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MASTER THESIS

NİĞDE ÖMER HALİSDEMİR UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

T.C.
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DEPARTMENT OF AGRICULTURAL GENETIC ENGINEERING

IDENTIFICATION OF TISSUE, DEVELOPMENTAL STAGE AND STRESS RESPONSE SPECIFICITY OF WRKY TRANSCRIPTION FACTOR FAMILY

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Supervisor

Assoc. Prof. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE

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Esra KARAKAŞ tarafından Zahide Neslihan ÖZTÜRK GÖKÇE danışmanlığında hazırlanan "Identification of Tissue, Developmental Stage and Stress Response Specificity of WRKY Transcription Factor Family" adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü Tarımsal Genetik Mühendisliği Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

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ONAY (CONFIRMATION):

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....../...../20...

Doç. Dr. Murat BARUT

MÜDÜR

THESIS CERTIFICATION

I hereby declare that this thesis has been written by me and that, to the best of my knowledge and belief. All information presented as part of this thesis is scientific and in accordance with the academic rules. Any help I have received in preparing the thesis, and all sources used, have been acknowledged in the thesis.

Esra KARAKAŞ

ÖZET

WRKY TRANSKRİPSİYON FAKTÖR AİLESİNİN DOKU, BÜYÜME EVRESİ VE STRESS CEVABI AÇISINDAN ÖZGÜNLÜĞÜNÜN TANIMLANMASI

KARAKAŞ, Esra
Niğde Ömer Halisdemir Üniversitesi
Fen Bilimleri Enstitüsü
Tarımsal Genetik Mühendisliği Anabilim Dalı

Danışman : Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE

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WRKY transkripsiyon faktör ailesi bitkilerde stres faktörlerinin etkili olduğu durumlarda önemli role sahip olan en büyük protein ailelerinden biridir. Bu transkripsiyon faktörü ailesi özellikle bitkilerde stres faktörlerine maruz kalındığı durumlarda protein fonksiyonlarının kaybolmaması adına oldukça önemlidirler. Bu tezde seçilmiş olan 3 monokot ve 1 dikot olmak üzere toplamda dört bitkideki (*Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* subsp. *japonica* ve *Brachypodium distachyon*) WRKY genleri belirlenmiş ve kuraklık, tuzluluk ve sıcaklık koşulları gibi başlıca abiyotik stres faktörleri altında ve bitkilerin farklı gelişim evrelerinde WRKY transkripsiyon faktörlerinin ifadelenme düzeyleri biyoinformatik yöntemler kullanılarak tespit edilmiştir. Her bitki için ayrı ayrı filogenetik ağaçlar oluşturulmuş, kromozomlar üzerinde WRKY genlerinin yerleri ve korunmuş bölgeleri belirlenmiş, yapılan *in silico* çalışmalarla ayrıca gen yapısı (ekzon/intron), gen ontolojisi ve hedef miRNA'lar tespit edilerek WRKY proteinlerinin annotasyonu amaçlanmıştır.

Anahtar Sözcükler: Abiyotik stres, biyoinformatik, WRKY, transkripsiyon faktörleri

SUMMARY

IDENTIFICATION OF TISSUE, DEVELOPMENTAL STAGE AND STRESS RESPONSE SPECIFICITY OF WRKY TRANSCRIPTION FACTOR FAMILY

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The WRKY transcription factor family is one of the largest protein families that has an important role in stress. Especially in plants they have a crucial importance to maintain the protein functions in situations where plant exposed to stress factors. In this study, in four plants selected as 3 monocots and 1 dicot, *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* subsp. *japonica* and *Brachypodium distachyon*, WRKY genes were identified and changes in WRKY transcription under different abiotic stress factors such as drought, salinity and temperature conditions were investigated. The expression levels of the factors were determined using bioinformatics methods. Phylogenetic trees were created for each plant separately, the location and conserved regions of WRKY genes on chromosomes were determined, gene structure (exon / intron), gene ontology and target miRNAs were determined and annotation of WRKY proteins was aimed with these *in silico* studies.

Keywords: Abiotic stress, bioinformatics, WRKY, transcription factors

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SYMBOLS AND ABBREVIATION

Abbreviation	**Description**
AP2	APETALA2
ABA	Abscisic Acid
BLAST	Basic Local Alignment Search Tool
bZIP	Basic Region/Leucine Zipper Motif
cDNA	Complementary Deoxyribonucleic Acid
DNA	Deoxyribonucleic Acid
EREBPs	Ethylene-Responsive Element Binding Proteins
H_2O_2	Hydrogen Peroxide
ID	Identification
JA	Jasmonic Acid
miRNA	Micro Ribonucleic acid
mRNA	Messenger Ribonucleic acid
MYB	MYB Transcription Factor
NAC	NAC Domain Containing Transcription Factors
NO	Nitric Oxide
NJ	Neighbor Joining
PEG	Polyethylene Glycol
RNA	Ribonucleic acid
RT–PCR	Reverse Transcription Polymerase Chain Reaction
ROS	Reactive Oxygen Species
SA	Salicylic acid
SPF1	Sweet Potato Factor1
TF	Transcription Factor
TTG2	Transparent Testa Glabra2
WRKY	Transcription Factor

CHAPTER I

INTRODUCTION

Transcription factors are *trans*-regulatory elements that are involved in a number of mechanisms most of which are responsible for plant growth, development and tolerance to abiotic and biotic stress factors. Global warming is known to have a very negative effect in all areas. Excessive increases in air temperatures, especially in agriculture, adversely affect plant growth and development. These negative effects caused some stresses on the plant. Environmental factors which have negative effects on plant growth and causes yield loss are known as abiotic stress. Drought, salinity and heat stresses are the major abiotic stress factors. It is known that these abiotic stress factors limit the distribution, growth and development of plants in nature, cause severe yield losses in agriculture and change general plant architecture by altering morphology, biochemical and molecular mechanisms of plant. Transcription factors come into prominence in molecular studies. Not all the functions of transcription factors have yet been reached, but bioinformatics that has been developing in recent years is helping to solve these problems gradually. Scientists have begun various studies to solve these kind of problems and bioinformatic studies have gained momentum because it has become easier to observe changes of abiotic stresses in plants.

This study will focus on genome-wide analysis of the WRKY transcription family. WRKY proteins are one of the largest known transcription factor families. WRKY transcription factors play an important role in the formation and regulation of defense mechanisms against abiotic stresses in plant interactions with the environment. The aim of this study is to determine the location, structure and bioinformatics of WRKY transcription factors in four plants, *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* and *Brachypodium distachyon* and determine the expression levels of WRKY transcription factors in different developmental stages of using bioinformatics approaches. In this study, *Arabidopsis thaliana* was chosen as the dicot plant. Since *Arabidopsis thaliana* is a model organism for dicot plants and many studies have been conducted in this plant, it is considered that it is the best example of which factors should be considered when comparing the transcription factor studies of monocot plants. *Oryza sativa* (rice) and *Zea mays* (maize) were selected as monocot because they are important in terms of food consumption in the world. *Brachypodium distachyon* is also model organism just like *Arabidopsis thaliana*. But it is known as monocot and model plants for grass family. The comparison of monocot and dicot plants was observed in this study. During comparison, Arabidopsis was selected as a dicot organism as a reference and would help to achieve healthy results.

This study will provide an insight into the functional characterization of the WRKY family to determine the best candidate gene/genes for improving abiotic stress tolerance using transgenic approaches. Overall this study will comprehensively examine WRKY transcription factor family proteins in the chosen four plants.

CHAPTER II

LITERATURE REVIEW

2.1 Transcription Factors

DNA-binding proteins play central roles in all living organisms. Replicating the genome, transcribing active genes and reparing damaged DNA can be the examples of the roles of DNA-binding proteins (Pabo and Sauer, 1992). In gene expression, transcription is an important step and transcription factors are the regulators of this process which have roles in activating and repressing the mechanism (Li et al., 2014). Transcription factors are the classes of DNA binding proteins and they display sequence-specific DNA-binding and regulating the gene expression thus they regulate the cell development, differentiation, and cell growth (Pabo and Sauer, 1992). Furthermore they have ability to control of activating or repressing transcription of multiple target genes (Wu et al., 2005) and also they have crucial roles in plant physiology, development and response to environment (Parent et al., 2009).

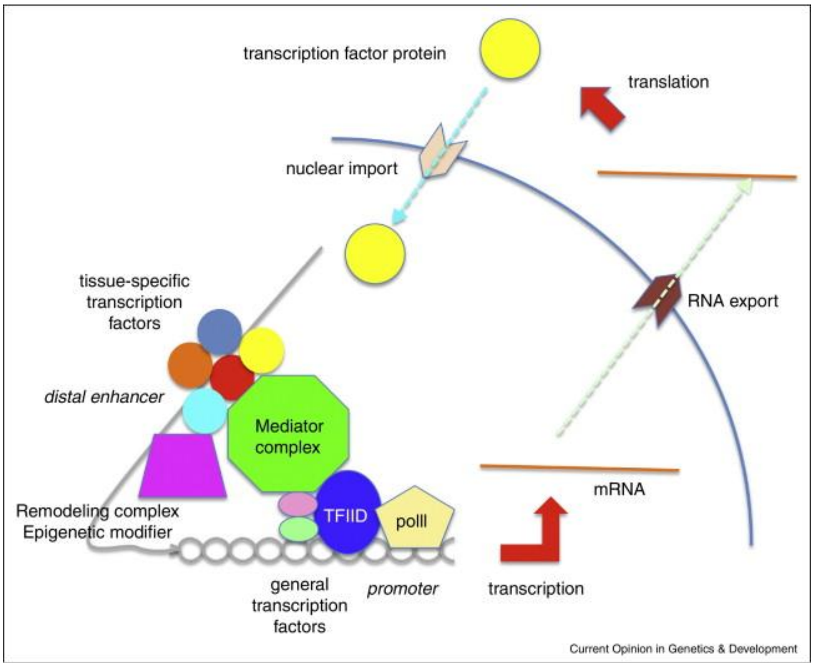

Figure 2.1. Eukaryotic transcription mechanism and function of transcription factors (Niwa, 2014)

bZIP (basic region/leucine zipper motif), AP2 (APETALA2) and EREBPs (ethylene-responsive element binding proteins), MYB (myoblast) and WRKY gene families are best known and the largest transcription factor families.

AP2 (APETALA2) and EREBPs (ethylene-responsive element binding proteins) are known as transcription factors specific to plants with AP2 DNA-binding domain binding properties. Although the AP2 / EREBP gene family is one of the largest families, it has key roles in the plant life cycle, for example in the various responses to abiotic and biotic stresses, the roles of AP2 sepal and petals in the identification and development of plant growth (Riechmann and Meyerowitz, 1998).

It is known that basic region / leucine zipper motif (bZIP) transcription factors play an important role in pathogen defense, light and stress signals, seed formation stages and development in biological processes in plant cells (Jakoby et al., 2002).

The MYB family is one of the largest protein families, with various plant-specific processes, capable of binding to DNA represented in all eukaryotes. It is known to responses to ultraviolet light, to control responses to biotic and abiotic stresses and to regulate metabolism (Singh et al., 2002).

2.2 WRKY Transcription Factor Family

2.2.1 Structure and members of WRKY proteins

WRKY transcription factors (TF) are one of the largest transcription factor families. The first WRKY TF was cloned from sweet potato by Ishiguro and Nakamura (1994), determined as sweet potato factor1 (SPF1) (Eulgem et al., 2000). WRKY TFs have one or two matchless DNA-binding domain (Wei et al., 2012). The name of WRKY originates from the WRKY domain which is prominent characteristic of this protein (Eulgem et. al., 2000). According to Ülker and Somssich (2004) WRKY proteins consist of conserved peptide stretch of about 60 amino acids, WRKY motifs have WRKYGQK sequence along with a zinc-finger-like motif C_2H_2 ($C–X_{4–5}–C–X_{22–23}–H–X–H$) or C_2HC ($C–X_7–C–X_{23}–H–X–C$) at the C-terminus (Babitha et al., 2013). The WRKY domain has a high binding affinity to W box (T/CTGACC/T), in the promoters of its target genes, which is distinct cis-acting DNA element. WRKY proteins can regulate the gene expression in the target promoters via W-box identification (Ülker and Somssich, 2004; Banerjee and Roychoudhury, 2015). On the other hand WRKY TFs can interact with the other genes and thus they can regulate the plant defence responses. WRKY transcription factors have the ability to act as a cellular response to internal and external stimuli, owing to its directing of the expression of genes and their action in concert with other components in the transcriptional structure (Wei et al., 2012). WRKY motif has shown in Figure 2.2.

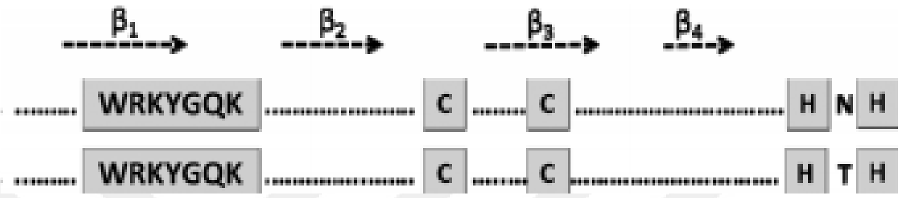

Figure 2.2. WRKY motifs (Bakshi, 2014)

The external and internal stimuli might be abiotic such as drought, salinity, heat, and cold or biotic such as insects, bacteria, viruses, parasites, and fungi (Wei et al., 2012). WRKY TFs are plant-specific so they have a crucial role in the control of plant-spesific regulation (Jiang et al., 2012). Latest studies showed that they are involved in plant growth, trichome and seed development, leaf senescence, dormancy, embryogenesis and stress signaling via genes (Jiang et al., 2017). In case of biotic or abiotic stresses in numerous plant species, WRKY genes are robustly and quickly upregulated and respond (Eulgem et al., 2000). The plants divide the vast majority of their genome capacities into transcription, for example *Arabidopsis thaliana* and *Oryza sativa* have more than 2100 and 2300 transcription factors, respectively (Chen et al., 2010). These transcription factors mostly belong to large gene families, and WRKY transcription factors are a large family of regulator proteins. Ülker and his colleaques reported (2000) that *Arabidopsis thaliana* has 72 WRKY genes and they can be divided into three groups depending on the number of the WRKY domains and the zinc finger motifs (Eulgem et al., 2000). While the first group involve two WRKY domains (C-and N-terminal) the other two groups involve only one WRKY domain. Group II proteins can be divided five subgroups (IIa + IIb, IIc, IId + IIe) based on the amino acid motifs outside of the WRKY domains and group III proteins has different zinc finger motif C_2HC while other groups have C_2H_2 (Wei et al., 2012). More than 100 WRKY genes also have been identified in rice genome through the medium of bioinformatics approach (Wu et al., 2005; Zhang and Wang, 2005).

2.2.2 Functions of WRKY proteins in plants

Abiotic stresses such as drought, heat, salinity, cold and flood affect plants processes like physiological and biochemical stages (Joshi et al., 2016) and sometimes these kind of effects can be harmful for plant development and growth. In order to adapt to such adverse conditions, plants have developed mechanisms that increase tolerance (Chen et al., 2012). The first steps of plant stress tolerance are as follows: stress signals are recognized, signals are transmitted to activate responses, and stress-dependent genes are regulated. Studies showed that trasncript profiling has deduced the possible involvement of WRKY TFs in an indirectly way, nevertheless the functional analyses showed more direct evidence. Even after identification of variety of WRKY genes relating to different species, only minor percentage of them have been functionally characterized. Because of continuous reporting of unique role of WRKY genes in different stress responses, detailed expression analysis were carried out by support of cDNA or oligo microarray, reverse transcription PCR (RT-PCR), or cDNA real-time PCR techniques at genome wide level under different stress conditions (Ramamoorthy et. al. 2008). For example, WRKY63/ABO3 regulates plant responses to drought tolerance and ABA in Arabidopsis (Ren et al., 2010). Under a series of phytohormone treatments and abiotic stresses, *Brachypodium distachyon* WRKYs (BdWRKYs) showed various expression levels and expression patterns, correlations between the differences in the WRKY domain and their temporal and spatial expression patterns in response to stress treatments was observed. For examples, BdWRKY81 showed a high up-regulation under salt and heat stresses and BdWRKY10, 233, 259, and 265 were up-regulated in both cold and heat treatments (Wen et al. 2014). Overexpression of the stress-induced OsWRKY45 casues an increase in drought tolerance in Arabidopsis (Qiu and Yu, 2008).

