regulated in response to drought conditions and repress the expression of its target ARF, leading to many physiological and morphological mechanisms, mainly by enhancing root and leaf development and this causes stress adaptation (Guilfoyle T et al., 2007). The target of miR164b is the NAC transcription factor. As reported previously in *Arabidopsis*, the overexpression of this NAC transcription factor produces larger leaves, thicker stems and more abundant roots (Xie Q et al., 2000). Another microRNA is miR166h, it is a member of the miR166 family and its target is the Class III HD-ZIP protein 4 gene which works by changing leaf polarity and controls cambium activity by controlling axial cell elongation and xylem differentiation (Juarez M et al., 2004; Ilegems M et al., 2010). Furthermore, miR169d which overexpressed the expression of its target, the CCAAT-box transcription factor (CCAAT-box TF). This can influence ABA-responsive transcription and lead to enhanced dehydration stress tolerance (Xingli et al., 2015). The putative target of miR444d.3 is encoding a translation initiation factor 3 (IF3) gene. MiR444d.3 was reported to be down-regulated in the drought-tolerant cultivar, proving that IF3 is also involved in dehydration stress tolerance in wheat (Xingli et al., 2015)

MicroRNAs are well conserved in plants and animals and are thought to be evolutionarily and vital ancient element of genetic regulations (Tanzer and Stadler et al., 2004). Conserved miRNAs have a nearly identical sequences and as a result have a common targets but the major difference between them is that their expression levels differs under different environmental stress (Ason and Darnell et al., 2006). A total of 11 conserved identified miRNAs in our study were also reported to be differently expressed under drought and salinity conditions in many different plant species (Table 4.2).

Table 4.2. Conserved miRNAs identified in *T. monococcum* and reported previously in other plant species (Jannatul et al., 2015)

miRNA Name	**Plant Species**	**Target Name and Functions**	**Reference**
miR159	*Ath, Rice, Ppe*	MYB and TCP transcription factors — ABA response, Nacl stress response, floral asymmetry and leaf development	Arenas-Huertero et al. (2009), Eldem et al. (2012), Jones-Rhoades and Bartel et al. (2004), Liu et al. (2008), Reyes and Chua (2007), and Zhou et al. (2010)
miR160	*Ppe, Pto, Ptc*	ARF 10, ARF 16 and ARF 17—seed germination and post-germination stages	Eldem et al. (2012), Jones-Rhoades and Bartel (2004), Liu et al. (2007), Ren et al., (2012), and Shuai et al. (2013)
miR164	*Mtr, Ptc, Tdi*	NAC domain TF—lateral root development	Shuai et al. (2013) and Wang et al. (201-
miR166	*Tdi, Gma*	HD-ZIPIII transcription factor—axillary meristem initiation, leaf and vascular development	Kantar et al. (2011), Li et al. (2011a,b), Sun et al (2012), and Williams et al. (2005)
miR169	*Ath, Tomato, Rice, Mtr, Ppe, Gma, Pto, Peu*	NF-YA transcription factor subunit A-3, NF-YA transcription factor subunit A-10, SlMRP1—Plant development and Flowering timing, response to different abiotic stresses	Eldem et al. (2012), Li et al. (2008), Li et al. (2011a,b), Qin et al. (2011), Ren et al. (2012), Trindade et al. (2010), Wang et al. (2011), Zhang et al. (2011), Zhao et al. (2007), and Zhou et al. (2010)
miR171	*Ath, Tdi, Rice, Mtr, Ppe, Pto*	GRAS transcription factors—response to abiotic stresses and floral development	Eldem et al. (2012), Kantar et al. (2011), Llave et al. (2002), Liu et al. (2008), Ren et al. (2012), Wang et al. (2011), and Zhou et al. (2010)

Table 4.2. Conserved miRNAs identified in *T.monococcum* and reported previously in other plant species (Continued) (Jannatul et al., 2015)

miRNA Name	**Plant Species**	**Target Name and Functions**	**Reference**
miR172	*Ath, Rice, Pto*	cDNA floral homeotic protein APETALA2, bZIP transcription factor family protein—flowering time, floral organ identity, cold stress response	Jones-Rhoades and Bartel (2004), Ren et al. (2012). and Zhou et al. (2010)
miR393	*Ath, Ppe*	TIR1 and AFB2 and AFB3—susceptibility to virulent bacteria	Liu et al. (2008), Navarro et al. (2006), and Eldem et al. (2012)
miR397	*Ath, Rice, Ppe, Pto*	Laccases—lignin biosynthesis, ion absorption and stress response	Abdel-Ghany and Pilon (2008), Ding and Zhu (2009), and Eldem et al. (2012)
miR398	*Mtr, Tdi, Mtr, Ppe*	Copper superoxide dismutases; cytochrome C oxidase subunit V—Copper homoeostasis, oxidative stress; enzyme involved in respiration	Ren et al.(2012), Sunkar and Zhu (2004), and Zhou et al. (2010)
miR399	*Mtr, Pto*	Phosphate transporter–role in response to phosphate starvation	Bari et al. (2006), Jones-Rhoades and Bartel (2004), Ren et al. (2012), and Wang et al. (2011)

* *Ath*, *Arabidopsis*; *Gma*, *Glycine max*; *Mtr*, *Medicago truncatula*; *Peu*, *Populus euphratica*; *Ptc*, *Populus trichocarpa*; *Pto*, *Populus tomentosa*;*Ppe*, *Prunus persica*; *Tdi*, *Triticum dicoccoides.*

5. CONCLUSION AND RECOMMENDATIONS

Wheat is one of the most important agricultural crop species in Turkey, which makes functional genomics studies of this crop very important. According to miRNA database (miRbase), large number of miRNA has been identified in many model crops but only few miRNAs are reported in wheat till date which is very less compared with the other plant miRNAs. We have identified 167 putative miRNA sequences expressed in *T. monococcum* under drought and salt stress. These are the first and the largest number of expressed miRNA sequences directly isolated, identified and characterized by a comparative genomics approach in *T. monococcum*. The identification of these sequences will greatly improve the miRNA database for wheat and research efforts focusing on epigenetic regulation in this model organism.

Drought and salinity are a significant problems affecting physiological, biochemical and molecular processes of plants and is predicted to become a larger problem in the coming decades. The detrimental effects of these abiotic stresses on plants can be observed at the whole-plant level or in the cellular level in terms of plant death and/ or decrease in productivity. One way in which plants respond to abiotic stress is by modifying their gene expression through the post transcriptional gene regulation. MiRNAs as gene regulators are an important components in the regulation of many drought and salt responsive genes.

Several studies have reported that drought and salinity stresses are responsible for altering the level of miRNAs expression in many plant species. Therefore, understanding how miRNAs regulate gene expression will enable researchers to explore the role of miRNAs in drought and salinity stress response in different plant species.

Currently, next generation sequencing and bioinformatics tools are widely used as a rapid, accurate and affordable method to identify miRNAs. This approach has been very effective in plants with sequenced genomes but limited for other plant species with unknown genome. Therefore, *T. monococcum* complete genome data set will be required for the identification of additional miRNAs for this model organism.

Furthermore, the identification of entire sets of miRNAs and their targets will provide the fundamental that is required to discover the gene regulatory networks of miRNAs during plant development and other physiological processes such as abiotic stress and will greatly increase our understanding of plant tolerance to abiotic stress. Despite the existing and increasing data relating the miRNAs, a lot more remains to be known in terms of the identification and characterization of miRNAs in different plant species under drought and salinity conditions.

Understanding miRNA mediated stress regulatory networks will provide new tools for the genetic improvement of salt and drought tolerance in plants. Although this field is still in its infancy, the idea that miRNAs can be used in the therapy of plant stress is certain. If miRNAs can be used appropriately, a new avenue of biotechnology aimed at achieving enhanced drought and salt tolerant plants will be opened.

There are few reports showing that manipulation of miRNA guided gene regulation can help in the engineering of stress resistant plants. In a recent study, artificial miRNAs (amiRNAs) were used as a target stress-responsive mRNAs and designed to knock down nuclear cap-binding protein 80 [CBP80; also known as Abscisic Acid Hypersensitive 1 (ABH1)] in potato (Pieczynski et al. 2013). The results of this study showed that transgenic potato plants exhibited a higher tolerance to drought stress, particularly due to an increase in leaf stomata and trichome density, ABA-hypersensitive stomatal closing, and compact cuticle structures containing a lower number of micro-channels (Pieczynski et al., 2013).