A single WRKY gene can react to several stress factors simultaneously with varied regulatory functions. Li et. al.(2009) indicated that AtWRKY25 and AtWRKY33 respond to both heat and salt treatments. By using bioinfiormatic tools, more than 100 WRKY members were reported in rice genome (Wu et al. 2005, Zhang and Wang, 2005). By Northern Blotting analysis, Qiu et al., (2008) concluded in rice that 10 of 13 OsWRKY genes were differentially modulated in response to abiotic stress factors polyethylene glycol (PEG), NaCl, heat and cold. Heterologous expression of OsWRK23 causes increase in dark-induced leaf senescence in Arabidopsis (Jing et al., 2009). Li et. al., (2011) has indicated that AtWRKY26 induced by heat stress, tolerance to heat stress as a function. Also Song et al., (2009) has showed that OsWRKY08 induced by drought and salinity and tolerance to osmotic stress as a function. In leaves of *Boea hygrometrica*, BhWRKY1 is referred as a tuner in an ABA-dependent signal pathway to modulate BhGolS1 expression. And according to Wei et al., (2008) TcWRKY53 induced by cold, salt and PEG treatments. Involvement of AtWRKY2 to regulate seed germination and post-germination arrest of development by ABA have also been reported (Jiang and Yu, 2009). The Arabidopsis *atwrky53* mutant shows a delay in leaf senescence whereas *atwrky53* overexpression resulted in quick senescence, proving that *atwrky53* functions as a positive regulator of leaf senescence (Miao et al., 2004; 2007). The Arabidopsis double mutant *atwrky54/atwrky70* shows a significantly enhanced senescence phenotype, proving that AtWRKY70 and AtWRKY54 act as negative regulators of leaf senescence (Besseau et al., 2012). Cold sensitivity of mature pollen is being controlled by male gametophyte-specific WRKY34 in Arabidopsis (Zou et al., 2010). These examples show us that WRKY genes are expressed under different abiotic stresses. In this way, plants encountering various disadvantages can participate in the control of signaling processes associated with transcriptional reprogramming. It may be useful for researchers to find clues about regulatory functions for specific stress conditions by looking at expression models under different abiotic stresses.

Due to global warming, extreme temperatures have begun to appear and these high temperatures cause to huge lost in agricultural crops. Each plant has optimal range temperature that can tolerate and when the temperature level exceeds the tolerance range of organism, it is regarded as a major abiotic stress (Jiang et al., 2017). Therefore new strategies should be developed to strengthen the plant this can prevent the crop loses. At this point WRKY TFs respond to high temperature and help to plants to gain tolerance against the temperature changes. Thanks to the work has been done in recent years, there is increasing evidence that the WRKY TFs are involved in response to heat and cold stresses, which is due to the emergence of the complex mechanism in the reactions of plants in extreme heat (Chen et al., 2012). Li et al. (2010) has showed that

AtWRKY39 responses to heat stress by regulating the cooperation between the SA- and JA- activated signaling pathways (Li et al., 2010). Chen et al. (2012) reported that AtWRKY25, AtWRKY26, and AtWRKY33 are involved in the regulation of heat stress resistance. It has been reported that *AtWRKY10* also known as *MINISEED3* is expressed in globular embryo, pollen and in developing endosperm (Rushton et al., 2010). It has been proved that AtWRKY44 is the first WRKY transcription factor which has function in trichome development (Johnson et al., 2002). Countless Arabidopsis WRKY genes are expressed in roots in a spesific location domain and they implicatively have a specialised role in cell maturation (Bimbaum et al., 2003). Trichomes are known to provide the physical protection for plant species. In a study Johnson et al. (2002) has reported that Transparent Testa Glabra2 (TTG2) gene encodes the WRKY families and this gene takes part the trichome development. WRKY family members have been shown to be the first family to control plant morphogenesis (Johnson et al., 2002).

Table 2.1. Examples of WRKY TFs functions

Gene	**Induced by abiotic factors**	**Function in abiotic stress**	**References**
AtWRKY25	Ethylene, NO, NaCl, mannitol, cold, heat, ABA, cold	Tolerance to heat and NaCl, increased sensitivity to oxidative stress and ABA	Li et al., (2011)
AtWRKY26	Heat	Tolerance to heat	Li et al., (2011)
AtWRKY34	Cold	Negative regulator in pollen specific cold response	Zou et al., (2010)
AtWRKY60	Wounding	ABA signaling, NaCl and mannitao tolerance	Chen et al.,(2010)
OsWRKY08	Drought, salinity	Tolerance to osmotic stress	Song et al.,(2009)
OsWRKY11	Heat, drought	Tolerance to xerothermic stress	Wu et al., (2009)
OsWRKY72	Salinity, heat, ABA, NAA, osmotic stress, sugar starvation	Negative regulator in ABA signaling and sugar starvation	Song et al.,(2010)
OsWRKY89	Salinity, ABA, UV-B, wounding	Tolerance to UV-B radiation	Wang et al.,(2007)

2.3 Abiotic Stress

All living organisms especially plants which are sessile organisms exposed to many adverse conditions, which can cause harmful impacts of development (Banerjee and Roychoudhury, 2015). These kind of adverse conditions are called stress for particular organisms, the most effective abiotic stresses are drought, salinity, heat and cold which inhibit the particular physiological growth of the plant system. Because of their potential effects on agriculture especially regarding yield loss, much attention has been given to these stresses. It is hard for the plant to adapt to this factors and try to sustain its life cycle. So plants had developed some defence responses against these stresses. At cellular stage closing the stomata and inhibition of vegetative growth, at the molecular level induction of the stress responsive genes can be given as an example (Matsui et al., 2008). Out of all the environmental factors drought stress have its own vital role that eventually cause disturbance in crop production and plant distribution (Zhu, 2002). Thereby it is important to understand the complex mechanism of abiotic stress tolerance for agricultural sustainability. Eventually this causes decrease in photosynthesis and also accumulation of some soluble materials as well as the occurence of the Reactive Oxygen Species (ROS) like ascorbate, glutathione, carotenoids and catalase. To decrease the negative effect of drought stress, plants have adopted some multifaceted ways, including biochemical , physiological and morphological adaptations (Ingram and Bartels, 1996; Xiong et al., 2002; Zhu, 2002; Shinozaki et al., 2003; Bohnert et al., 2006). These strategies can be divided into two main categories, either to avoid drought stress by reducing water lose or increase in uptake of water, other way is to protect plant cells from destructive damage when water becomes deficient and tissue damage becomes inescapabale (Verslues et al., 2006). Moreover, the coordination of adaptive responses against drought stress is more important at cellular, molecular and whole plant levels (Yu et al., 2008). Loss of transpirational water through stomata is a key and vital factor of tolerance against drought (Xiong et al., 2002). Turgor-driven change volume in guard cells function as a mediator to control opening and closing of the stomata (Yu et al., 2008). Calcium ions, potassium ions, light, NO, H2O2, malate and phytohormones are the main factors to change turgor pressure of guard cells sense and initiate environmental signals to regulate stomatal movement in drought stress response (Assmann and Wang, 2001; Schroeder et al., 2001; Assmann, 2003; Nilson and Assmann, 2007; Shimazaki et al., 2007). Because of the complicated nature of drought stress response, the molecular mechanisms which are essential for plant tolerance to drought stress are yet to be unveiled. In the event of these responses be sufficiently robust, plant survival is guaranteed. These kind of plant defence responses to environmental stresses occurs at molecular level by altering the expression level of genes with the help of signaling pathways (Babitha et al., 2013). Such environmental stresses obligate the plant adaptation which allows to development of the individual plant and also allows to stabilize the successive generations (Banerjee and Roychoudhury, 2015). Transcription factors such as WRKY, NAC (NAM, ATAF1/2, CUC1/2), ERF (ethylene-responsive factor)/AP_2($APETALA_2$) are quite effective at plant-spesific regulation stage (Jiang et al., 2017). According to microarray analysis WRKY genes are induced by various abiotic stresses. It has been reported that WRKY TFs play key role in drought resistance (Tripathi et al., 2014). When the plants are exposed to various stresses WRKY transcription factors is induced to express (Jiang et al., 2017). This expression is quite fast and tissue spesific. For example, several WRKY genes are among the families induced under stresses such as drought, cold, hot and high salinity in *Arabidopsis thaliana*. Chen et al. (2010) has reported that AtWRKY18, AtWRKY40 and AtWRKY60 proteins have complex function in the plant responses against abiotic stress. Role of WRKY TFs in abiotic stress has shown in Figure 2.3. According to this figure when the abiotic stresses such as drought, heat, cold, salt and light affect the plant some kind of signal pathways (ABA and MAP kinase) activate and these signals affect the WRKY TFs. The numbers are represent the WRKY numbers for example WRKY21 is responsible to show drought tolerance to drought stress. At the end WRKY TFs show response against to abiotic stress.

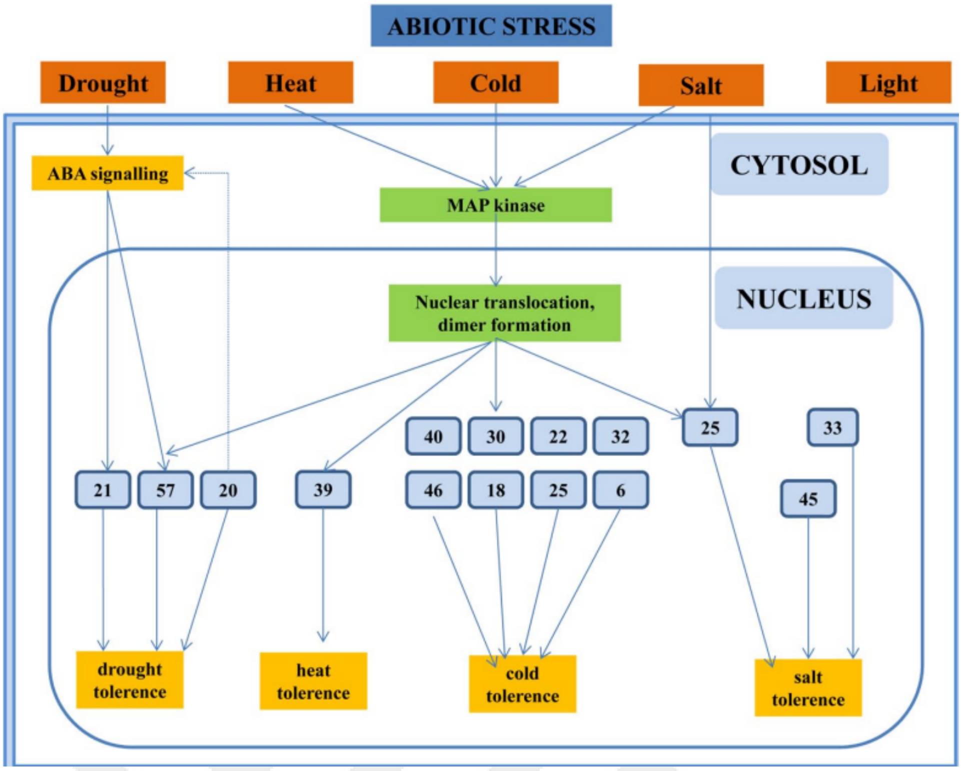

Figure 2.3. Role of WRKY proteins in abiotic stress (Bakshi, 2014)

2.4 Bioinformatic Analyses

2.4.1 Phylogenetic tree construction

All organisms are related and when it goes back to all living things have a common ancestor. There are phylogenetic trees which represent relationship among organisms and evolutionary history. Trees help us to better understand how new species emerged from common ancestors (Feng and Doolittle, 1990).

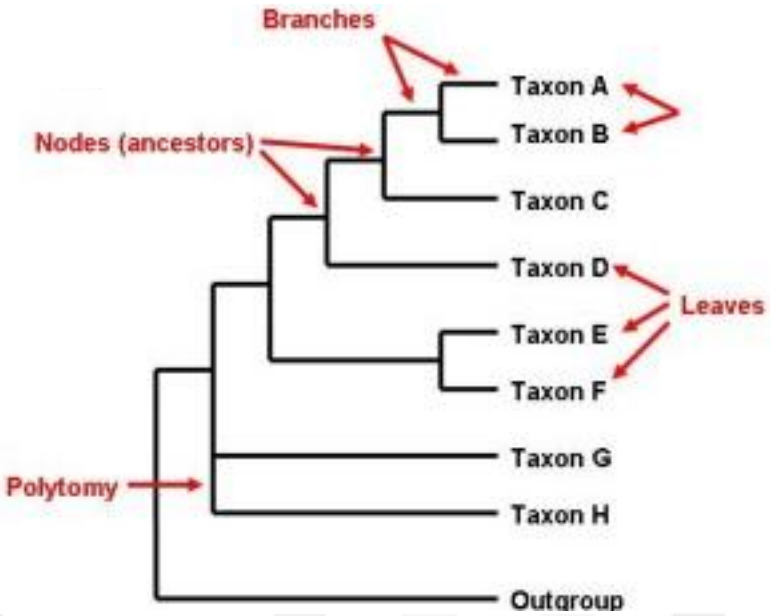

Figure 2.4. Phylogenetic tree construction (Anonymous, 2008)

A and B are the sister groups C is the outgroup to A and B. The nodes (branch points) are where separation and speciation occur from a common ancestor. The labels as a taxon of a phylogeny can correspond to individual organisms, to species, or to sets of species, as long as each taxon makes up a separate branch on the tree of life. Essentially, the taxons can even correspond to individual genes. However, some general terms for the items represented by these taxons include "terminals," "terminal taxa," or "taxa"; in more mathematical circles, they may also be called "leaves." As opposed to taxons, the branching points within a tree, which correspond to inferred speciation events, are called nodes. Each node represents the last common ancestor of the two lineages descended from that node. Internal branches or internodes connect two nodes, whereas external branches connect a tip and a node. Polytomy is a section in which the relationships cannot be fully resolved to dichotomies, thus presenting an unlikely picture of many apparently simultaneous temporally based branches. Hypotheses that give information about the relationship of taxa to each other are called phylogenetic trees. These hypotheses are derived from information previously obtained by observing morphological or genetic features. These data collected previously are used to define homology, where the similarity comes from the common ancestor. There are some tools (MEGA, clustal W, and Geneious) to produce phylogenetic trees and these tools are using some methods. These methods are including neighbour joining, maximum parsimony, UPGMA, Bayesian phylogenetic inference, maximum likelihood and distance matrix methods. Bootstrap, a standart technique, is a reconstruction technique with srtucturing of minor values when creating phylogenetic trees. In scientific studies generally 1000 bootstraps are prefered to take a better results (Pattengale, 2010).

2.4.2 Gene structure and chromosomal location prediction

The gene structure is formed by bringing together the sequence elements in the gene. In order to produce proteins, a gene is first transcribed from DNA to RNA. When this gene is transcribed and RNA is generated, not all of the DNA sequence is transcribed. The encoded regions are called exons and the non-encoded regions are called introns. In molecular studies, gene structure is important for understanding the function of the gene. Some bioinformatic tools have been developed to find these exon intron regions. Gene structure display server is one of these tools and coding sequnce and genomic sequnce is loaded into the program provides access to the exon-intron region.

Genes are located at specific loci on chromosomes. Many genes are carried on each chromosome. In order to determine the properties of genes, their position on the chromosome must be determined. There are some tools (RCSB, and mapchart) created for this purpose. The mapchart consists of a vertical bar with map locations and location names, a group diagram showing graphs of connection groups (Voorrips, 2002).

2.4.3 Gene ontology

Gene ontology is used to understand the representation of gene and gene product characteristics. Experimental data can be easily evaluated and functional interpretation in all aspects using gene ontology. In order to illustrate the relationship between gene products, GO terms are positioned as follows;

(i) biological process; They are functions programmed by the organism to achieve specific objectives, enabling multiple molecular activities to occur. For example cellular process or biological regulation.

(ii) cellular component; subcellular localization. It is the place where the gene product forms the function in the organism, such as, nucleus, ribosome, and chloroplast.

(iii)molecular function; activity of the gene product at the molecular level. For example, transcription regulator activity, ADP binding, and zinc ion binding.

There are some tools to detect the gene ontology such as Blast2GO and GeneOntology.

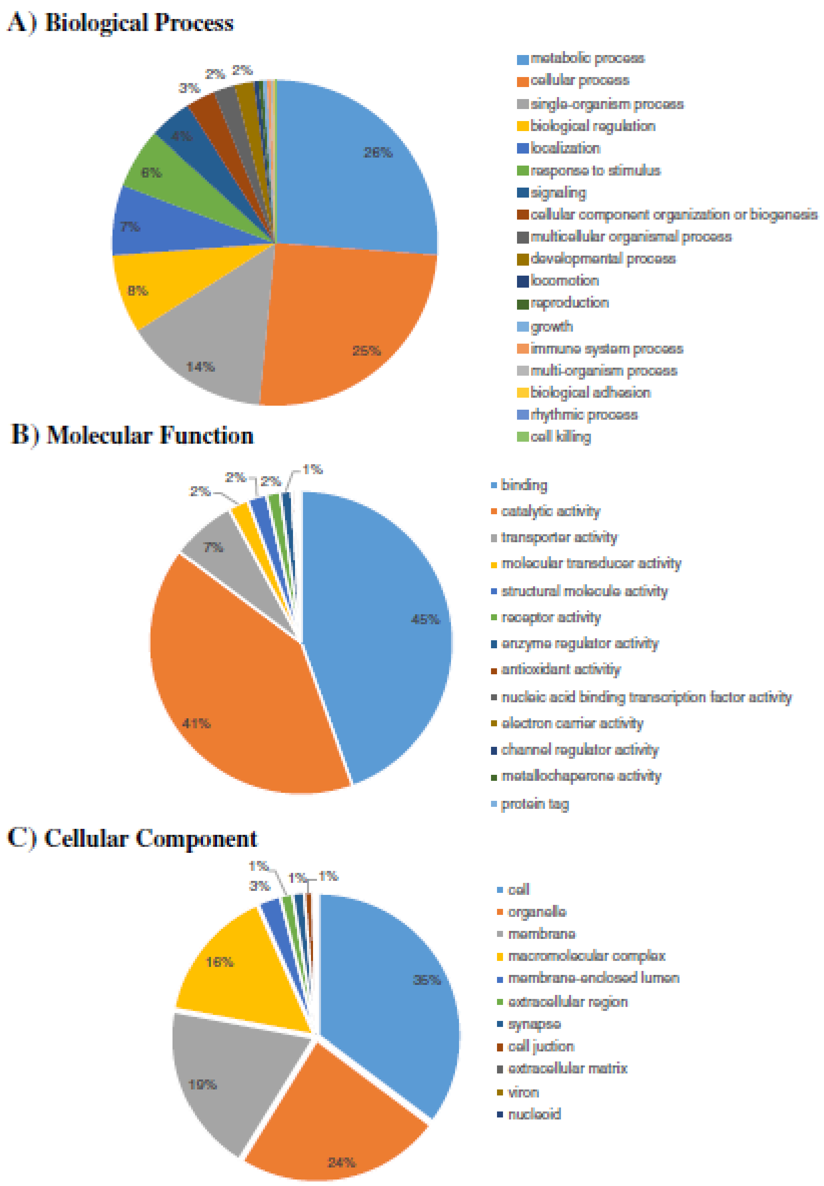

Figure 2.5. Example for gene ontology (A) biological process, (B) molecular function, and (C) cellular component (Lee, 2015)

2.4.4 MiRNA detection

MicroRNAs (miRNA) are the non-coding, 20-24 nucleotide lenght small RNAs in plants. miRNAs play essential role in cellular process and they also repress the gene expression. It has been reported that miRNAs have play important role in regulation of biotic and abiotic stresses. miRNAs used for the better understand the expression level and the changes of gene level. For that reason scientists focused on the determination of miRNA, lately (Liu et al., 2017).

2.4.5 *in silico* expression profiling

DNA, which carries information about vital functions, undergoes processes such as transcription and translation, and the information it carries becomes specific expressions. Changing environmental conditions cause changes in the expression of cells. Recently, In order to understand these changes in the gene, information technologies such as microarrays and large-scale gene expression (transcriptome) profiling have started to spread (Alba et al., 2004). There are some tools such as GeneCluster (Reich et al., 2004), Genevestigator (Zimmermann et al., 2004), expression atlas which has investigated to see the expression level of genes or proteins. These tools help to better understand the expression levels and also give heat map or logartihmic graphs with values. Gene expression patterns can provide important clues for gene function. Therefore, the expression of the gene is tried to be understood.

CHAPTER III

MATERIALS AND METHODS

3.1 Identification of WRKY Genes in Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa subsp. japonica and Zea mays Genome

Peptide sequences of WRKY transcription factors (TF) of the plants (*Arabidopsis thaliana, Zea mays, Brachypodium distachyon, Oryza sativa*) were downloaded from Plant Transcription Factor Database (PTFDB http://planttfdb.cbi.pku.edu.cn/) (Pérez-Rodríguez et al., 2009). Each sequences were compared with all the plants using a BLASTP query in the database PHYTOZOME v12 (https://phytozome.jgi.doe.gov). The peptide sequences of *Arabidopsis thaliana, Brachypodium distachyon and Oryza sativa* were searched in the database PHYTOZOME and the probable WRKY proteins were detected. The peptide sequences of *Zea mays* were searhed in the NCBI database and the probable WRKY proteins of *Z. mays* were detected. Thesis workflow was summarized as flow chart given in Figure 3.1.

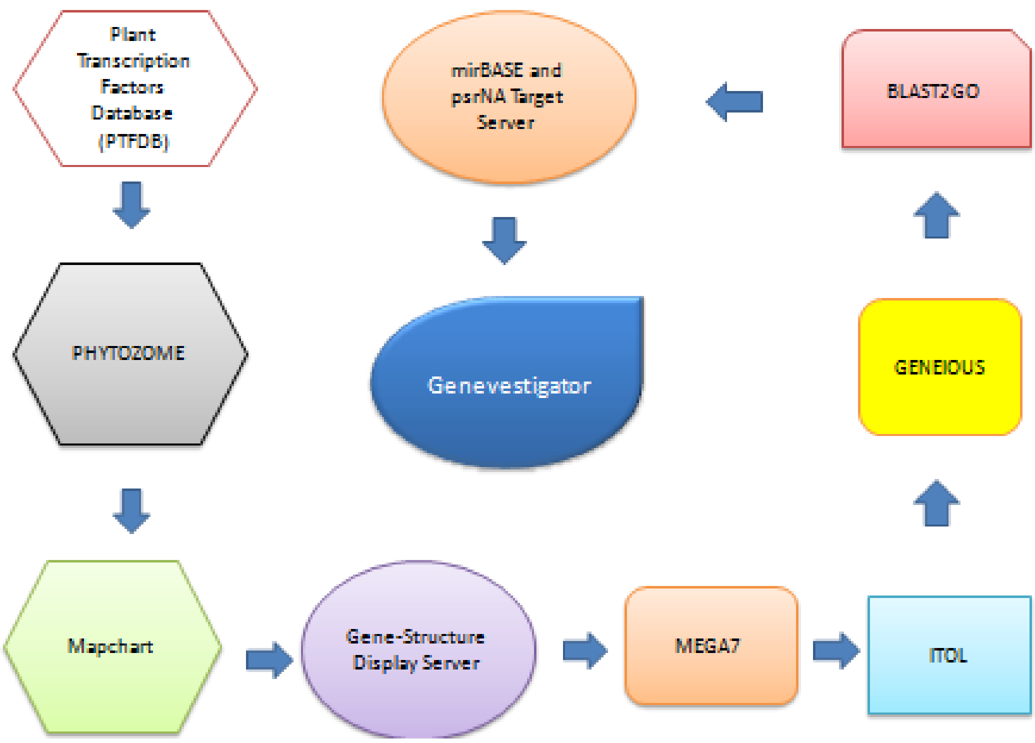

Figure 3.1. Flow chart

3.2 Determination of Chromosomal Location of WRKY Genes and Estimation of Gene Structure

Exon-intron organizations were evaluated for the determination of the structure of the WRKY protein genes. For this purpose, the coding sequences and related genomic sequences which are taken from PHYTOZOME database were obtained by using Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/) (Guo et al., 2007). Gene Structure Display Server search was done using default settings. Chromosomal location of genes encoding *Arabidopsis thaliana, Zea mays, Brachypodium distachyon,* and *Oryza sativa* WRKY proteins were determined by using PHYTOZOME database. Visualization of chromosomal locations was performed by using MapChart (Voorrips, 2002) software. Mapchart search was done using default settings.

3.3 Sequence Alignment, Phylogenetic Analysis and Determination of Conserved Motifs

The amino acid sequences of the WRKY proteins of the *Arabidopsis thaliana, Brachypodium distachyon, Zea mays,* and *Oryza sativa* plants were loaded into the MEGA7: Molecular Evolutionary Genetics Analysis (Kumar et al., 1994) software and using ClustalW (Du and Lin, 2006) algorithm with a gap open and gap extension penalties of 10 and 0.1 multiple sequence alignments were aligned, respectively (Thompson et al., 1997). An unrooted phylogenetic tree based on the neighbor joining method was constructed by using this alignment file (Saitou and Nei, 1987). After bootstrap analysis for 1000 replicates, using Interactive tree of life (iTOL; http://itol.embl.de/index.shtml) the tree was exhibited (Letunic and Bork, 2011). Geneius program has used to make alignment and after that conserved motifs of protein sequences were identified. Geneius search was done using default settings.

3.4 Gene Ontology Analysis

Functional analysis of WRKY proteins belonging to *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa* and *Zea mays* plants were carried out through the online gene ontology program (Blast2GO) (Conesa and Götz, 2008). The amino acid sequences were loaded into the program; molecular functions, cellular components and biological processes were determined. Gene ontology search was done using default settings.

3.5 Comparison of Data for Evaluation of The Tissue, Growth and Stress Responses

First of all the goal was identified the WRKYs for four plants. By using PHYTOZOME and NCBI tools were determined the WRKYs. In this part GENEVESTIGATOR programme was used to clarify how WRKY TFs effect the four chosen plants under drought, heat and salinity stresses separately. For this purpose gene ID's were taken from Plant Transcription Factors Database (PTFDB) and drought, heat and salinity stresses were chosen. As a result, it was determined which WRKY played a role in which plants development stage.

3.6 *in silico* Identification of miRNAs Targeting WRKY Genes

Sequences of all known miRNAs from plants were obtained from miRBase v21 (http://www.mirbase.org/) for use in the detection of miRNAs targeting transcripts of *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa* and Zea *mays* WRKY proteins. The transcripts of the *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa* and *Zea mays* WRKY proteins targeted by the miRNA sequences obtained were determined using the Plant Small RNA Target Analysis Server (psRNATarget, http://plantgrn.noble.org/psRNATarget) online tool. Plant Small RNA Target Analysis Server search was done using default settings.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Determination of Chromosomal Location of WRKY Genes and Estimation of Gene Structure

Determination of WRKY transcription factors was performed by using PHYTOZOME online tool and NCBI tool. To investigate the relationship between genetic divergence within the AtWRKY family in *Arabidopsis thaliana*, BdWRKY gene family in *Brachypodium distachyon*, OsWRKY family in *Oryza sativa subspecies Japonica* and ZmWRKY gene family in *Zea mays,* chromosomal locations of AtWRKYs, BdWRKYs, OsWRKYs and ZmWRKYs has determined by using Mapchart tool. Scientists were identified 72-74 WRKY TFs, in this study at the end of analysis it was identified that 72 *Arabidopsis thaliana* genes out of 90 protein sequences encoding the WRKY domain (Figure 4.1) on 5 chromosomes. In other studies 86 BdWRKY genes were identified but in this study 60 *Brachypodium distachyon* genes out of 134 protein sequences encoding the WRKY domain (Figure 4.2) on 5 chromosomes. 102 WRKY genes were identified in *Oryza sativa* subsp. *japonica* in different studies but in this study 101 *Oryza sativa subsp. japonica* genes out of 128 protein sequences encoding the WRKY domain (Figure 4.3) on 12 chromosomes. 136 WRKY genes were identified in *Zea mays* in a study but in this study 44 *Zea mays* genes out of 161 protein sequences encoding the WRKY domain (Figure 4.4) on 10 chromosomes. These genes were named after the At, Bd, Os and Zm prefix and the name of the WRKY family, indicating the name of the organisms (*Arabidopsis thaliana, Zea mays*, *Brachypodium distachyon, Oryza sativa*) according to their location on the chromosomes. In the light of all this information, the numbers of the genes encoding the families of WRKY proteins that we have identified, overlapped the results for *Arabidopsis thaliana* and *Oryza sativa* which were previously made, and it was seen that the results were almost overlapping with *Zea mays* and *Brachypodium distachyon*. *Zea mays* WRKY proteins were determined using the NCBI tool. In order to reach the most probable results, *Zea mays* WRKY nomenclatures were made considering the highest query cover values. Although the protein sequences of *Zea mays* were quite high, 44 WRKY genes could be identified as a result of attempting to obtain WRKYs with the highest query cover corresponding to these protein sequences.

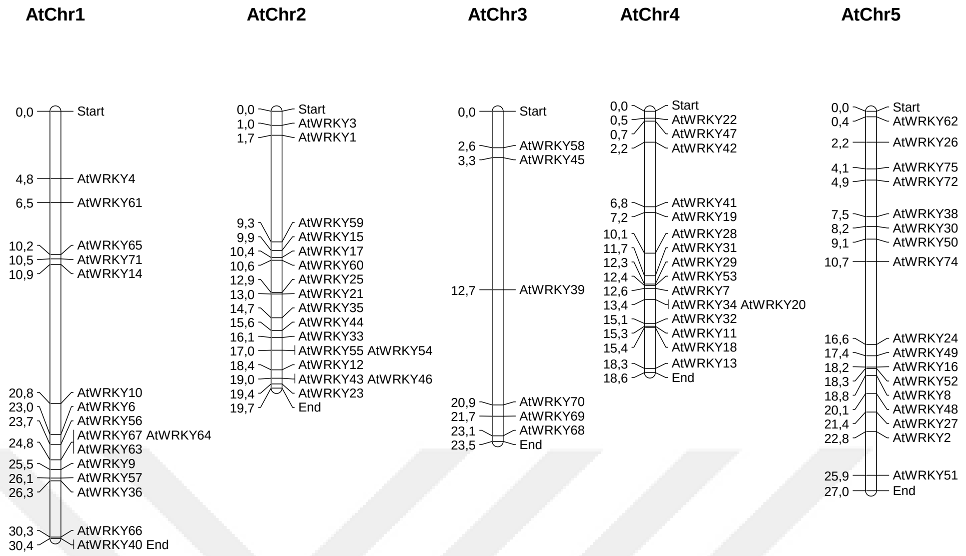

Figure 4.1. Distribution of WRKY TFs on *Arabidopsis thaliana* chromosomes

Totally *Arabidopsis thaliana* has 72 WRKY genes. Chromosome 2 and chromosome 5 had the highest density of WRKY genes with 17 members while chromosome 3 had the least numbers with only 6 members.

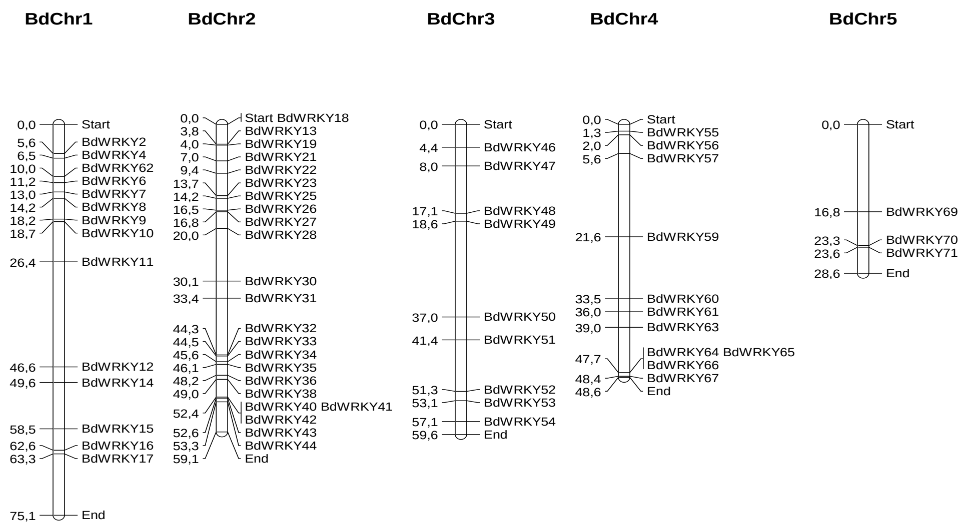

Figure 4.2. Distribution of WRKY TFs on *Brachypodium distachyon* chromosomes

Totally *Brachypodium distachyon* has 60 WRKY genes. Chromosome 2 had the highest density of WRKY genes with 23 members while chromosome 5 had the least numbers with only 3 members.