Nevertheless, understanding of miRNA evolution is just at the starting point for elucidating their complex regulatory roles. As our understanding of the roles of miRNAs during salt and drought stress deepens, the possibilities for using miRNA mediated gene regulation to enhance plant stress tolerance will become enormous. Therefore, the identification and characterization of miRNA homologs in *Triticum monococcum* will provide much deeper understanding of miRNAs regulations and serve as a point of departure to investigate the mechanism of stress responsive miRNAs and for possible future use of miRNA mediated gene regulation to enhance wheat stress tolerance.

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APPENDICES

7. APPENDICES

Table S1. Raw data after miRNA sequence analysis

	miRNA Name	Sequence ID	Mature miRNA Sequence	S.L	C.N
1	miR6300	ESU_188	GUCGUUGUAGUAUAGUGG	18	1
2	miR894	ESU_312	UUCGUUUCACGUCGGGUUCA	20	2
3	miR5048	ESU_999	UAUAUUUGCAGGUUUUAGGU	20	1
4	miR6478	ESU_1153	CCGACCUUAGCUCAGUUGGU	20	1
5	miR716	ESU_1634	CGAGCCCGGGCGGAGCGGC	19	1
6	miR5062	ESU_1667	UGAACCUUGGGGAAAAGCCG	20	2
7	miR167-3p	ESU_2966	UCAUGCUGGAGUUUCAUC	18	13
8	miR159	ESU_2993	UUUGGAUUGAAGGGAGCUCUG	21	1
9	miR396	ESU_3211	UCCACAGGCUUUCUUGAACU	20	1
10	miR168-5p	ESU_3218	UUGCUUGGUGCAGAUCGGG	19	1
11	miR456-5p	ESU_3536	UGCACUGCCUUCAGAGUG	18	1
12	miR1273	ESU_4014	AAUGAUUCGAUCUCGACUCACU	22	1
13	miR1120	ESU_4283	AUUUUUAUAUUAUGAGAC	18	1
14	miR5368	ESU_4299	GACCCGCGGGCCAAGGGA	18	1
15	miR166	ESU_4331	UCGGACCAGGCUUCAUUCCCC	21	1
16	miR6203	ESU_4984	AGGGAUUGCAGGUCUUCUUAA	21	1
17	miR6198	ESU_5192	CGGCUCUGUCUUGGAUGGUCAU	22	2
18	miR168-3p	ESU_5229	CCCGCCUUGCACCAAGUG	18	13
19	miR444	ESU_5662	UGCAGUUGCUGUCUCAAGC	19	1
20	miR5054	ESU_5940	AACCACGUGGCCGUGGGU	18	1
21	miR4995	ESU_6284	CAUAGGCAGUGGCUUGGUUAA	21	6
22	miR8155	ESU_6363	ACCUGGCUCUGAUACCA	17	6
23	miR319-3p	ESU_8347	CUUGGACUGAAGGGUGCUCCC	21	46
24	miR1133	ESU_8555	AAGUUUUUUCGGACGGAG	18	1
25	miR5073	ESU_9648	GUUUGGUGAAUCGGAAACAAUUU	23	1
26	miR171-5p	ESU_10224	UGGUAUUGUUUCGGCUCAU	19	1
27	miR395-5p	ESU_10245	UGAAGUGUUUGAGGGAAC	18	1
28	miR168	ESU_11772	UCGCUUGGUGCAGGUCGGGAA	21	2
29	miR5067	ESU_12254	UUCAUAUUAGUUGUCGCU	18	1

Table S1. Raw data after miRNA sequence analysis (continued)

	miRNA Name	Sequence ID	Mature miRNA Sequence	S.L	C.N
30	miR5538	ESU_18681	ACUGUUGAGUAACGGCAGCAAG	22	1
31	miR827	ESU_18892	UUAGAUGACCAUCAGCAAACA	21	1
32	miR390-3p	ESU_18966	GCUAUCUAUCCUGAGCUCC	19	2
33	miR1136	ESU_20921	ACUUGUCGCAGGUAUGGAUAUA	22	2
34	miR1135	ESU_22024	CCGUUCGGAAUUACUUGUCGCAG	23	1
35	miR1436	ESU_22210	AUUAUGGGACGGAGGGAGU	19	1
36	miR5083	ESU_22605	UAUUUAGUGUUGACCAAAUU	20	1
37	miR-B6-3p	ESU_24038	CGUCUCCGGCGCCGGGU	17	1
38	miR5523	ESU_24413	UAACUAGUAAAUAUGUUCC	19	1
39	miR5072	ESU_24437	UUCUGGGUUCGUUCCCCAG	19	4
40	miR5141	ESU_24712	CCGUCAGUCGCGUCGGG	17	1
41	miR393	ESU_25179	UCCAAAGGGAUCGCAUUGAUC	21	46
42	miR99	ESU_27634	CACCCGUAGAACCGACCUUGC	21	1
43	miR6981-5p	ESU_28260	AGAGGAGAAGGAAGAAGCUGAA	22	1
44	miR466-5p	ESU_30084	AACACACACACACACACAC	19	1
45	miR319	ESU_30821	UUGGACUGAAGGGAGCUCC	19	2
46	miR3711	ESU_31042	GCCCUCCUUCUAGCGCCA	18	3
47	miR619-5p	ESU_31360	GCCUCGGCCUCUCAAAGUGCUG	22	1
48	miR1285	ESU_31832	CAGAGGUUGCAGUGAGUGGA	20	1
49	miR5106	ESU_34202	GGGUCUGUAGCUCAGUUG	18	1
50	miR166-3p	ESU_34590	UCGGACCAGGCUUCAAU	17	1
51	miR171	ESU_34608	UUGAGCCGUGCCAAUAUCACG	21	3
52	miR156-3p	ESU_35145	GCUCACCCUCUCUCUGUCAGC	21	16
53	miR398-3p	ESU_35460	GUGUUCUCAGGUCGCCCCUG	20	1
54	miR167	ESU_36962	UGAAGCUGCCAGAAUGAUCUGA	22	1
55	miR6173	ESU_37472	AUGGGAUUAGAGACCCCAGU	20	1
56	miR2538-5p	ESU_38638	AUCCUCUAUUAUUUUAGU	18	1
57	miR159-3p	ESU_38693	UUUGCAUGACCGAGGAGC	18	1
58	miR156	ESU_38830	UGACAGAAGAGAGUGAGCAU	20	5
59	miR169	ESU_44650	AGCCAAGGAUGACUUGCCA	19	1
60	miR6219-5p	ESU_48455	UGUAAGAACCGGGACUAA	18	1
61	miR479	ESU_49645	UGAGCCGAACCAAUAUCACUC	21	1

Table S1. Raw data after miRNA sequence analysis (continued)

	miRNA Name	Sequence ID	Mature miRNA Sequence	S.L	C.N
62	miR164	ESU_51471	UGGAGAAGCAGGGCACGUGCA	21	7
63	miR5079	ESU_55792	UAUAAUUUGGAUUUGUUAUUUU	22	1
64	miR5585-3p	ESU_61480	CCAGGCAAGGUGGCGGGCACCU	22	1
65	miR11214	ESU_64517	UAGUGAUCUAAACGCUCUUA	20	1
66	miR1273-3p	ESU_66448	GUCCUGCUCUGUCACCCA	18	1
67	miR160	ESU_68248	UGCCUGGCUCCCUGAAUGCCA	21	1
68	miR528-5p	ESU_71805	UGGAAGGGGCAUGCAGAGGAG	21	2
69	miR5084	ESU_75342	GUGAUCCUCUGCAGUACUGU	20	1
70	miR5096	ESU_76359	AGACAGGGUUUCACCAUGUUG	21	1
71	miR706	ESU_77016	CCAGGGCUAUACAGAGAAACAC	22	1
72	miR396-3p	ESU_80403	GGUCAAGAAAGCUGUGGGAAG	21	4
73	miR6199	ESU_81522	CCACAGAAUUCUCACAGU	18	1
74	miR5082	ESU_85872	GCGAUGAUGGCCGCGCGGG	19	2
75	miR3682-5p	ESU_91792	AGGAUAACACAGGUAGAA	18	1
76	miR1122	ESU_93304	GUCUAGAUACGGAUGUAUC	19	1
77	miR172	ESU_94151	GAAUCUUGAUGAUGCUGCA	19	2
78	miR1137	ESU_95565	AGUUAGUACAAAGUUGAG	18	1
79	miR1117	ESU_96037	UUAGUACCGGUUCGUGGCA	19	1
80	miR169-3p	ESU_96600	GGCAGUCUCCUUGGCUAGC	19	4
81	miR5064	ESU_100842	UGAAUUUGUCCAUAGCAUCA	20	38
82	miR5174-5p	ESU_101659	CAAAAACGCUGUUAUAUUA	19	1
83	miR6253	ESU_109761	AGGAAAGUGGGCAGUUGGG	19	1
84	miR1139	ESU_117049	AUGUUACUAGUGUAUGUUACUC	22	1
85	miR6204	ESU_120188	AGAAAUGGAAAGGAGAAUAAUU	22	1
86	miR166-5p	ESU_122546	GGUUGUUGUCUGGUUCAA	18	2
87	miR5076	ESU_124276	UCUUUUUCCUUAAAUGGGAGC	21	1
88	miR5532	ESU_136944	UAUGGAAUAUAUGACAAAGGUG	22	1
89	miR529	ESU_138405	AGAAGAGAGAGAGUACAGCCC	21	1
90	miR1432-5p	ESU_140524	UCAGGAGAGAUGACACCGAC	20	2