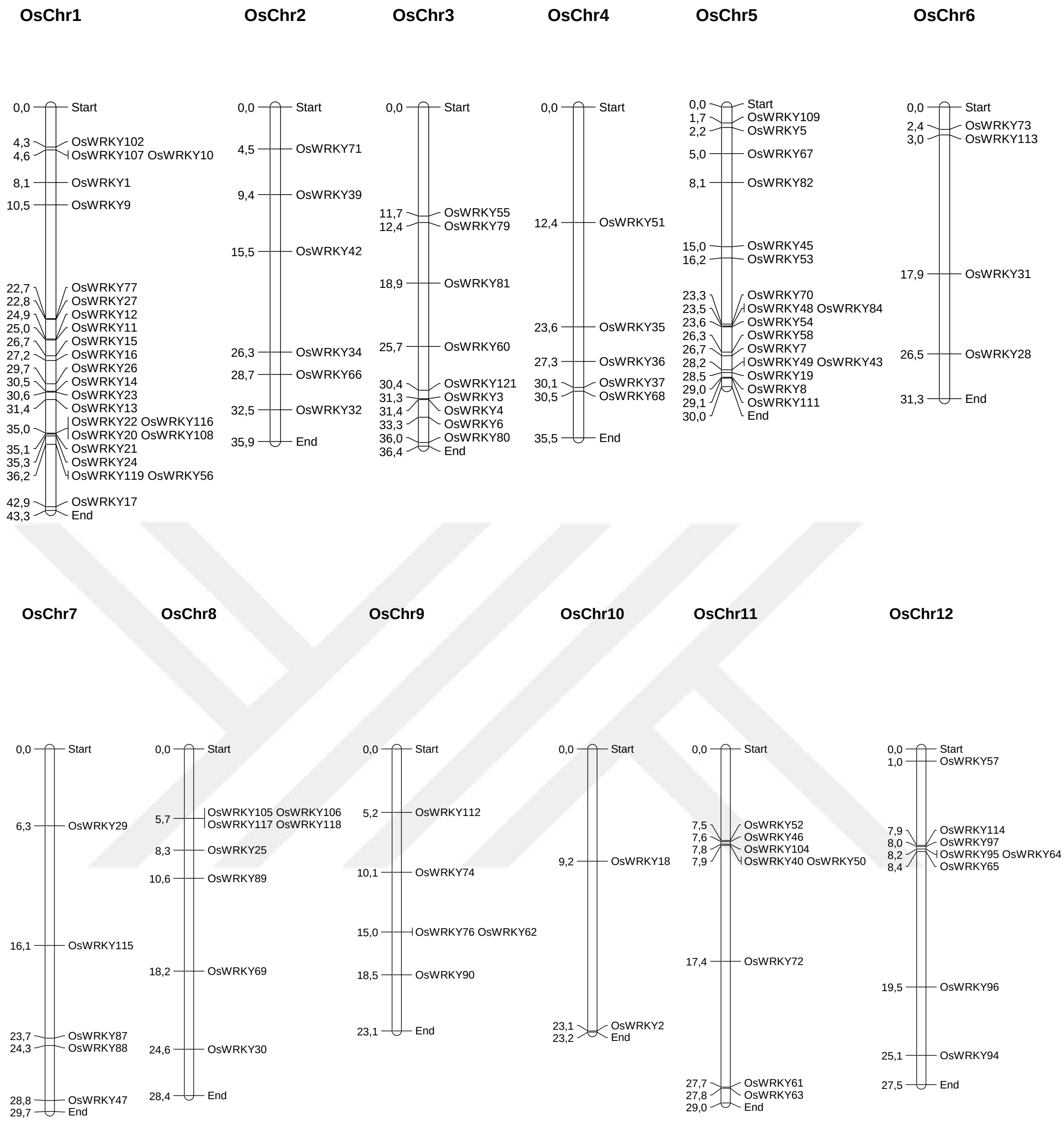

Figure 4.3. Distribution of WRKY TFs on *Oryza sativa subsp. japonica* chromosomes

Totally *Oryza sativa* has 101 WRKY genes. Chromosome 1 had the highest density of WRKY genes with 24 members while chromosome 10 had the least numbers with only 2 members.

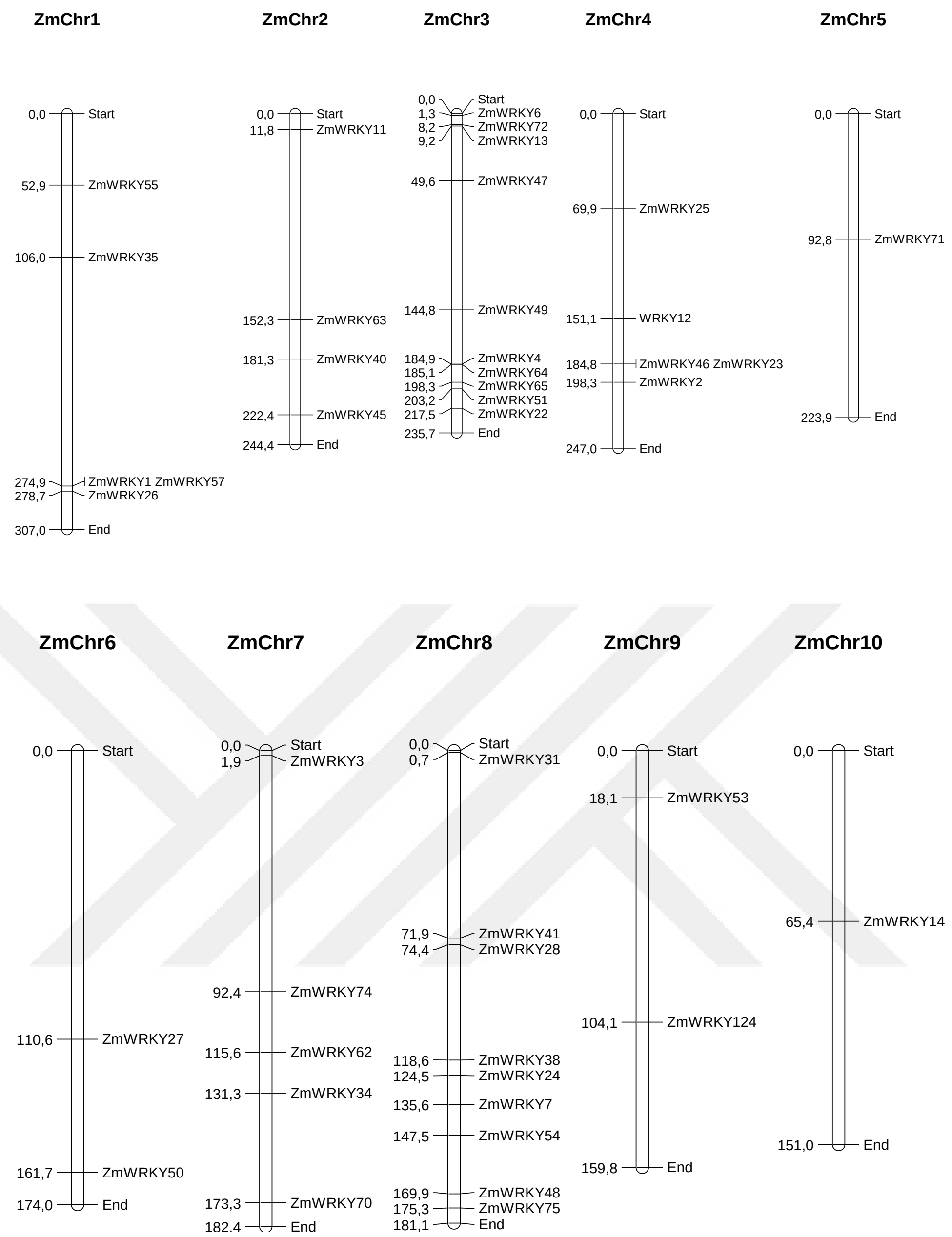

Figure 4.4. Distribution of WRKY TFs on *Zea mays* chromosomes

Totally *Zea mays* has 44 WRKY genes. Chromosome 3 had the highest density of WRKY genes with 10 members while chromosome 10 had the least numbers with only 1 member.

4.2 Sequence Alignment, Phylogenetic Analysis and Determination of Conserved Motifs

By using Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/) *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa,* and *Zea mays* exon-intron organizations were determined (Figure 4.5, Figure 4.6, Figure 4.7, Figure 4.8). Structures of the *AtWRKY* genes were evaluated by looking at exon-intron organization. It was determined that the AtWRKY19 gene is the most common site with the intron region (13 pieces) (Figure 4.5). Interestingly, OsWRKY10, OsWRKY25 has no intron regions (Figure 4.7). Phylogenetic trees performed from amino acid sequences of abiotic stress responsive WRKY domains based on an alignment of *Arabidopsis thaliana, Brachypodium distachyon, Zea mays* and *Oryza sativa.* The consensus unrooted phylogenetic trees was generated after alignment of *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa* and *Zea mays.* To further investigate the evolutionary relationships among the WRKY domains from different species phylogenetic trees have been calculated by using MEGA 7.0.21 software. The phylogenetic trees were generated with Clustal W alignment with a gap open and gap extension penalties of 10 and 0.1 multiple sequence alignments by using Neighbour joining (NJ) method. The phylogenetic trees were inferred by using MEGA 7.0.21 software. The numbers at the nodes indicate that 1000 bootstrap replicates. By using ITOL program circular trees were generated and the branch lines and nodes of subtree were coloured indicating different WRKY subgroups (Figure 4.13, Figure 4.14, Figure 4.15, Figure 4.16). Exon-intron regions were put the right sides of the phylogenetic trees as well (Figure 4.9 , Figure 4.10, Figure 4.11, Figure 4.12).