Table S1. Raw data after miRNA sequence analysis (continued)

	miRNA Name	Sequence ID	Mature miRNA Sequence	S.L	C.N
91	miR444-3p	ESU_143911	UGCAGUUGCUGCCUCAAGCUU	21	8
92	miR3630-3p	ESU_157727	AUGGGAAUCUCUCUGAU	17	31
93	miR30-5p	ESU_163881	UGUAAACAUCCUCGACUGGA	20	1
94	miR5059	ESU_166069	CGAGCCUGGGCAGCACC	17	1
95	miR1584	ESU_169449	AGGAUCAAGGGAAUCGGG	18	1
96	miR6181	ESU_169510	UGCUCUUCAUGGACUGCGGCGC	22	1
97	miR398	ESU_176762	UGUGUUCUCAGGUCACCCCU	20	1
98	miR3885-5p	ESU_182073	UGCUGAGCGGCGGCCGCCG	19	1
99	miR5387	ESU_183241	CGAACCGGUGCUAAAGGA	18	1
100	miR827-5p	ESU_184639	UCUGAACUUGUUUUGCUGGUUG	22	1
101	miR395	ESU_189670	UGAAGUGUUUGGGGGAACUCC	21	1
102	miR1125	ESU_189686	AAAUUUAACCAACGAGACCAACUG	24	1
103	miR5571-5p	ESU_201403	AUGUGAACCAAGCAAUUCUCA	21	1
104	miR5049	ESU_209639	AGCUGAGACACUUAUUUUGGGAC	23	1
105	miR928	ESU_217281	GUGGCUGUGGAAGCUGG	17	1
106	miR399	ESU_223279	UGCCAAAGGAGAAUUGCCCUG	21	3
107	miR6191	ESU_231217	CUUAGAUUUGUCUAGAUA	18	1
108	miR5181	ESU_249726	AACUGCGACACUUAUUAUG	19	1
109	miR159-5p	ESU_261500	AGCUGCUUGUUCAUGGUUCCC	21	1
110	miR22	ESU_261767	AAGCUGCCAGUUGAAGAACUGU	22	1
111	miR394	ESU_265170	UUGGCAUUCUGUCCACC	17	5
112	miR-I5-3p	ESU_265941	GGAUGAAGAAGACGACGA	18	1
113	miR319-5p	ESU_266923	AGAGCGUCCUUCAGUCCACU	20	3
114	miR5056	ESU_267588	UCGGGAGGAAGAACCGGUAAU	21	1
115	miR3887-3p	ESU_271959	GGAGAGAUGGCUGUGGAA	18	1
116	miR530	ESU_275164	CUGCAUUUGCACCUGCACCU	20	1
117	miR7757-5p	ESU_281562	CACAAAACCUUCAGCUA	17	2
118	miR171-3p	ESU_282400	UUGAGCCGUGCCAAUAUCAC	20	2

Table S1. Raw data after miRNA sequence analysis (continued)

	miRNA Name	Sequence ID	Mature miRNA Sequence	S.L	C.N
119	miR6250	ESU_300797	UGCCGCCAAUCUUCUCGGGG	20	1
120	miR156-5p	ESU_301338	UGACAGAAGAGAGCGAGCAC	20	1
121	miR7398-3p	ESU_304756	CGUAAGAGAAGGGAGAA	17	1
122	miR2916	ESU_307499	CAAGAACGAAAGUUGGGGAC	20	1
123	miR5174-3p	ESU_308525	UUAUGGAACGGAGGGAGUA	19	1
124	miR414	ESU_309351	GAGGAUGAUGAGGAUGA	17	1
125	miR5049-3p	ESU_312503	CAAGUAAUAUGGAUCGGAGG	20	1
126	miR6874-3p	ESU_313582	UUUACCUAGUUCUGCUGU	18	1
127	miR6621-5p	ESU_339819	AUCUGGUACAACAGCCUGU	19	1
128	miR845-5p	ESU_347171	ACCUUGCUCUGAUACCAAU	19	1
129	miR2478	ESU_352300	AGAGGGCGUGGGUUCAUA	18	1
130	miR5050	ESU_353841	UUUUGCUGGUUGAACGA	17	1
131	miR2673	ESU_359076	CUUUUCUUCCUCUUCCUC	18	1
132	miR6182	ESU_361898	GAGUGUGUGAUGGAUGGCUUU	21	1
133	miR650	ESU_378616	CCAUGGUGGAGAUGUCCUGAG	21	1
134	miR165	ESU_396141	UCGGACCAGGCUUCAUCCCCC	21	1
135	miR6177	ESU_403410	CCAUGGACAGAAGGCACUUA	20	1
136	miR157	ESU_409496	UUGACAGAAGAUAGAGAGCAC	21	1
137	miR181-5p	ESU_415468	AACAUUCAACGCUGUCGGUGAG	22	1
138	miR7116-3p	ESU_422547	UCCUUUUUCCUUUGCCUU	18	1
139	miR3348	ESU_424743	CCUCGCCGGGAGGCUCG	17	1
140	miR2525	ESU_431642	UUUGAUCCACUUCGCUGUC	19	1
141	miR6188	ESU_434825	GGAGGAUCGAUGAACCCGG	19	1
142	miR5658	ESU_435864	GAUGAGAUGAUGAUGAUGA	19	1
143	miR7042-3p	ESU_436189	GUAUCAAGAGAGAAAACA	18	1
144	miR408	ESU_438606	UGCACUGCCUCUUCCCUGGCU	21	1
145	miR4922	ESU_461374	UAAAUUGUAUCAUUUUUC	18	1
146	miR172-5p	ESU_465742	GCAGCACCACCAAGAUUCACA	21	1
147	miR390	ESU_483231	AAGCUCAGGAGGGAUAGCGCC	21	1
148	miR1131	ESU_488177	CUUUAGUACCGGUUCGUG	18	1
149	miR5503	ESU_497712	AAUGCCUCUAGAAAGAUCCGAA	22	1
150	miR1878-3p	ESU_515097	AUUUGUAGUGUUCGGAUUGAGUU	23	2

Table S1. Raw data after miRNA sequence analysis (continued)

	miRNA Name	Sequence ID	Mature miRNA Sequence	S.L	C.N
151	miR2120	ESU_524874	GAACCGGGACUAAAGAUC	18	1
152	miR767-5p	ESU_528256	UGCACCAUGGUUGUCUGAGCAUG	23	1
153	miR2111-5p	ESU_566515	UAAUCUGCAUCCUGAGGUUUA	21	1
154	miR6214	ESU_572103	ACGACGACGACGAGCACGAC	20	1
155	miR2919	ESU_573267	CCUGCCGUCGCUGUGCUUC	19	1
156	miR3944-3p	ESU_610168	ACCUUCGGGCUGGCCUGC	18	1
157	miR6244	ESU_625280	CCUUGUGGUCGUGGGUUCG	19	1
158	miR394-3p	ESU_640939	GUGGGCAUACUGCCAAUG	18	1
159	miR5021	ESU_652277	CUACAAUUUCUUCUUCUU	18	1
160	miR397	ESU_652715	UUGAGUGCAGCGUUGAUGAA	20	12
161	miR5169	ESU_655228	UUGACCAAGUUUGUAGAA	18	1
162	miR182	ESU_659722	GUGGCACUAGUGGAAUUC	18	1
163	miR1520	ESU_661017	CCCAUCACGUGUCAUGUU	18	1
164	miR529-3p	ESU_676363	GCUGUACCCUCUCUCUUC	18	1
165	miR393-3p	ESU_687112	GAUCAGUGCAAUCCCUCUGGAA	22	1
166	miR1207-5p	ESU_726078	GGGGCAGGGAGGCAGGGA	18	1
167	miR535	ESU_731460	UGACAACGAGAGAGGGCACGCG	22	1