Figure 4.5. *AtWRKY* genes exon-intron organizations

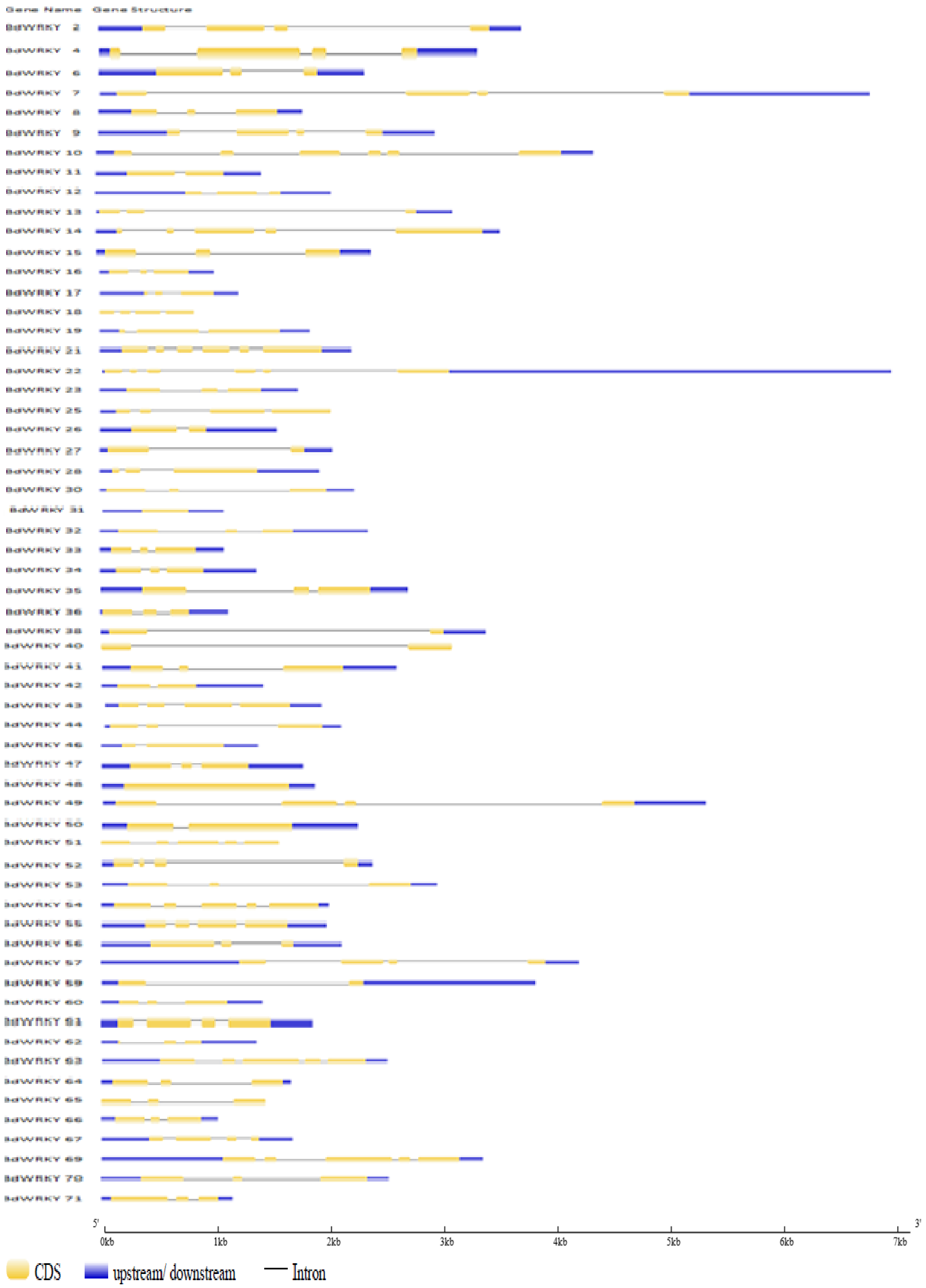

Figure 4.6. *BdWRKY* genes exon-intron organizations

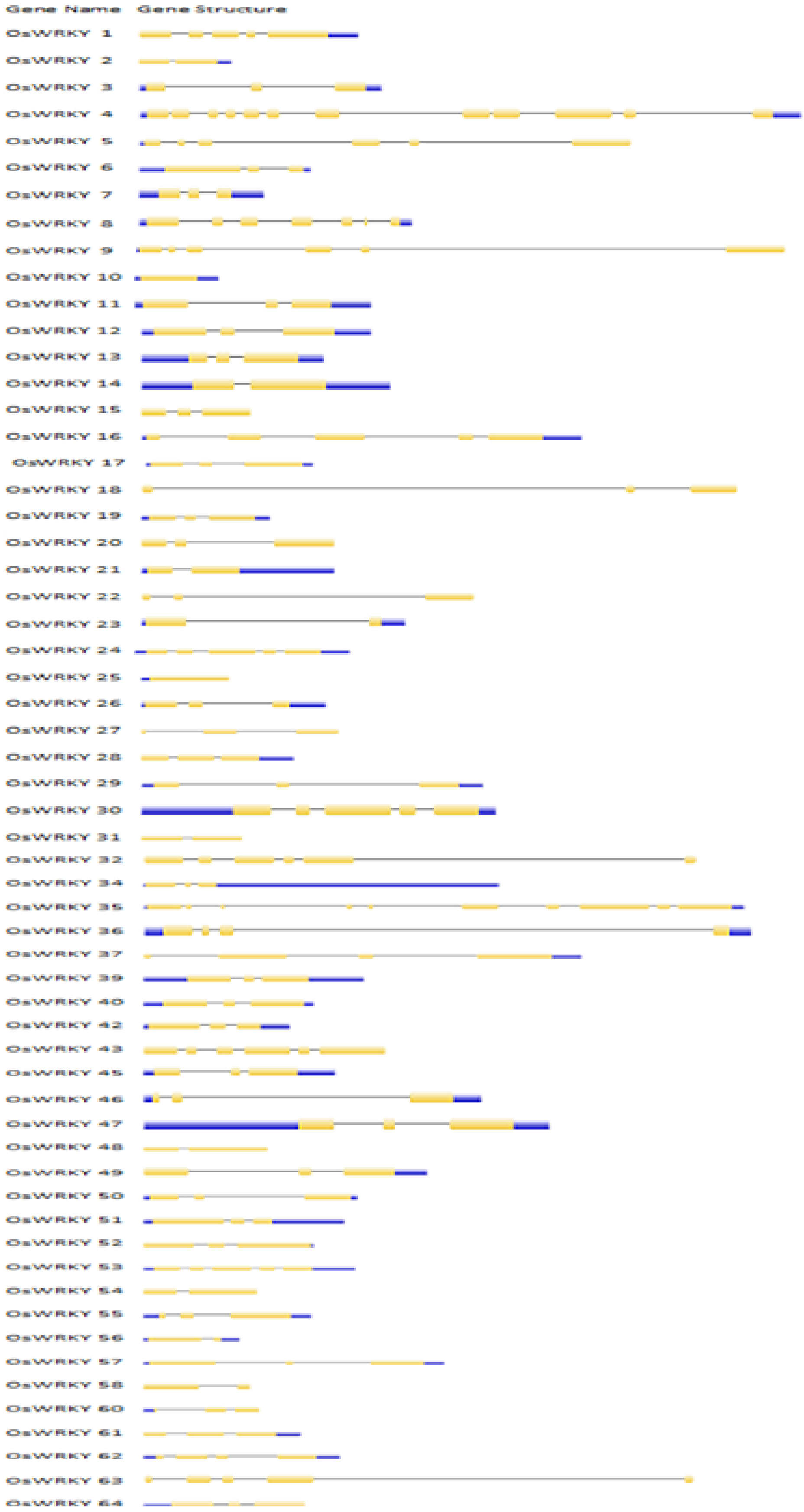

Figure 4.7. *OsWRKY* genes exon-intron organizations

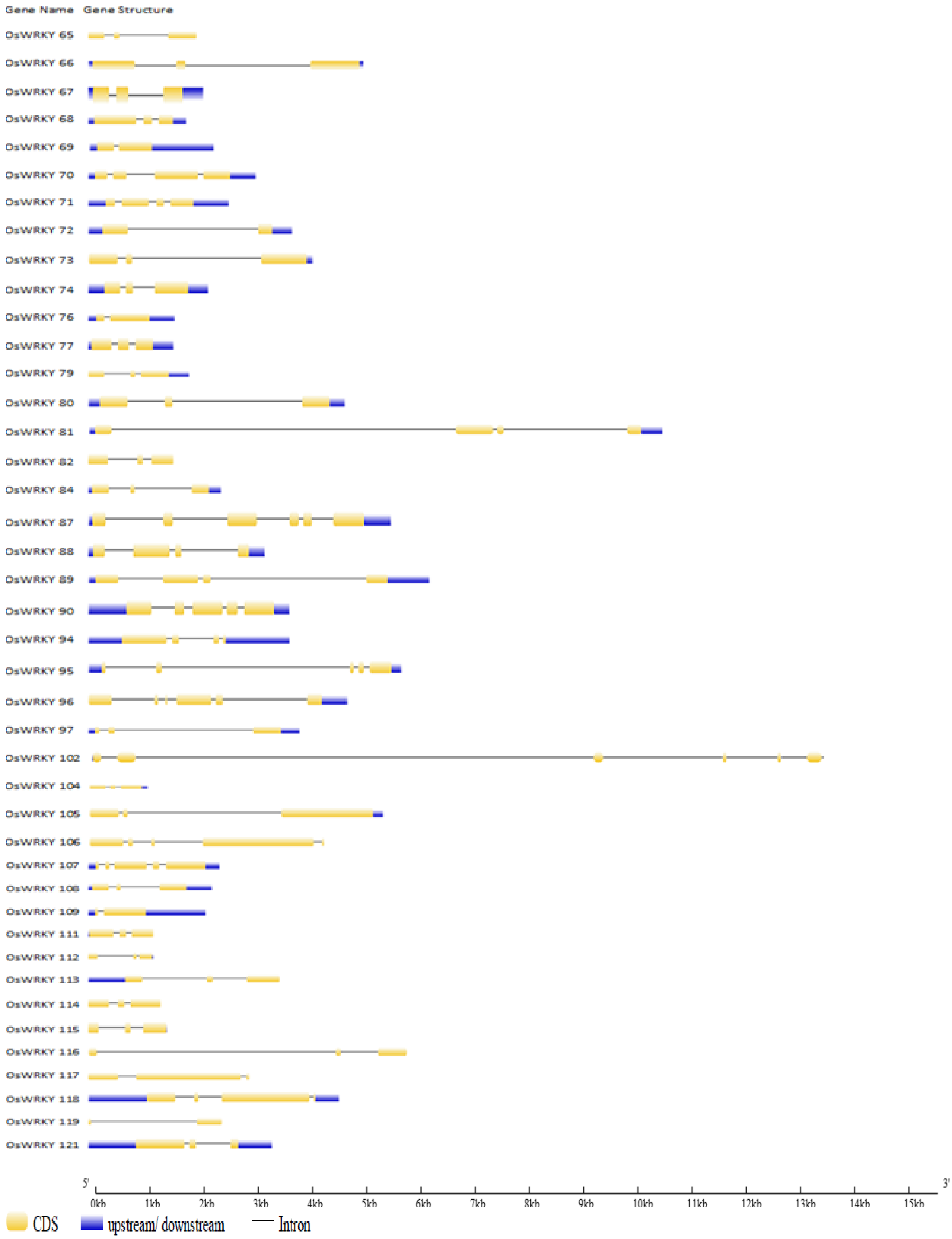

Figure 4.8. (Continuation) *OsWRKY* genes exon-intron organizations

Figure 4.9. *ZmWRKY* genes exon-intron organizations

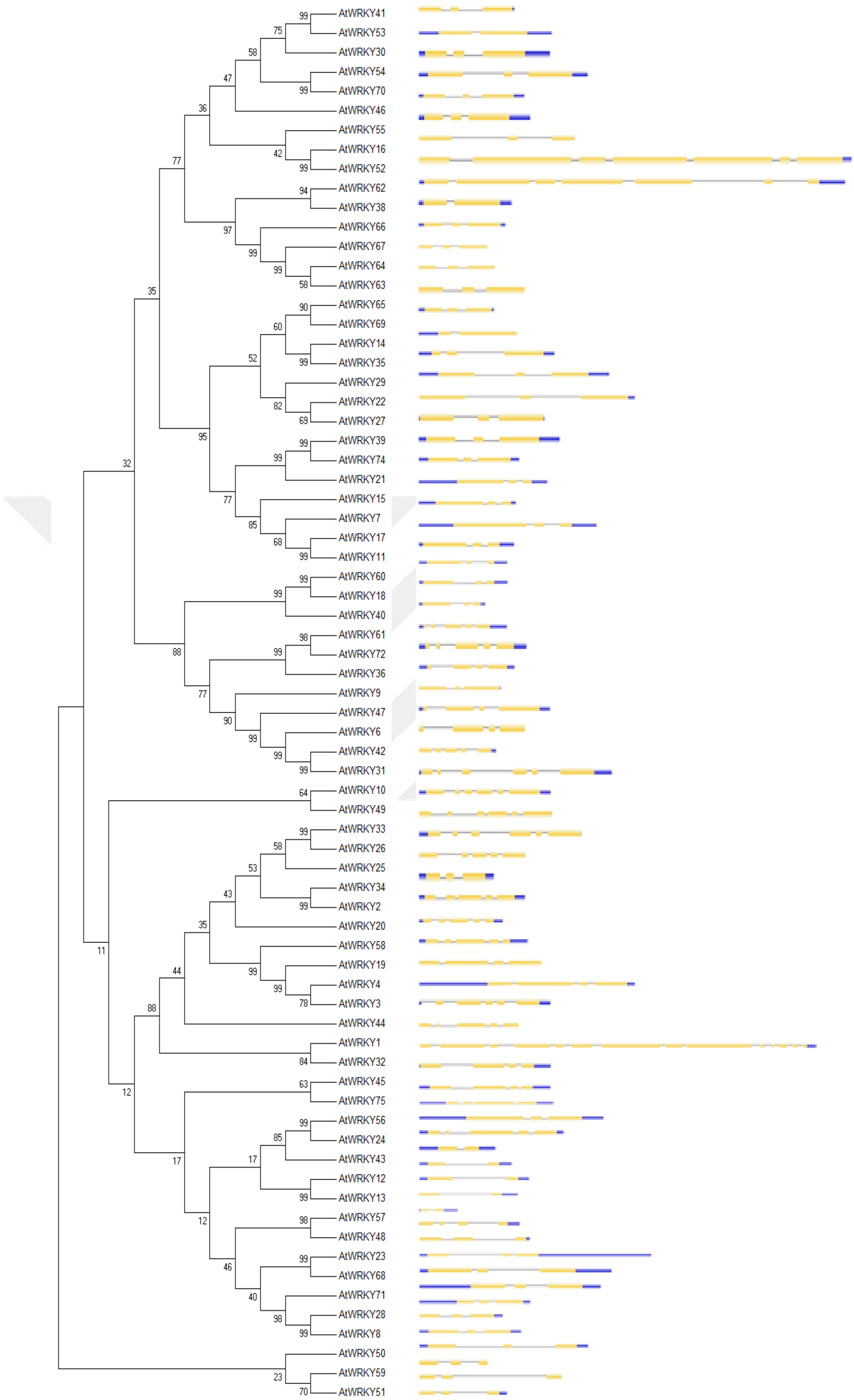

Figure 4.10. *AtWRKY* phylogenetic tree and exon-intron regions

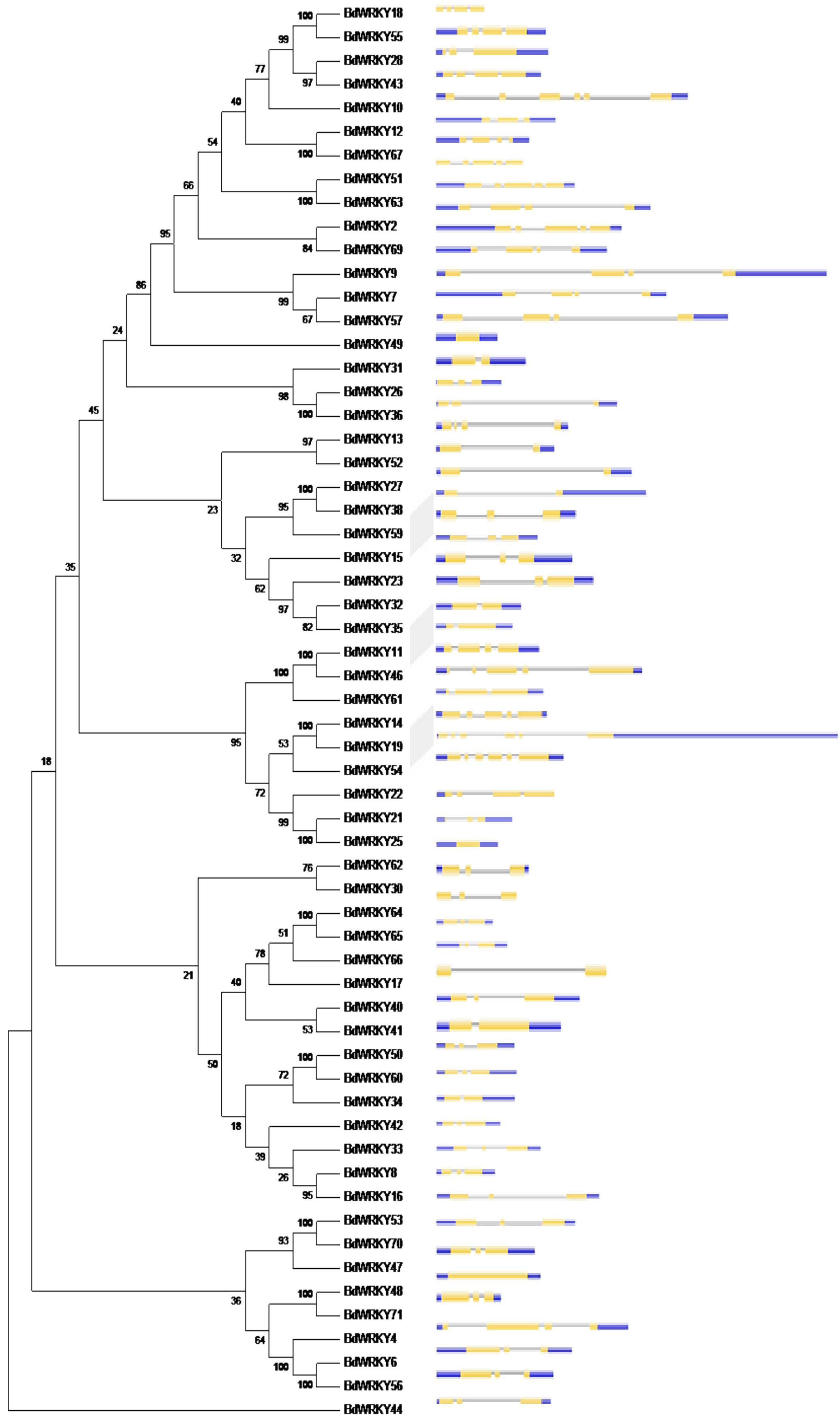

Figure 4.11. *BdWRKY* phylogenetic tree exon-intron regions

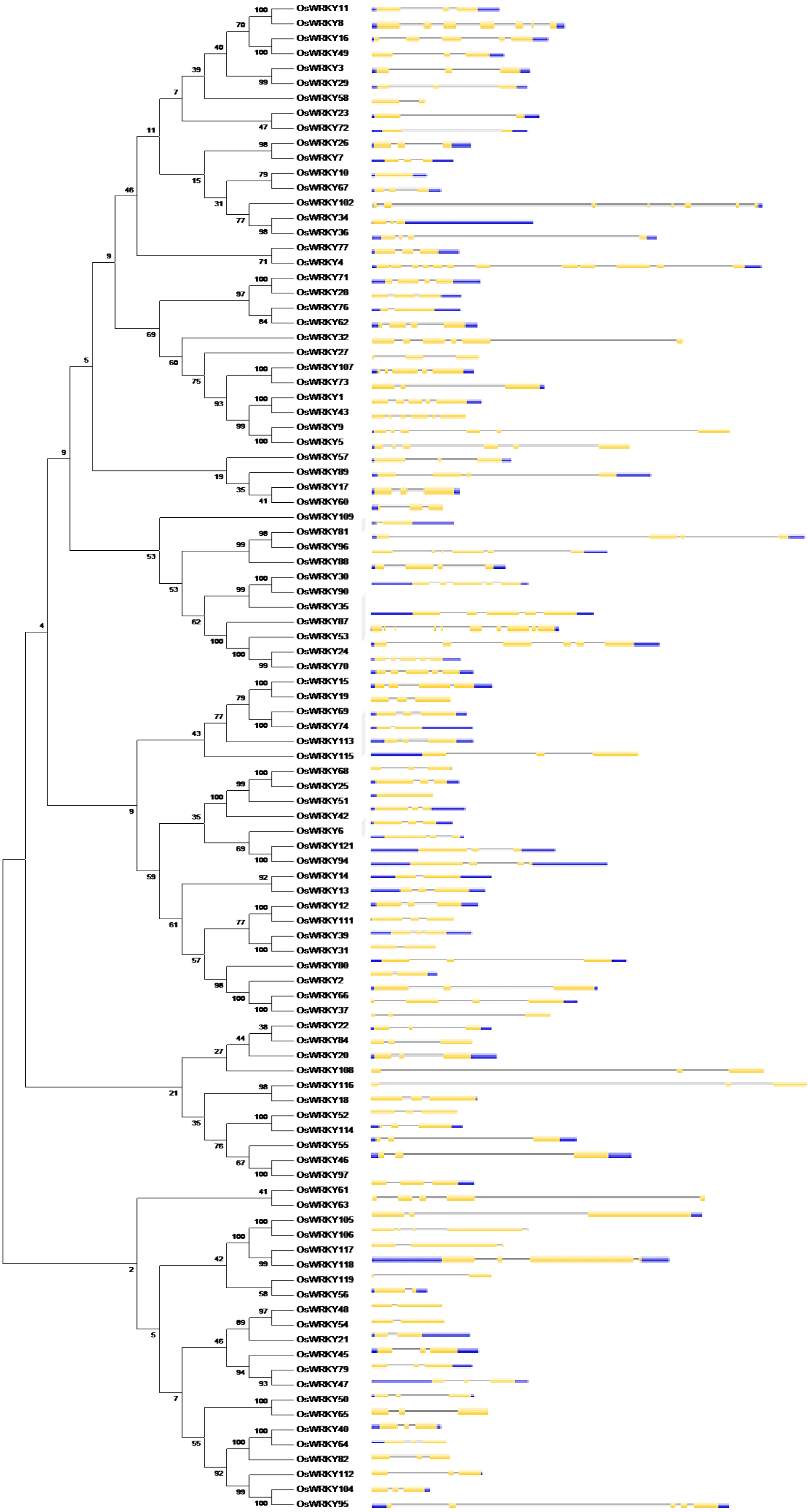

Figure 4.12. *OsWRKY* phylogenetic tree and exon-intron regions

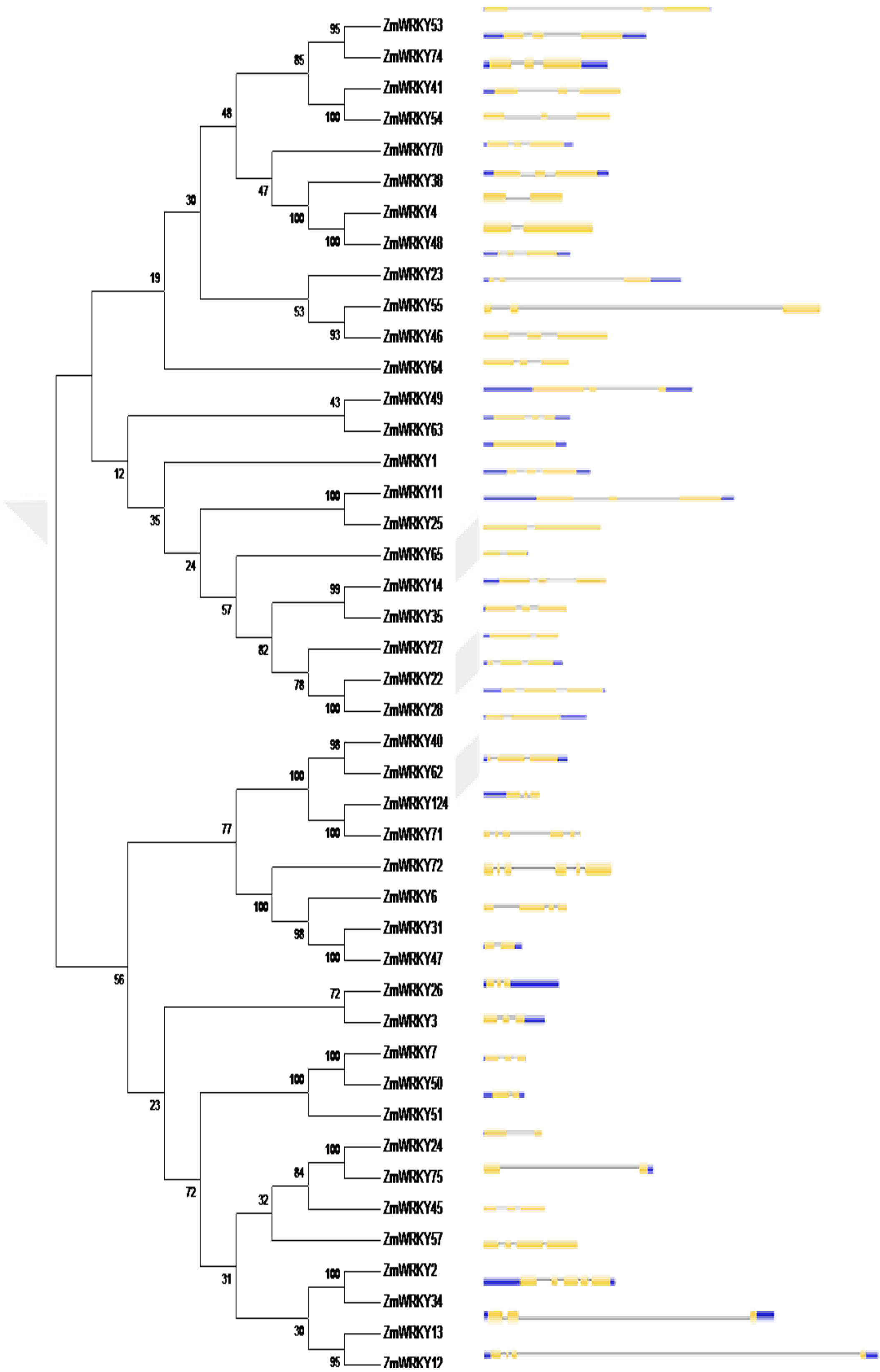

Figure 4.13. *ZmWRKY* phylogenetic tree exon-intron regions

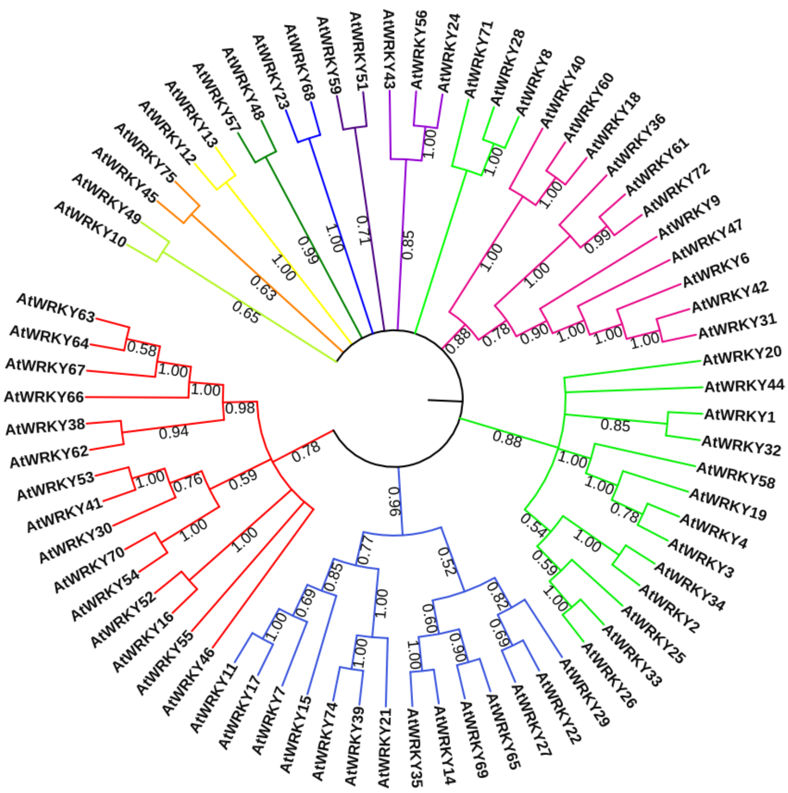

Figure 4.14. *AtWRKY* itol circular tree

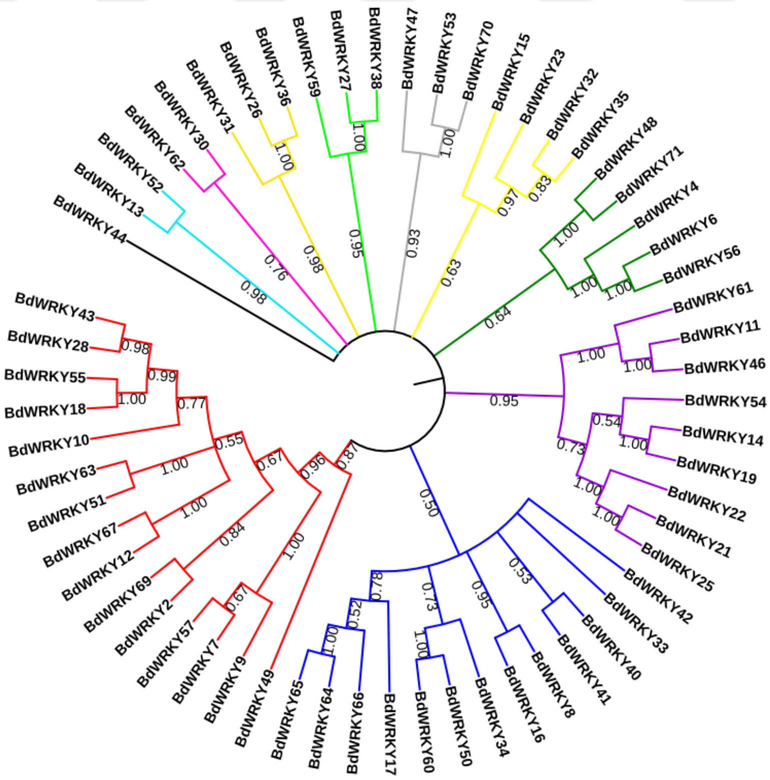

Figure 4.15. *BdWRKY* itol circular tree

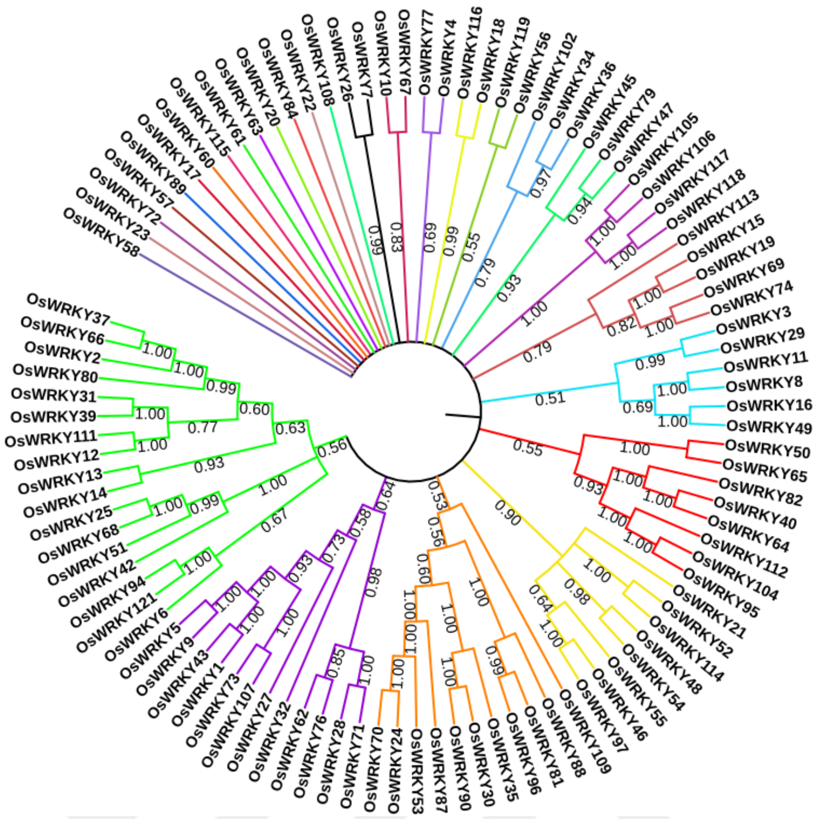

Figure 4.16. *OsWRKY* itol circular tree

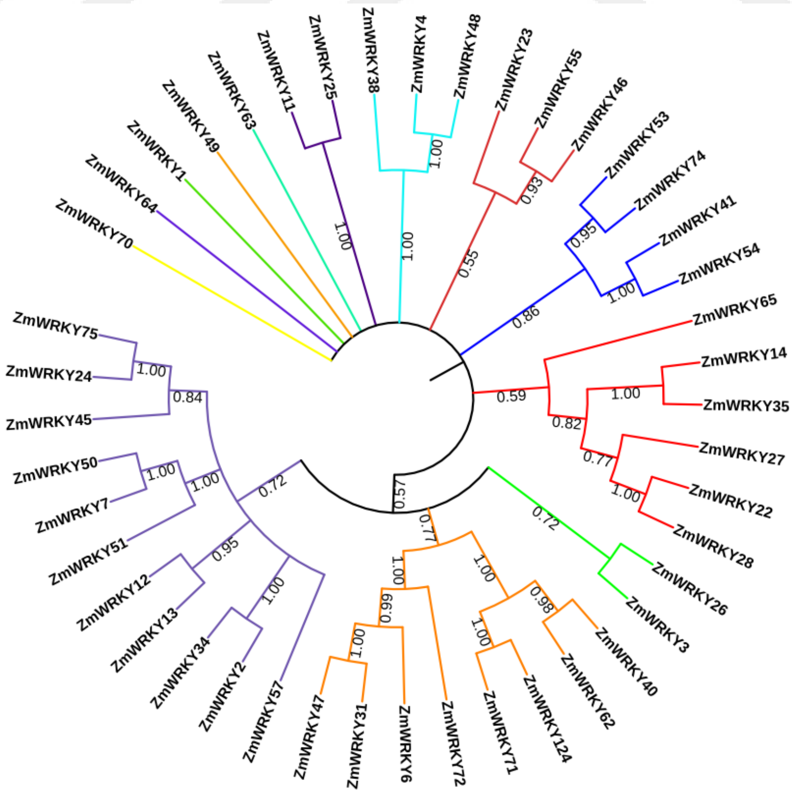

Figure 4.17. *ZmWRKY* itol circular tree

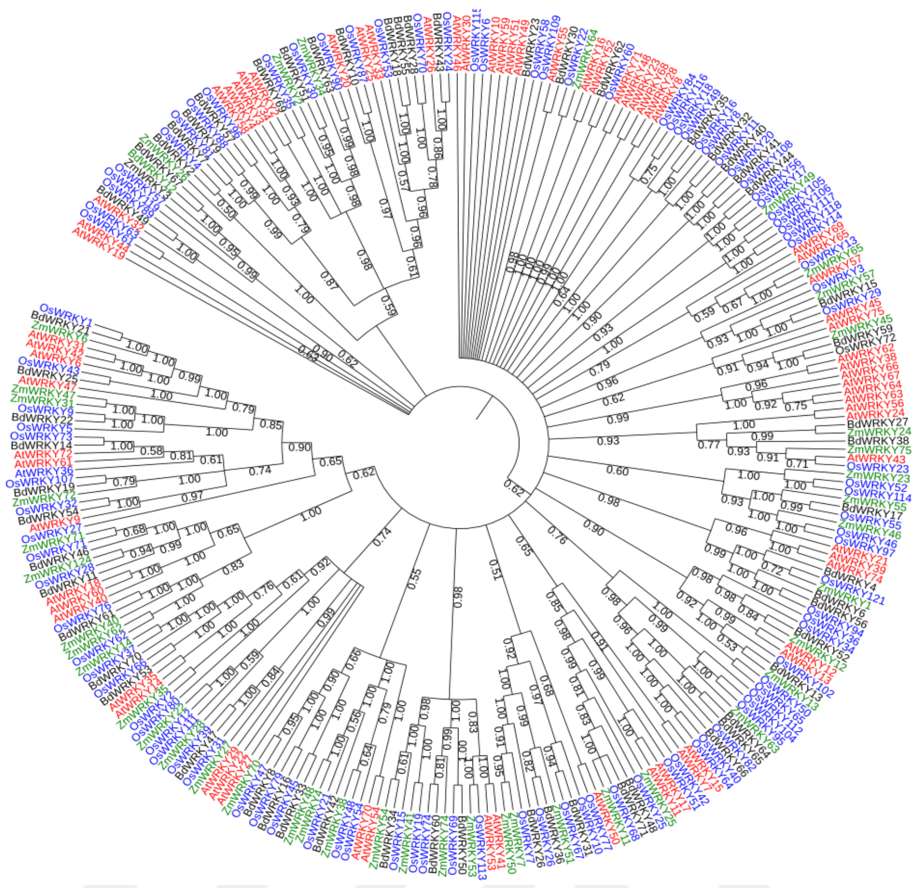

Figure 4.18. Nj analysis from *AtWRKY, BdWRKY, OsWRKY and ZmWRKY* TFs containing 277 plant WRKY proteins