* SL: Sequence Lenght, CN: Copy Number

Table S2. Family names for identified putative mature miRNA sequences

1	**Family Name**	**miRNA Name**
2	miR6300	miR6300
3	miR894	miR894
4	miR5048	miR5048
5	miR6478	miR6478
6	miR716	miR716
7	miR5062	miR5062
8	miR167	miR167
		miR167-3p
9	miR159	miR159
		miR159-3p
		miR159-5p
10	miR396	miR396
		miR396-3p
11	miR168	miR168
		miR168-3p
		miR168-5p
12	miR456	miR456-5p
13	miR1273	miR1273
		miR1273-3p
14	miR1120	miR1120
15	miR5368	miR5368
16	miR166	miR166
		miR166-3p
		miR166-5p

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
17	miR6203	miR6203
18	miR6198	miR6198
19	miR444	miR444
		miR444-3p
20	miR5054	miR5054
21	miR4995	miR4995
22	miR8155	miR8155
23	miR319	miR319
		miR319-3p
		miR319-5p
24	miR1133	miR1133
25	miR5073	miR5073
26	miR171	miR171
		miR171-3p
		miR171-5p
27	miR395	miR395
		miR395-5p
28	miR5067	miR5067
29	miR5538	miR5538
30	miR827	miR827
		miR827-5p
31	miR390	miR390
		miR390-3p
32	miR1135	miR1135

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
33	miR1136	miR1136
34	miR1436	miR1436
35	miR5083	miR5083
36	miR-B6-3p	miR-B6-3p
37	miR5523	miR5523
38	miR5072	miR5072
39	miR5141	miR5141
40	miR393	miR393
		miR393-3p
41	miR99	miR99
42	miR6981	miR6981-5p
43	miR466	miR466-5p
44	miR3711	miR3711
45	miR619	miR619-5p
46	miR1285	miR1285
47	miR5106	miR5106
48	miR156	miR156
		miR156-3p
		miR156-5p
49	miR398	miR398
		miR398-3p
50	miR6173	miR6173
51	miR2538	miR2538-5p

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
52	miR169	miR169
		miR169-3p
53	miR6219	miR6219-5p
54	miR535	miR535
55	miR479	miR479
56	miR164	miR164
57	miR5079b	miR5079
58	miR5585	miR5585-3p
59	miR11214	miR11214
60	miR528	miR528-5p
61	miR5084	miR5084
62	miR5096	miR5096
63	miR706	miR706
64	miR6199	miR6199
65	miR5082	miR5082
66	miR3682	miR3682-5p
67	miR1122	miR1122
68	miR1137	miR1137
69	miR1117	miR1117
70	miR6253	miR6253
71	miR5064	miR5064
72	miR1139	miR1139
73	miR6204	miR6204

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
74	miR1432	miR1432-5p
75	miR5076	miR5076
76	miR5532	miR5532
77	miR3630	miR3630-3p
78	miR30	miR30-5p
79	miR5059	miR5059
80	miR1584	miR1584
81	miR6181	miR6181
82	miR3885	miR3885-5p
83	miR5387	miR5387
84	miR928	miR928
85	miR399	miR399
86	miR6191	miR6191
87	miR5181	miR5181
88	miR1125	miR1125
89	miR5571	miR5571-5p
90	miR160	miR160
91	miR172	miR172
		miR172-5p
92	miR5174	miR5174-3p
		miR5174-5p

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
93	miR529	miR529
		miR529-3p
94	miR5049	miR5049
		miR5049-3p
95	miR22	miR22
96	miR394	miR394
		miR394-3p
98	miR5056	miR5056
99	miR3887	miR3887-3p
100	miR530	miR530
101	miR7757	miR7757-5p
102	miR6250	miR6250
103	miR7398	miR7398-3p
104	miR2916	miR2916
105	miR414	miR414
106	miR6874	miR6874-3p
107	miR6621	miR6621-5p
108	miR845	miR845-5p
109	miR2478	miR2478
110	miR5050	miR5050
111	miR2673	miR2673
112	miR6182	miR6182

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
113	miR650	miR650
114	miR165	miR165
115	miR6177	miR6177
116	miR157	miR157
117	miR181	miR181-5p
118	miR7116	miR7116-3p
119	miR3348	miR3348
120	miR2525	miR2525
121	miR6188	miR6188
122	miR5658	miR5658
123	miR7042	miR7042-3p
124	miR408	miR408
125	miR4922	miR4922
126	miR1131	miR1131
127	miR5503	miR5503
128	miR1878	miR1878-3p
129	miR2120	miR2120
130	miR767	miR767-5p
131	miR2111	miR2111-5p
132	miR6214	miR6214
133	miR2919	miR2919

Table S2. Family names for identified putative mature miRNA sequences (continued)

	Family Name	miRNA Name
134	miR3944	miR3944-3p
135	miR6244	miR6244
136	miR5021	miR5021
137	miR397	miR397
138	miR5169	miR5169
139	miR182	miR182
140	miR1520	miR1520
141	miR1207	miR1207-5p

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome

	Predicted miRNA	**Extracted sequence**
1	tmo-mir-156-3p	TGAGCACACAGCGGCAGACTACGTCTGGTAA AGAGGACGGATGGATGCGAGGAGAGATATA GACGCAGCTGCTGTTGCGT**GCTCACCCTCTC TCTGTCAGC**TCTCTCTCCCCCTTGGGACTTGT TCCGCCATCAATCTATCCATCCATCTCCCCTT GCCGTTCCCATCGCTTCGCAGCCAT
2	tmo-mir-156-5p	CGAGCAGCACCGGCAGTATGTTTGTTTGTTCT ACTCTGGTACGTGGTGTGCTGGTTCCATCCGC CGTGCCCGTGGGAGGC**TGACAGAAGAGAGC GAGCAC**ACGGCGGAGGCCCGCGTCGTGGAG ACGCCACCCCCGACACGTGATCCCTCCTCTTT CCGTCGGCCTCCGGCTCATCGGCA
3	tmo-mir-159	TCCCATCTCCATTGAAAACGAGGAGATCGGC TTGTGGTTTGCATGACCGAGGAGCCGCTTCGA TCCCTGGCTGACCGCTG**TTTGGATTGAAGGG AGCTCTG**CATCTTGATCTACTGTCACTTAGTT TTATCTGCTGCCTATTGAACGCTAGCCGCTGC AAATCTTGAAAATGCTCCACAGT
4	tmo-mir-159-3p	CTATCGAAGGGTTTGTGCAGCTGCTTGTTCAT GGTTCCCACTATCCCATCTCCATTGAAAACGA GGAGATCGGCTTGTGG**TTTGCATGACCGAG GAGC**CGCTTCGATCCCTGGCTGACCGCTGTTT GGATTGAAGGGAGCTCTGCATCTTGATCTACT GTCACTTAGTTTTATCTGCT
5	tmo-mir-159-5p	ATCTGTGTGGTTTGTTTTGGATTTGGTTTTGAG GTGGAGCTCCTATCATTCCAATGAAGGGTCTA TCGAAGGGTTTGTGC**AGCTGCTTGTTCATGG TTCCC**ACTATCCCATCTCCATTGAAAACGAG GAGATCGGCTTGTGGTTTGCATGACCGAGGA GCCGCTTCGATCCCTGGCTGACC
6	tmo-mir-160	GGAACTATGGATGGATGGATGGTGCAGCCCG GCTTGATGCAAAAGGTTAGTGCATGTATGGCT GGCTGGCTGGTGCCGCT**TGCCTGGCTCCCTG AATGCCA**TCCGAGAAGCGCGCCGCTGTGGGA GGCGTCCCTCCTGGTTGGCATTGAGGGAGTCA TGCAGGCCTCGGCTCCTCCTTCCT