Neighbour joining analysis of 277 WRKY proteins from *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa and Zea mays* suggested that BdWRKY and ZmWRKY domains are evolutionarily more closely related to *O. sativa* WRKY domains than those of *A. thaliana* (Figure 4.17).

All protein sequences of *Arabidopsis thaliana* blasted for other three plants on NCBI. After blasting the sequences, some WRKYs like WRKY2, WRKY12, WRKY22, WRKY27, WRKY31, WRKY47, WRKY50, WRKY54, WRKY57, WRKY65, WRKY70 and WRKY71 of *Arabidopsis thaliana* matched with other three plants because *Arabidopsis thaliana* WRKY's protein sequences and the other three plants WRKY protein sequences are highly matched to each other. *Arabidopsis thaliana* WRKY17 has matched with WRKY51 of other three plants (*Brachypodium distachyon, Oryza sativa and Zea mays*) while *Arabidopsis thaliana* WRKY41 matched with WRKY53 of other three plants. On the other hand *Arabidopsis thaliana* WRKY14 matched with WRKY35 of *Brachypodium distachyon* and *Zea mays* but WRKY4 of

Oryza sativa (Table 4.1). After blasting all sequences were aligned by using MEGA7.0.21 and phylogenetic tree was also constructed by using bootstrap method after the alignment (Figure 4.18). Itol circular phylogenetic tree was also performed by usign bootstrap method (Figure 4.19).