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	**Extracted sequence**
7	tmo-mir-164	GTGCATCACGCTCCTCAGTGGGAAGAAAGAC AGACCACACTGGAAGAAACACTCGGAAGGAT CAGCGAGAAGGACCGCGC**TGGAGAAGCAGG GCACGTGCA**TGCATGCGAGCGCGCTCGATCT CCTCCTGCCTCCGTCGGCGGGATCGGAGCTGC TAGCCCTGCACTGCACGTGTTCTTCT
8	tmo-mir-166	TTCTTGGTTCCGCGTCATGGTTGTCGAGGGGA ATGACGCCGGATCCGAAAGAGAGACGCATGG CGTGCGTGTGGCGCGTT**TCGGACCAGGCTTC ATTCCC**CATGACACCATCATGGTTCAGGTTCA CGGACTCGGCCTCGGCGGGTGTTCTTGTGAGC TCCGCCTGTGCTTCCCTCCGTGT
9	tmo-mir-166-3p	GGGGGTTGTTGTCTGGTTCAAGGTCTCCACAT ACATCATATACATGGAGAGCATGATATGGATT GTATGATTTGGTGACC**TCGGACCAGGCTTCA AT**CCCTTTAACCAGCATCTGCACAATTCGCAC GCCTCATGCATATGATAGTTCCAGCGGCGGTG GTTTGCTTCTAAGGTAAG
10	tmo-mir-166-5p	TCCATGGCGAAGTAGCTTCACCGGCCTTTTGC TGGTGGGTTTGTTTGGACTTCATGTCCTGGTTG AAGTTAGGTTAAGGG**GGTTGTTGTCTGGTTC AA**GGTCTCCACATACATCATATACATGGAGAG CATGATATGGATTGTATGATTTGGTGACCTCG GACCAGGCTTCAATCCCT
11	tmo-mir-167-3p	TCAAGGTGCACCACAAGCTGGTGAAGCTGCC AGCATGATCTGATGACCTAACTCATGGATCAG AATCCATGTCAATCAGG**TCATGCTGGAGTTT CATC**TGCTGGTCGGAGCACAACGAAAACCCT AAAATCCCATGAAATCTATCCATGGACGGGTG GACCATCTACCCTTCCTGCAG
12	tmo-mir-169	ATAGCTGAGAGACAAGAGAAGTCAGAGCGAA GAGTTCACTGCCGCAAGAGCCATGCACAAAG AGGCGGAGAGTGGAATGC**AGCCAAGGATGA CTTGCCA**GCAGCACAGAACAAGGCGGTAATC TTTGATACGATTGCCATTTGCCCGGCAAGTCC ATCCTTGGCTACACCTTACTCTCT

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
13	tmo-mir-169-3p	AGCCAAGGATGACTTGCCTGTGGCTGTGAAT GGGATCTCTTTCTCTCTGGTGGATCGGTCTCT CACTACTATGCACATCA**GGCAGTCTCCTTGG CTAGC**CCGAGTGGCTCTTACCCTTCATGCCAG CCTCTCATCTCTTTTCATACTCAATTCATTCAT CACAACCGGGAAATTGTTCC
14	tmo-mir-171	TGCGAGGGGAGTGAACGCGGTATTGGTGCGG TTCAATCAGAGAGCTGGTGCCCCAGGAGACA AGGGGTTCCGCCCTTCGA**TTGAGCCGTGCCA ATATCACG**CGGTTTCTCTTGACTCGCATCCTC CTTCCATGCGCATGCATGCGCGCCAATTCCAT GGTTTCAATTAAGGTAGCTATCCG
15	tmo-mir-172	TCTCTCTCTCTCTCTCTGCCAAGCAGTTTGTTT GTTTCCAAATGAGATATATAGATGCATATAG AACATACTTTGGATGA**GAATCTTGATGATGC TGCA**TCCGCACACAAGCGCTTCCCTTTGTCCT ATACATGATCAGGTGAGACCTTTGATTCATCC TGTTTCTTTTATCTCGTTGC
16	tmo-mir-172-5p	TCTCGCCATGGAAATACATGGGAGGAATTTC ATGGCCGGAGCAGAGGGAAGATATCAGATAA CAGTCGGTTCTTGCAGGT**GCAGCACCACCAA GATTCACA**TCGGATCCGTCGTCGTAAATTAA ATTTATGCGACGCGACAGGTGATGAGAATCT TGATGATGCTGCATCAGCAGGCACTC
17	tmo-mir-319-3p	GAGCAAAGTTATTTGCCGAGGAGCTTAGCAG GTGAGTGAATGAAGCGGGAGGTAAAGTCTTT GATCTCGCACCGTCGTTG**CTTGGACTGAAGG GTGCTCCC**TCCTCCTCTCTAAGCCATGTTATG TAGCACGGCACCTGCTTGATCTGCTCGTTTCT TCGGGCGCCATACCGCCTGATCGA
18	tmo-mir-384-5p	CAAGGAGAGCATGAGCAAGCCGCTGTTGCCC AGGCAGGTCATCCTCATGTGCTTACCGTAGAA TTCTAAGCACCAGATAT**TTGGCATTCTGTCC ACC**TCCTTCCAGAGGAGAGTCATCTTCTGCAA CTCCCAGATGCAGACGCAGGTTGCTGCGTTCT TGCACAGCAGACCCACAAG

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	**Extracted sequence**
19	tmo-mir-393	GAGAGAAATTGAGAAATAGAGAGAGAGAG AGTGAGGGAGGGAGGGAGGGGGAGAGAG AGCAACAATGCCTTGGGGAAGCA**TCCAAA GGGATCGCATTGATC**CTTCCATCCATGGT GTTGATGGCTGTCATCAATCAACCAGCTCG ACGGATCATGCGATCCTTTTGGAAGCTTCC TCTAGC
20	tmo-mir-393-3p	TAAGCTTTCAGGAAGCTAGTGGAGGATTCC AAAGGGATCGCATTGATCCATCTCTCCGTA ACCGGCGCTCAAGATCGATG**GATCAGTGC AATCCCTCTGGAA**TTCTCCGCTTGCCTCCG CCTCCGGCCGTCCATCTCTTCCGTCCGACCT GGCCATGCCAGGAGGTAATTAAACAGAGA TGG
21	tmo-mir-396-3p	CTCTGTCTCTCCCTTTCTCTCCGTTGGTAGA GAGAGACATGAATGGGGAGGGAGAACAAG AGCAGGAGCAGCAAGAAGAG**GGTCAAGA AAGCTGTGGGAAG**AAATGGCATATCCAGG ACTTTCAATTGCGACTAATTTCCAATTGCTG GGAGGGGTGGGAGATTTCATGTTAATCTCC ACG
22	tmo-mir-397	TACGGAAGCTAAGCTAGCCAGATGCAACCA GACACTACTACTGGGGAAGACGAAGAGGA GGAAGAGACGCAAAGGTGCCA**TTGAGTGC AGCGTTGATGAA**CCGTCCTACTCCTCCGAG GCCGGAGCGGTTTGCACACAATGACGGCTC TGCTTTCTCCGGCGAAACAGCAAAGCAAGC CT
23	tmo-mir-398	GAAGAGAAGCCGATCCAGAGGAGTGCCAC TGAGAACACAAGCGCGGGCTCCTTTGTCAG GTGCTTCGGATCGCATGCGAT**TGTGTTCTC AGGTCACCCCT**TTGGGTTGCTTCCCTTGAT

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
24	tmo-mir-399	TTGATCGACGTGTCTCCCTTGGCATGGTGG CATGCATGTACCGGTAAGTGTGCGGCGTGC AGTTTCTGGCAAGCCGTGCG**TGCCAAAGG AGAATTGCCCTG**CCATTCGCCTCAGCTTCT CTGAACTCTGGTCGACCAGAGTTCATTTGT TCCTGAGACTAACCATGCTTACGTTTGGCT
25	tmo-mir-414	GTGGTGAGTTGATCAGCTTCTCCTTCAGAG CTGAAAGACCCAAGCTGGTCGTGATTTATG TCAACAAAGCAGAAGATTAT**GAGGATGAT GAGGATGA**TGACGACCCACTTGGTGATGC CATCGTAGCTCAAAGAATGAAGTTGAGCGA GGAGGAGGTGTGCAACCTATGGGACATCA
26	tmo-mir-444-3p	TCCGCTTATATTAATTGGTGGTTTATTTTCT TTGCAGAATTAATAGAAGATCACCATACTT GTGGCTTTCTTGCAAGTCG**TGCAGTTGCTG CCTCAAGCTT**GCTGCCTCCCTTTGCCAAAG CTATCAGAAAAAACATAAAGTTCTGTTAGT TTCCCTAAACTTTAAAACATGTTCTACAAA
27	tmo-mir-466-5p	CATGCAACTGGGGACGCTCGACAAACACA AACACCCGTAGTTGCGAGTCGATGGGTGCC ATGCAACTGGGGGCCCCGACA**AACACACA CACACACACAC**CTAGTTGTGAGTCGATGG TTGGCTTTCAATTGGGGGCCCTCGACAAAC ACAAACATCCTTAATAGCGAGTCAATGGTT GG
28	tmo-mir-528-5p	GCTCCCGCCTCCCGCCGATGGAAGCAGCAC TCTCACCGGCGTGTGTGCTTGGGTGTTTAAT TTGGCCGGAGCAGCAGCGG**TGGAAGGGGC ATGCAGAGGAG**CGGCCATGGGAGCTTTGC TTGCCTCTAGGCTCTGGCTCTCTCCTGTGCC TGCCTCTTCCATTCCTGCGGCTGATGGCCGT
29	tmo-mir-530	GAGAGCATACTTGGTTTGCATGTCAGGCAA GGGAAGCTAGAGAGCCAGCAAAGTGCACA GCAAAGGGTTATGGGATAGAG**CTGCATTT GCACCTGCACCT**AGAGAGGAAGAAAGGT CGAGGGCTTGGAACTTGGCCATGCATGCG

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
30	tmo-mir-716	CGACCCACACCCGGCCATCTGGGCGAGTG CCATGCCCCAATGAGTAGGAGGGCGCGGC GGCCGCTGCAAAACCCGGGGCG**CGAGCC CGGGCGGAGCGGC**CGTCGGTGTAGATCT TGGTGGTAGTAGCAAATATTCAAATGAGA ACTTTGAAGGCCGAAGAGGAGAAAGGTTC
31	tmo-mir-827-5p	AGCTCTGGCTAGGCTCCGTGTGTTTCTTCC CTTCTCGTCTCTCTCGCCACTCTCAATCGT ATATCTCTAGTTCATGCAGC**TCTGAACTT GTTTTGCTGGTTG**TCATCTAACCATCGTT CAGTCTGCCGTCTGTAAGCTTGGTGCATGG ATGGGTGCCTGAGTGGGTGCATGGCGATT
32	tmo-mir-845-5p	CATGTCACTTTCTTCATACCTGCGGTCGAC ATAACTAAAACTCCAGGCGGTTAAGCCAA AATCACACAGAACAAGTGAGT**ACCTTGCT CTGATACCAAT**TGAAAGTGTGTTATATCG ACTAGAGGGGGGGTGAATAGGCAATTTTT ACGAAATTCTTCACTGAGGAATTTGCCGGT
33	tmo-mir-1117	ATTTCCATTATTTTCTTTTATTTTTTTTAAA ACTGAAAAGGCGATCCACCGGGGGGGGGT AGAGTTTGAAAATGGGACCT**TTAGTACCG GTTCGTGGCA**CGAACCGGGACTAAAGGT CTCAACCCCATTAGTCCCTGTTCGTGCCTC AAACCGGGACTAAAGGTCTAATCTTTAGT
34	tmo-mir-1120	CGTGGTGGACAGCGTGCCGGGCCACAAGT ACTACTGCTGAGAGTACTACTCCCTTCGTT CTATAATATAGTTTTAAGAAT**ATTTTTATA TTATGAGAC**AGAGTGAATAGTACACAGCT CCCACGGTCGGCGCTTGTGCAGTTTTATTT TTAAACGGAACACGCGTTTCATTCCTCTTC
35	tmo-mir-1122	CGTATAAAATGGTTTATGGTAAAGCTTGTC ACTTACCTCTCGAACTACTCCCTCCGTTCT AAATTACTTGTCTTGGATTT**GTCTAGATAC GGATGTATC**TAGACTCATTTTAGTGCTAG ATACATCCGTATCTTGACAAATCCAAGGC AAGTAATTCGGAACGGAGGGAGTAGAACA TA

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
36	tmo-mir-1125	TTGTGACCCTCTTTTTTCAAAGTTACTCCC TCCATTTCAAAATATAGTGCGCCCGCTTT CCGAGGTCCAACTTTGACCAT**AAATTTAA CCAACGAGACCAACTG**TGGCGGGAGAA AAAAATTATATAATTGGAAACTTCTTTTG AATACGAATTCATTGATATAA
37	tmo-mir-1131	ACATTTCCATTATTTTCTTTTATTTTTTTTA AAACTGAAAAGGCGATCCACCGGGGGGG GGTAGAGTTTGAAAATGGGAC**CTTTAGT ACCGGTTCGTG**GCACGAACCGGGACTA AAGGTCTCAACCCCATTAGTCCCTGTTCG TGCCTCAAACCGGGACTAAAG
38	tmo-mir-1133	GAGAAAACTTATCCCAAGCTTGTCCCTCA AACAGATGTATCTAACACTAACTTGATGT TAGATACATCCATTTAAGGGAC**AAGTTT TTTCGGACGGAG**AGAGTATCTGCTTGTG TGCTTCCTAGCTTTGCATATACGTGCGTT GTGCTTGGTAGCACATAAATC
39	tmo-mir-1135	ATATCCCAACTAAGTTAGCCTGTTAATAA TTTTAAAAGCATGGTTAGCATTGTGAATG GCGTAGATATTTTATACTCCCT**CCGTTCG GAATTACTTGTCGCAG**AAATGGATGTAT CTAGACGTATTTAGTTCTAGATACATCCA TATCCGAGACAAGTAATTCCGAACGGAG GGAGTAGAATG
40	tmo-mir-1136	TGCAGGTCATCTCTGTCACCAGCGCCAGC TACGACCTCGAGCTAGCCTTGTGAAAAAT TGTACTTCCTTCGTTCTGAATT**ACTTGTC GCAGGTATGGATATA**TCTAGATGTATTT TAATTCTAAATATATCCATTTCTGCGACG AGTAATTTGGAACGGAGGGAGTAGTGAT CTAGGGCTTT
41	tmo-mir-1137	TTCTTTAGCCCCATTTAGACATTCATATA CTCCCTCTGTCCCAAAATAAGTGTCTCAA GCTTAGTACAACTTTGTACTAA**AGTTAGT ACAAAGTTGAG**ACACTTACTTTGGGACG GAGGGAGTAGTTACAAGTGGAATTAGCA

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	**Extracted sequence**
42	tmo-mir-1432-5p	ACACAGAGACAGACAGAGAGACAGACAT ACCTTTGAGCAGATGAGCGCTTGAGCTGT TCTTGTGGGTTAGGGTCCTGTGA**TCAGGA GAGATGACACCGAC**GCCGATCGGATGG GTCGGCTTAACCAGGCCATGCATCCGTCT GGCCGGAGTTGGTGTCACCTCGCCTGAAC
43	tmo-mir-1436	CCCTCCTTCCTCATGTACTCCGTCCGTCCC ATAATATAAGAGCGTTTTTTACACAACAC TAGTGTCAAAAACGTTCTTAT**ATTATGG GACGGAGGGAGT**ACCATAGTGTTGACTA TAAGGTTGGCTATAAGGTCACAACTTTTT CTCACTTTCTCTCTATCTATTTCCTCTCAA
44	tmo-mir-1584	CGGCGGAGTGGGAGGTGCTGGCATGGGG AGCTCCTCTCCTTGTGTGATGCGTGCGGC GGCGCTAGGAGAATGAGAGGGAG**AGGA TCAAGGGAATCGGG**TAGGAGAGACGGG GGATCAAGCGGATGGGGTGCTGCGAAGG AGATGGGTGCGTTGCGGGAAGGAGAGAG
45	tmo-mir-1878-3p	TCAATCTTACAAACCACTTTTGCAAACTT AGTCTTGGCACTATAAATTTAAAATGTGC ATGTGAAATATGCTCATTATAA**ATTTGTA GTGTTCGGATTGAGTT**TATAATAGTCGT TCTAAGATTTGTTTGTGAACATAGTAATA TTGTTAAAATAAGGTTCTTGTTGGGAGCT
46	tmo-mir-2120	TACCAACCGGGACCAAAGGCCCTCGTGC CTGGGCTCGCCGGAGCGGCCACGTGGAG GCCCATCTGTCCCGGTTAGTATAA**GAACC GGGACTAAAGATC**TAGGGCATTAGTAAC GACCCTTTAGTCCCGGTTCCCAACCGGGA CAGATGGGCCTTATGAACCGGGACAAAT
47	tmo-mir-2538-5p	CTTTTCCACCTTTTTTCATTTGAGAGAGCA GTGTCCTTCGTCTTCATCTAATTGGATTTC GAAGGTCCCATTTTTTCAGA**ATCCTCTAT TATTTTAGT**TCTTCCACCCATGCAATAGA GAGGGAATGGGAAAAGAAGGGTTACTTT TATTTGCATTTTTTCCTTAAAAGATAGAC TTT