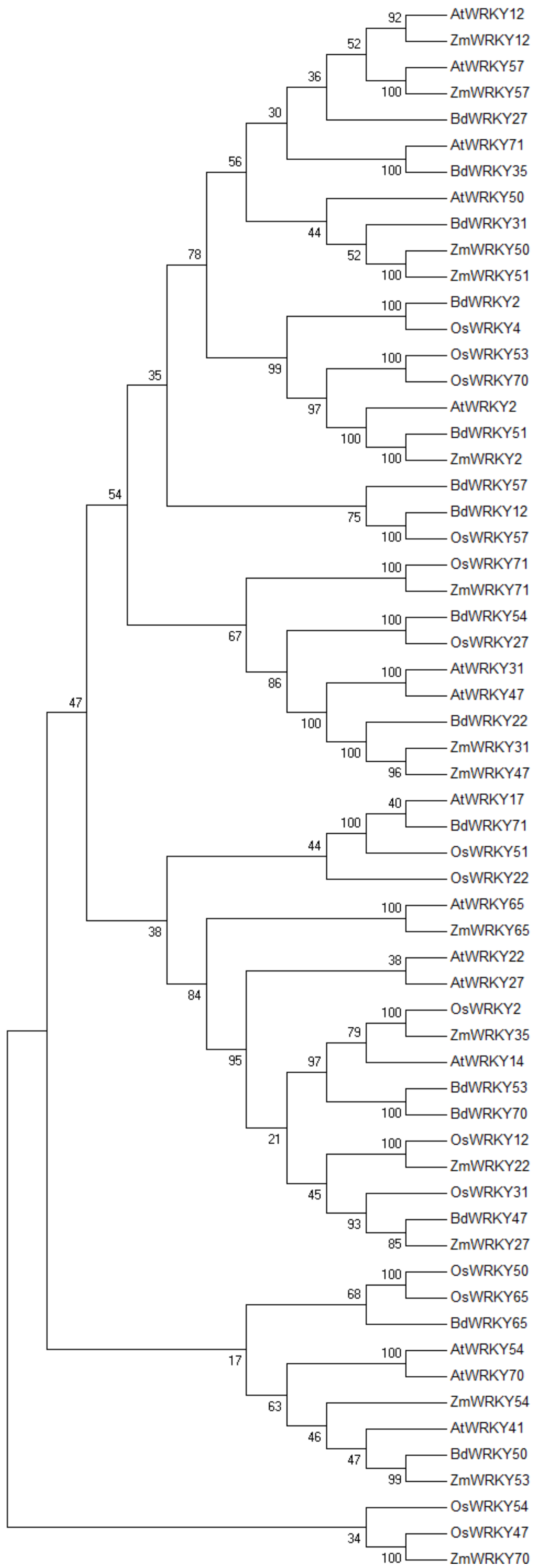

Figure 4.19. Comparison of WRKY TFs of Arabidopsis wtih other TFs in other plants phylogenetic tree

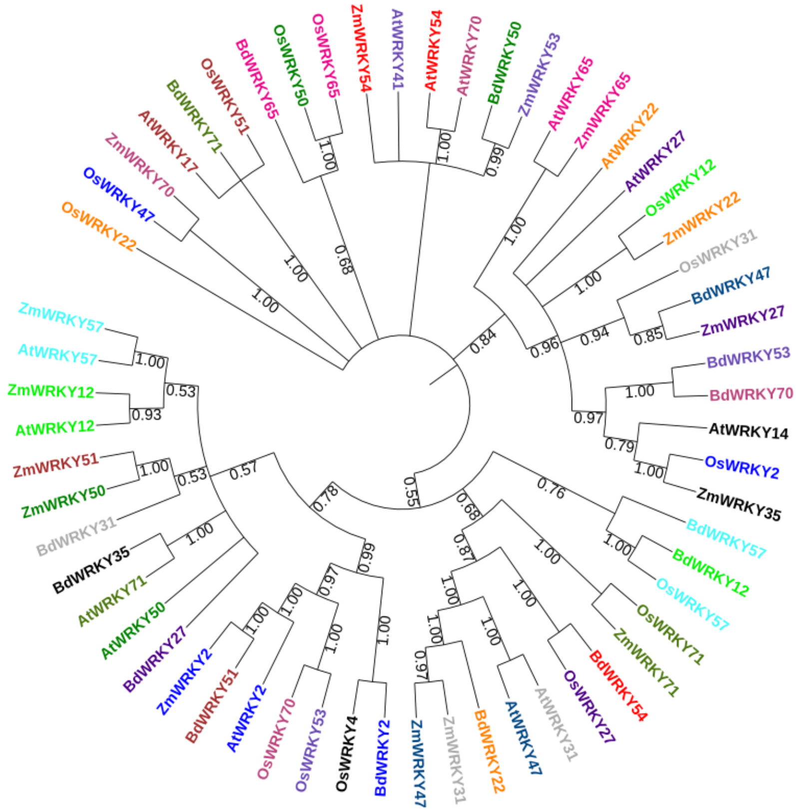

Figure 4.20. Comparison of WRKY TFs of Arabidopsis wtih other TFs in other plants itol circular Tree

WRKY2 OsWRKY4 WRKY12 AtWRKY14 AtWRKY17 WRKY22
WRKY27 WRKY31 WRKY35 AtWRKY4 WRKY47 WRKY50
WRKY51 WRKY53 WRKY54 WRKY57 WRKY65 WRKY70
WRKY71

Arabidopsis thaliana protein sequences blast results for other three plants showed in Figure 4.19 as a phylogenetic tree and Figure 4.20 as an Itol phylogenetic tree. There are result differences between Table 4.1 and Figure 4.20 such as WRKY2 is the same for all four plants but in phylogenetic tree OsWRKY2 is in different branch while AtWRKY2, BdWRKY2 and ZmWRKY2 is in the same brach.

AtWRKY17, AtWRKY41 and AtWRKY14 did not match with the same WRKY in *Brachypodium distachyon*, *Oryza sativa* and *Zea mays* (Table 4.1). AtWRKY17, AtWRKY41 and AtWRKY14 are also taking place in different branch in phylogenetic tree. *Arabidopsis thaliana* is dicot but *Brachypodium distachyon*, *Oryza sativa* and *Zea* *mays* are monocots this differences might be originate from that. It needs to be studied in more detail evolutionarily. For that reason, it is better to perform Ks / Ka analysis and evaluate duplications. There could be differences in sequences but it is clear that there might be functional similarity. Since Genevestigator could not allow to take information about the expression level of *Brachypodium distachyon*, functional expression levels could not be compared. Based on the heat map results in Genevestigar, it was concluded that AtWRKY17 had almost the same expression levels in drought, salinity and heat stresses, and OsWRKY51 had almost the same expression levels in drought, salinity and heat stresses (Figure 4.31, Figure 4.32, Figure 4.33, Figure 4.34, Figure 4.35, Figure 4.36, Figure 4.37 and Figure 4.38). However, it was observed that ZmWRKY51 showed very low expression in heat and drought stresses. As a result of this low expression, we can say that *Zea mays* does not show the same protein properties as AtWRKY17. Expression level of AtWRKY41 couldn't find in Genevestigator so the comparison was done for OsWRKY53 and ZmWRKY53. Based on the comparison OsWRKY53 has highly expressed level in drought, salinity and heat stresses but ZmWRKY53 has low expression level in drought and heat stresses. As a result of this low expression, we can say that ZmWRKY53 does not show the same protein properties as OsWRKY53. AtWRKY14 had almost the same expression levels in drought, salinity and heat stresses, and OsWRKY4 had almost the same expression levels in drought, salinity and heat stresses but ZmWRKY35 has low expression level in drought and heat stresses. As a result of this low expression, we can say that ZmWRKY35 does not show the same protein properties as OsWRKY4 and AtWRKY14.

Table 4.1. Comparison of WRKY TFs of Arabidopsis wtih other TFs in other plants

WRKY TFs	Arabidopsis Thaliana	Brachyodium Distachyon	Zea Mays	Oryza Sativa subsp. Japonica
WRKY2	AT5G56270.1	Bradi1g07970.1.p	GRMZM2G027972_P01	LOC_Os10g42850.1
WRKY12	AT2G44745.1	Bradi1g47690.1.p	GRMZM2G377217_P01	LOC_Os01g43550.1
WRKY22	AT4G01250.1	Bradi2g11170.2.p	GRMZM2G105140_P01	LOC_Os01g60490.1
WRKY27	AT5G52830.1	Bradi2g19070.1.p	GRMZM2G156529_P01	LOC_Os01g40430.1
WRKY31	AT4G22070.1	Bradi2g33540.1.p	GRMZM2G092694_P01	LOC_Os06g30860.1
WRKY47	AT4G01720.1	Bradi3g09810.1.p	GRMZM2G327349_P01	LOC_Os07g48260.1
WRKY50	AT5G26170.1	Bradi3g34850.1.p	GRMZM5G863420_P01	LOC_Os11g02540.1
WRKY54	AT2G40750.1	Bradi3g57710.1.p	GRMZM2G411766_P01	LOC_Os05g40080.1
WRKY57	AT1G69310.1	Bradi4g06690.1.p	GRMZM2G018721_P01	LOC_Os12g01180.1
WRKY65	AT1G29280.1	Bradi4g44360.1.p	GRMZM2G141299_P01	LOC_Os12g02470.1
WRKY70	AT3G56400.1	Bradi5g20290.2.p	GRMZM2G381378_P01	LOC_Os05g39720.1
WRKY71	AT1G29860.1	Bradi5g20700.1.p	GRMZM2G120320_P01	LOC_Os02g08440.1
	AT2G24570.1 (WRKY17)	Bradi3g39340.2.p (WRKY51)	GRMZM2G073272_P01 (WRKY51)	LOC_Os04g21950.1 (WRKY51)
	AT4G11070.1 (WRKY41)	Bradi3g52420.1.p (WRKY53)	GRMZM2G003551_P01 (WRKY53)	LOC_Os05g27730.1 (WRKY53)
	AT1G30650.1 (WRKY14)	Bradi2g45900.1.p (WRKY35)	GRMZM2G383594_P01 (WRKY35)	LOC_Os03g55164.1 (WRKY4)

The individual protein sequences of the four selected plants were loaded into the Geneious program. Alignment was performed and highly conserved motifs were identified after the alignment. Results of the conserved regions were exported from the program (Figure 4.20, Figure 4.21, Figure 4.22, Figure 4.23, Figure 4.24 and Figure 4.25). *Brachypodium distachyon* and *Zea mays* were found to have conserved areas accumulated in 2 different locations. Therefore, 2 different conserved regions of these two plants were exported from the program (Figure 4.21, Figure 4.22, Figure 4.24 and Figure 4.25). The motifs were shown in colored letters. Dark-colored regions in protein sequences are the most conserved regions, while light-colored regions are least conserved regions and gray-colored regions are moderately conserved regions. The region called "sequence LOGOS" is the region in which the motif contains stacks of letters and represents the letter that each motif emerges in the possible position. Size of the letters refers to the conserved region in the aligned sequences. As shown in the Figure 4.21, Figure 4.22, Figure 4.23, Figure 4.24, Figure 4.25 and Figure 4.26 the WRKYGQK domain is conserved in all plants. It is also proven in these figures that the conserved regions where the transcription factor's WRKY name originates from the WRKYGQK domain. According to the results in our study we proved that our WRKY proteins have WRKY domain, WRKYGQK Some figures such as Figure 4.21 has gaps among the protein sequences this gaps sources from the length of protein sequences, some protein sequences are short while some are long. Due to the study focused on the conserved motifs some of the short sequences weren't shown.