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
48	tmo-mir-2673	TTCTTTCCCCCCTCCTCCATCCCATCCATC CCCTTCTCCTCCCGCTTCCGCTTCCGCATC CCCTCCATCACATTCTCCTC**CTTTTCTTC CTCTTCCTC**CATGGAGGGGCGGAGCAAG GATTTGACCATGTCCAAGGCTTCGAGCAG TTGGCGGCCGTCGGACGGGCGACGGCGA
49	tmo-mir-3348	CATAAAGTTTCTACTCCTCACTGCATTGA GATGATTGGTTATGTTTTATTGTAGTCCA GTCCACAATAGCTCCCCGCGAT**CCTCGC CGGGAGGCTCG**CTGGATTCCAAGGCCAT GGCCAGCGATTTACCGCCAACCGCTCACT CCCCCAAGGCCAAGGATGATGCGGAGGT GCTGTC
50	tmo-mir-3630-3p	TTGGGGATCCATGGAAACAAGTGATGAT ACCAAGACAGGGACATCAGAACCCTTCG GGGTTATGACAGACTACGTCTATA**ATGG GAATCTCTCTGAT**GTTTTTGATTTCCCAA GCCAAAGTGATGTGTTTTTGAGTCGCTAT TTTTCTTCCTCTCTTAGATCTGCGACTCTT TTCTG
51	tmo-mir-3682-5p	GCATCTAGTGCCACCCCTGGTTGGTTTTG GAGTATTGACGACAAACCTGGTTGAGGG ACTAATGTGTTTGTGAGAATTGT**AGGATA ACACAGGTAGAA**GTCCCTCATTGATTCG GTTTTCCTACCAGAGATGACCCCTAAAAA TGTATGAAGACGTTGAAGTCAAAGGTGG
52	tmo-mir-3711	CATCCTTATAAACCAGAGCCGCTGAGGTC GCGGGTGCTGTTGTTGTGGTCCTGTGGTC GCGGGCACTGCTGCTGGTTCGA**GCCCTC CTTCTAGCGCCA**TCTCGGGTCAGCTCGC CTCTGCTGCGGTGGTCGCGGGCGCGTCCA CGATCCGCCGGCCGTTAACCAAAAATTGT
53	tmo-mir-4995	GAAACCGTGCTGAGCTCCCAAGCAGTGG GAAGGGAAAGTGATCTCTGACCGTGTGC CTGTTGAAGAATGAGCCGGCGACT**CATA GGCAGTGGCTTGGTTAA**GGGAACGGAA CCCACCGGAGCCGTAGCGAAAGCTAGTC TTAATAGGGCGATTGTCACTGCTTATGGA CCCGAACCTGGGT

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	**Extracted sequence**
54	tmo-mir-5048	CTGGCTTGACTTTGCTTTTCTTCAAAGCATCTT GACAGACCAAAATCCGTAATTTTTGGCACCAT GTTTTCGTCTAGCAA**TATATTTGCAGGTTTTA GGT**CTAAGTGAAGGATATCGATCTCTGTTAAT ATATTGTAAACCTTCACAAATTCCCTTGATTAT TTCATAGCGCGCTATTTT
55	tmo-mir-5049	TAGACATGATACTGAACTGCTTGGAAACCAGC TTTGATAAAGTATACTCCCTCCCTCCCAAAATA AGTGTCTCAAACTAA**AGCTGAGACACTTATT TTGGGAC**GGAGGGAGTAACAATTAGAGGAAG CAATTCAGTCATGCATTTATAGGCAAAATTCA GTTTACATTCACACCCGATCTCTC
56	tmo-mir-5049-3p	ATTCAAACCGACACTAATGTGCTTCACATTTG ACCCTTTTTCTACTAGTGTATCAATGCTAGATA CATCTATTTGAGCGA**CAAGTAATATGGATCG GAGG**GAGTATCAATTTTATACAATGCTCTTAA GACCAATCGGCTTAATAAGTTCCATTTCATCAT TTTCTGTTTATGTACCTGT
57	tmo-mir-5050	AGGTGTCCACCTCGTACCACCTCGTAGGTCCT GCGCCTCAGTGTCCCGTGAGGGCTTGGGCGCG ACCGGGCCCGCCGCCG**TTTTGCTGGTTGAAC GA**CCTCATCATGGCACGAGCAGATCTCCCTGG CACTTGTCGAGGAAGAGGCGGTCGACATGGTG AGGTCGTTCAACCGGCAA
58	tmo-mir-5054	ACGTGTGGCGTTTCTTTGGACTATATGGAAGC CAAAAACAATGACATTTTAATCCAAATTTTTC ACTTGAAGGCCAACCT**AACCACGTGGCCGTG GGT**TCGATCCCCACGGTCGGCGTTCTTTTTTGT GTTTGCCGTACTTATTTTTCTCCGGCAAAAATA AAATGTACATACTGTAC
59	tmo-mir-5064	TTTGTACGATTCTGAATTGAAAATTCTTTAGAA GGTCAGAAAGCTCCACTATGTATCAGTAGAAC TGAAAATCCTCTGGT**TGAATTTGTCCATAGC ATCA**TCCATCCTACCATTCAATGGTGGCTGAT GCTGCCCAACGGTGGGTAGGTGGCTGGTGCTA TGGATAGCTTCAACCCGATG

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
60	tmo-mir-5067	TCATGGCATTTACCGAGGTACTTGACTGAAA ATTTGGGACCGGCTACTACAGTATTTTGCTAG TCAAGTACTCCTTTCGT**TTCATATTAGTTGTC GCT**CAAACGGATGTATTTAGCACTTAAATAA GTCTAGATACATCCATTTCAGAAACAACTAAT ATAGAACTGAGGGAGTATGA
61	tmo-mir-5073	GATAAATTTTGGAAAAATTAAGAATTTCAGT ATTTTTTCTTATTCTTTTTATTTATTCTTTCAG AAAGAGATAAGAATTG**GTTTGGTGAATCGG AAACAATTT**TATAGAAAAATTGAAGTAAAAA TTGAAGTAAAAATTGCAATTTCTTTTCTTATG GAACATACATATCAATATGCATGGGT
62	tmo-mir-5076	GCTTTTCCCGGCGGTCTGGAGAAAGCAGCAA TCAATAGGACTTTCCTAATCCTCCCTTCCTTTC AGGAAGAACGTGAAAT**TCTTTTTCCTTAAAT GGGAGC**AGAGCAGGTTTGAAAAAGGATCTT AGAGTGTCTAGGGTTGGGCCAGGAGGGTCTC TTAACGCCTTCCTTTTTCTGCCCAT
63	tmo-mir-5079	TTCGCAATTTATTTACATAGTAGAACAAATCC AAATTGGAAGGGTACGAATTCTGCACTTATA GCTTCGATAGGATTTCT**TATAATTTGGATTT GTTATTTT**GGTATAAATCTATTAGGAATAGG CTTACATAGTTATGGTTCGTTTACATTAACAC CTATATGATTACATAAAATGAAATC
64	tmo-mir-5082	GTCCGGCGGCGCCGCGGCGGGCGAGGTGGGG CTTCGCTGCCGGCGGCGAGCGATGACGACTG CCCGCGGTCGGAGCGCGC**GCGATGATGGCC GCGCGGG**CTCACCCAACAAACAAAACAATC TCATTACTTTCTTGCTTTCTTCGAAGGGTTTTC ATCTTTTTTTGATCGATTTCTTTT
65	tmo-mir-5084	TTTTGTCAGAAAAAAAGAAAGAAAAAAAAAC ACTTGGTTTTCACCTTCTCTCTGAGACAATCA TAGATTCATAGATGGTT**GTGATCCTCTGCAG TACTGT**ATGATGCGCTACAGTAGAGGATCCT AACCCCACTGATGGATGGTTGCGATCATAAT GGCATGCCACGTCACCAGATTCGT

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	**Extracted sequence**
66	tmo-mir-5141	CTCCATTACAGATGGACAAATCTGTTTGGAAA TAGAGCTCTTTTATCGCGGAATTAGACCACCT ATTAACGTTGGCTTAT**CCGTCAGTCGCGTCG GG**TCTGCCGCTCACTTGAAAGCTATGAAACAA GTGTGCGGTAGTTCAAAACTGGAATTGGCACA ATATTGCGAAGTGGCCGC
67	tmo-mir-5174-5p	TGCCAGTGGTTTCATGCTTAACCTAATAACTAC TCCCTCTGTCCCATAATATAAGAACGTTTTTGA CATTAGTGTAGTGG**CAAAAACGCTGTTATAT TA**TGGAACAGAGGGAGTATCGTCTTTTGTTGA TGCCATATTTTATCCACCTTAACATAGCTAAGT TTCTCTGTTTGCAGCAT
68	tmo-mir-5181	GACAACCAATAAATTGTACTCCATCCGATCCA AAATAAGTGTCGTGGTTTTGTACTAAGGTTAG TTCAATCTTAGTTCAA**AACTGCGACACTTATT ATG**GATCAGAGGGAGTACTAAAATACAAAAC CATGCGCATATATTTATACGAAACAACAAGCC AACAACTAAGTTCAGAAGTA
69	tmo-mir-5368	GAAGAAGGCCCCCTTCCGGGGGGGCCCGAGC CATCAGTGAGATACCACTCTGGAAGAGCTCGG ATTCTAACCTTGTGTCA**GACCCGCGGGCCAA GGGA**CAGTCTCAGGTAGACAGTTTCTATGGGG CGTAGGCCTCCCAAAAGGTAACGGAGGCGTGC AAAGGTTTCCTCGGGCCAGA
70	tmo-mir-5387	CGTAAGGCCCTTTAGTGCCGGTTTGGTAACCG ACACTAAAATGTAGGCACTAAAGCCCCCCCTT TAGTACCGTTTCAGCA**CGAACCGGTGCTAAA GGA**CAACCATGTGGCACGAGCCAGCTTCGGG GGCAGGGAGCCCTTTAGTACCGGTTGGTGTCA CCAACCGGTATTAAAATGTT
71	tmo-mir-5523	ACTCGGGTTAGTTACCTATCTCAATAAAGTAT ATGATTGGTTTGAGGAACGTCTTGAGATTCAG GCAATTGCAGATGATA**TAACTAGTAAATATG TTCC**TCCCCATGTCAACATATTTTATTGTTTAG GGGGAATTACACTTACTTGTTTTCTAGTACAA GTCGCTACCGGTTTTGCTA

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
72	tmo-mir-6173	TTGAGATCGAAAGAACACCAACGGCGAAAGC ACTCTGCTGGGCCGACACTGACACTGAGAGA CGAAAGCTAGGGGAGCAA**ATGGGATTAGAG ACCCCAGT**AGTCCTAGCCGTAAACGATGGAT ACTAGGTGCTGTGCGACTCGACCCGTGCAGT GCTGTAGCTAACGCGTTAAGTATCCC
73	tmo-mir-6177	GTAGTGTGCCTTATTGATCTGTTTTCTGCCTG ATCCTCAGGGATCTTCACTGTCGCGGGCATCA TCGACTCGTTCATATA**CCATGGACAGAAGGC ACTTA**AGAAGAAAATGGAGATAGGCAAGTA CAGATGAAACCTAGGCAATATTTTCCAAGTT GTGTGGCTTGTAAGGAGGTGGATC
74	tmo-mir-6182	AAGATGCACTGTTACCACAGCCTGACCGTGTT ATGGAGTTCTGCAACAACAATGACCTACAGT TAATTGTGCGAGCACAC**GAGTGTGTGATGG ATGGCTTT**GAGCGTTTCGCCCAAGGTCACTT GATCACCCTCTTCTCGGCAACAAACTACTGTG GTATGGATATGTTATTCTTGCATAG
75	tmo-mir-6191	TTTCTTCATTGACAAAACAGGGCACCTGAGG ACTTCAGTCATTAGCATTAGTTTTACTCCATC CGTCCCAAATTACTTGT**CTTAGATTTGTCTA GATA**TGGATGTATCTAACACTAAAACGTGTC CAGATACATCCAAATCTAAGAGAAGTAATTT GGGACAGAGATTGTACAAATTA
76	tmo-mir-6198	TTCGATTATTCGAGGAGCTGTGGGCATCAAA AACCCACTCCCCCCTCTTTGAGAACCTTTTCA TTCTCACCGAGTATTGA**CGGCTCTGTCTTGG ATGGTCAT**TCTGTTGAGTGATAACCAGAGGA GCCTGACTTCATTGGTGGGGGTTGATCTGGTG AAAGAAGATGGTGGAGCTAGGTTGT
77	tmo-mir-6203	CCCTTATTTATATCCCATGCAAAAGAAGAGA ATCCAAGGCAAAGGCGAGAACAATAAAATTA AAATTTCTTCTTTTTCGG**AGGGATTGCAGGT CTTCTTAA**TCGTCTATTCGGCACAAGAAAAA GCCTTTCTCTTGTGCCGATTCTTCTTGTATCAA AATAAGAATCCTTTTTCTTCCTAA

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
78	tmo-mir-6219-5p	AAAAAATTTCAAAAAAAATCATAAAATTTCCT TTAGTCCCGGTGGAGCTCCACGTGGCTGCGCC CTTTAGTCCCGGTTCG**TGTAAGAACCGGGAC TAA**AGGGGAGAGGTATTAGTACCGACCCTTTA GTCCCGGTTCGAAAACCGGGACTAAAGGGGCC TTACCAACCGGGACAAAAG
79	tmo-mir-6250	CCTCTGACTTTTTTTTATAAATCGAAATCCTGC TTACTTGCGTTTTCCTCTCTCGATTCTTGATAA GTTGGCGGCTCCCA**TGCCGCCAATCTTCTCG GGG**AGGGGATAGATCGACGCGTCAAGAAATC GTTTCAATTGCCATGGCTTCCAGTAGGCAATA TGATTGTTGCTTGCGTACAA
80	tmo-mir-6300	GTTCTGGACTTCTGGCCATGCCGGCCTTCCGCA AATGTGCATTGCGCCTTAAAAATGGCAAGTAA TAATTATTCAAACAA**GTCGTTGTAGTATAGT GG**TAAGTATTCCCGCCTGTCACGCGGGTGACC CGGGTTCGATCCCCGGCAACGGCGTATTTTTTT AACTCTATTTTTTTCTA
81	tmo-mir-6478	GCGTCGCTAGAGAACGCCACGTGAGGAAAAA GATAAATAGAGCGATTGTATGTAGTTTAGCTA ATTAAATCATTCACATC**CCGACCTTAGCTCA GTTGGT**AGAGCGGAGGACTGTAGTATGTTGC AGCTAAATCCTTAGGTCACTGGTTCGAATCCG GTAGGTCGGATTATTCCTTTTTA
82	tmo-mir-6874-3p	TTTTGAGGTTCCCAGTGATGCTTTAACCATGCT AATACTGAAATTATCTGACGAGCAATATATTT TTACTGAGGGAACCA**TTTACCTAGTTCTGCT GT**TTTGACTTCCAGAAACATTGAATTTTTCATT TTATTTGTTCCCTTTTCAAATTTGTGCAATTGC ATCCTGTTCTGAATTT
83	tmo-mir-7398-3p	GACGTATAAGCTACAGGATAAGCTTAGAGAA ATGTTCATTTGCTGAGATACTGGATGCTTACCG AAAGAGGGGAATTTCA**CGTAAGAGAAGGGA GAA**TCTTCATGGACTGGCTTCCACCAATCGGA GTTTCGCGCATTTCAGATGGTGGTAAAGTGAT ACCACATAAGGAGCTCTTC

Table S3. Extracted miRNA precursor sequences from *T. urartu* genome (continued)

	Predicted miRNA	Extracted sequence
84	tmo-mir-8155	TGACATGCTGGTAATCAGTATGACTATTATCA CCCACAACTCATTTGTGTTCTACTCGTGCATG TAACATCTACATATAG**ACCTGGCTCTGATAC CA**CTATTGGGGAACGCAGCAATTTCAAAAAA ATTCCTACACACATGCAAGATCTATCATGGTG ATGCATAGCAATGAGAGGG
85	tmo-mir-B6-3p	AAGTGGGGCACGGACCCGGACCCCGACCGGC GGGTCGAGATCGTCGACGGCGGCCGGCTGGT GACGCTCACCCTCAACAA**CGTCTCCGGCGC CGGGT**TCCAGTCCCGGGACGCCTTCCTCTTC GGCGAGTTCACCATGGAGATGAAGCTCGTGC CCGGCGACTCCGCCGGCACGGTC
86	tmo-mir-I5-3p	GTGAGGACCTCCGGGTACCAAGGTCGAAGAA AACGTTGGGGACGGCGTTGTGGTTCTTCTCAA GTTGCCTGGCTCGACGT**GGATGAAGAAGAC GACGA**TGCAGAGCTCGGGGACTCATCGGCAG GGAGTGGAGTGGCCGGTGGCCATGGCTACGG CGAGCGGCGGCGACGAAGCCGTT

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