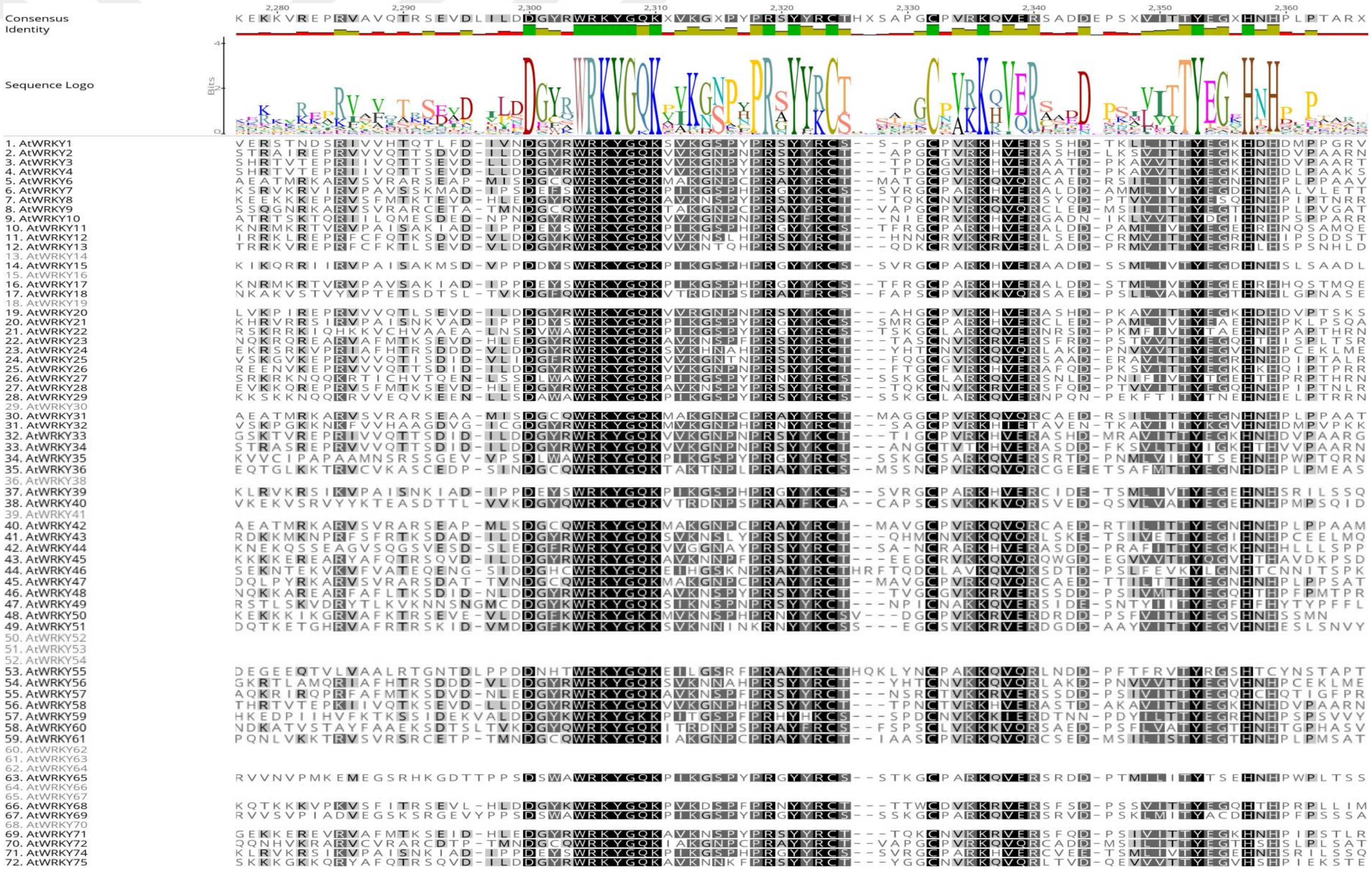

Figure 4.21. AtWRKY conserved motifs by geneious

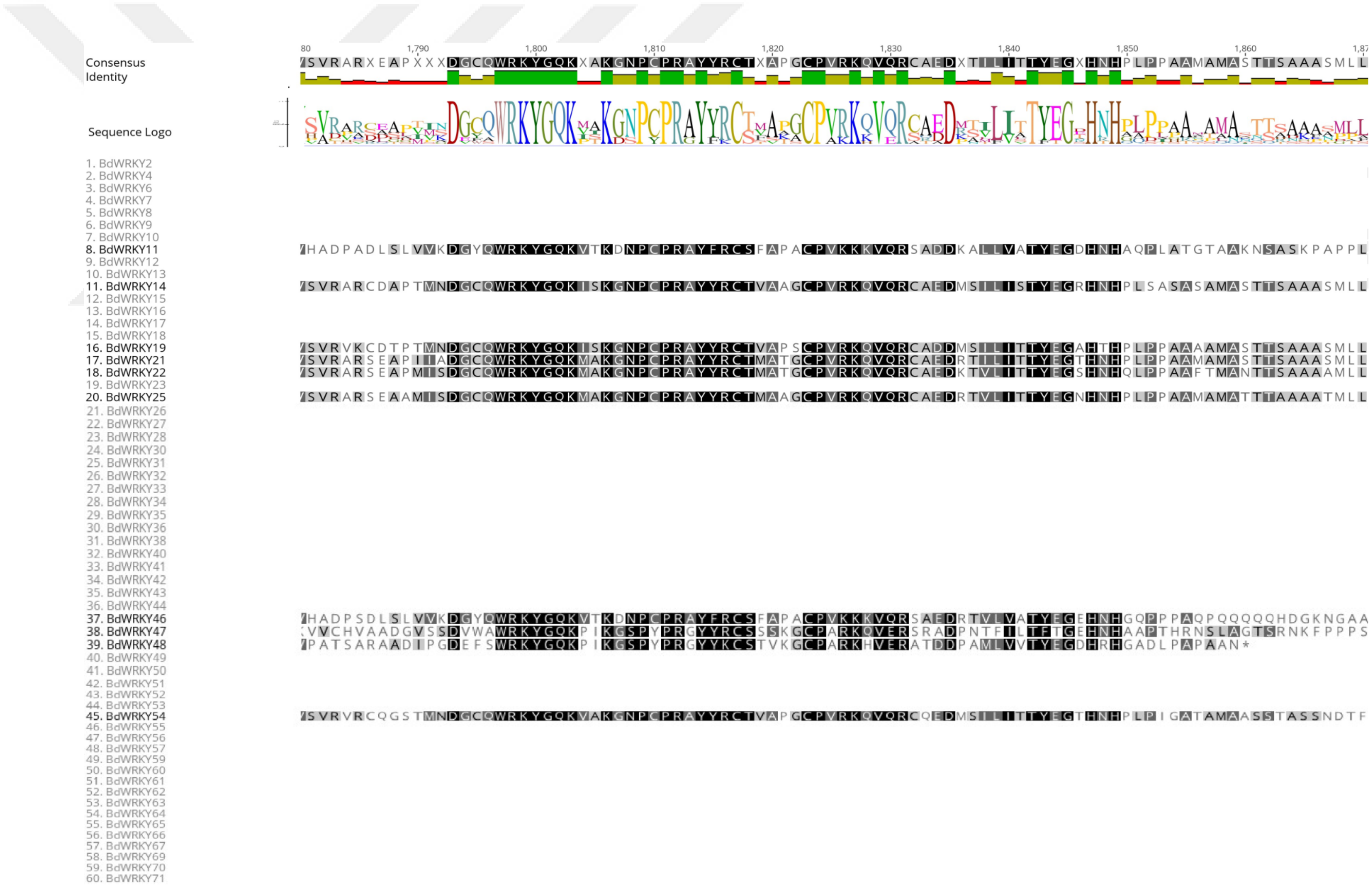

Figure 4.22. BdWRKY first part conserved motifs by geneious

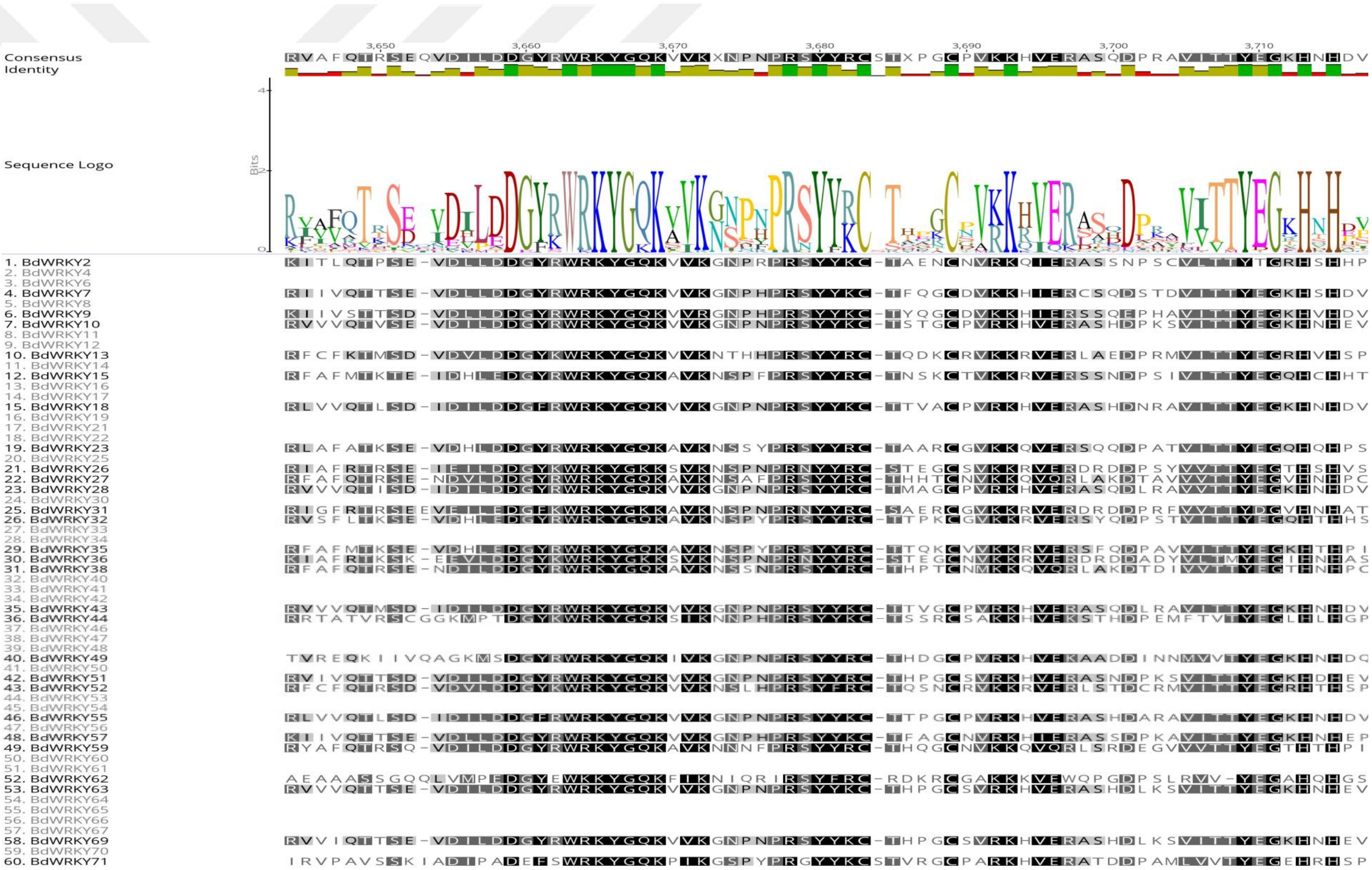

Figure 4.23. BdWRKY second part conserved motifs by geneious

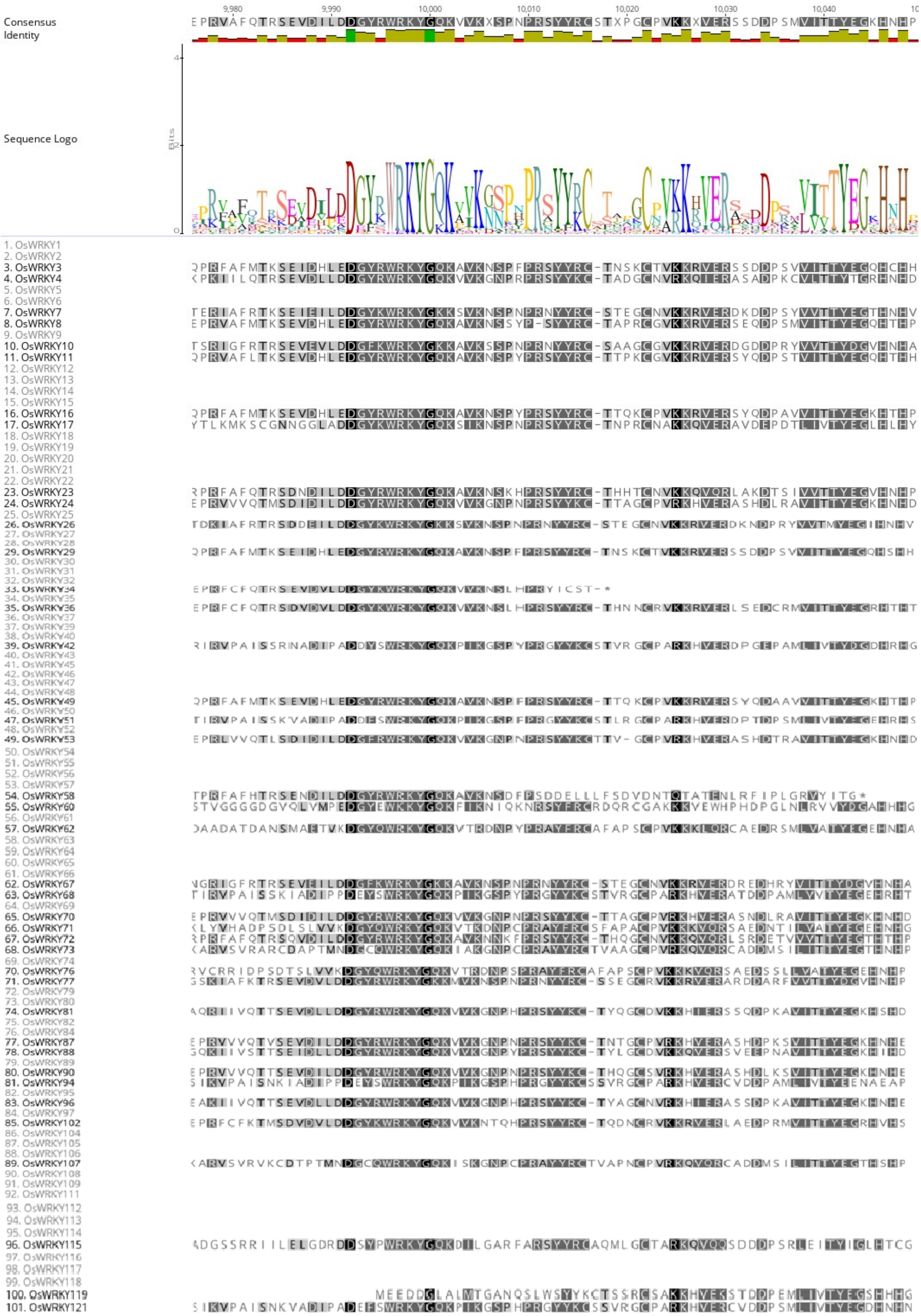

Figure 4.24. OsWRKY conserved motifs by geneious

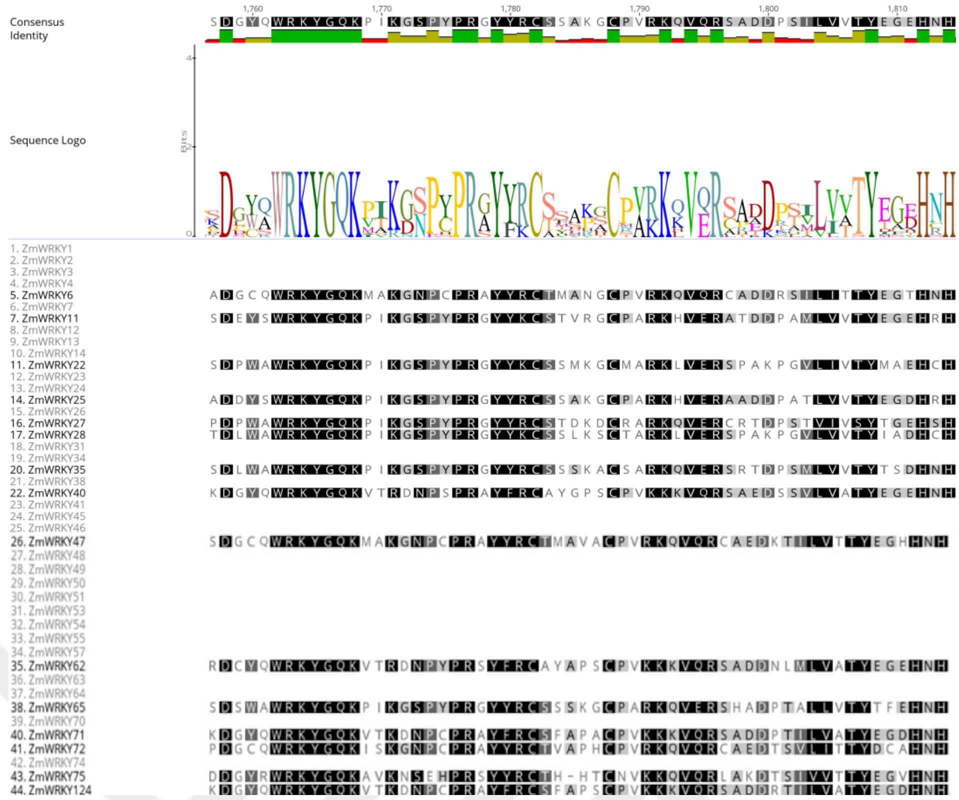

Figure 4.25. ZmWRKY first part conserved motifs by geneious

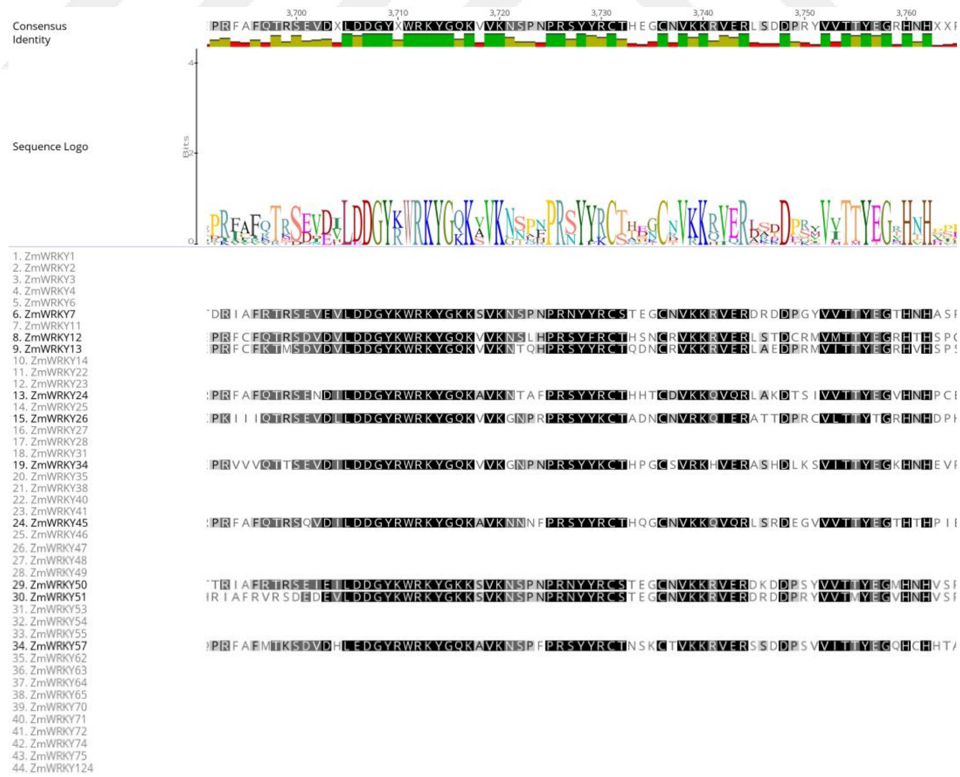

Figure 4.26. ZmWRKY second part conserved motifs by geneious

4.3 Gene Ontology Analysis

Gene ontology bioinformatics tool was used to combine the representation of gene and gene product characteristics separately for four plant species. By using the Blast2GO gene ontology program, the data was processed, thereby facilitating the interpretation of the sample, additional explanations of the gene and gene products were shown, and the controlled classification of the properties of the gene and gene products was provided. Ontology is the representation of the things we can represent or directly observe, and the representation of the relationship between them. The gene ontology program used in this study also provided an ontology of defined terms representing gene product properties. In this study, three different pie charts were performed for each plant:

(i) Biological process: These are processes that are essential to the survival of the living organism and are formed by shaping their capacity to interact with their environment, and the process of the interconnected living units. For example, the cellular process shown in this study refers to the process performed at the cellular level, but not limited to a single cell, such that cell communication between multiple cells occurs at the cellular level. Multicellular organismal process is also shown as any biological process that occurs at plant level. Developmental process is known as the biological process in the formation of an anatomical structure (cell, tissue or organ) or organism over time from the initial state to the next state.

(ii) Cellular component: The cellular component can be defined as locations relative to the cellular structures in which the gene product performs a function. In this study, the nucleus or chloroplast envelope WRKY proteins shown in the cellular component pie chart are concentrated in these cellular parts of the plant.

(iii) Molecular function: is molecular level activities of gene products using GO molecular function terms. Molecular function products correspond to the activities performed by the individual gene products, ie protein or RNA. In GO molecular functions, the word "activity" is used to avoid confusion between gene product names and molecular functions (transferase activity, DNA-binding transcription factor activity etc.). Due to transcription factors are used in this study, binding functions of transcription factors and binding activities of DNA binding regions are shown in pie chart.

In biological process of *Arabidopsis thaliana* multi-organism, cellular, metabolic, biological and developmental process and response to stimulus are known. In cellular component of *Arabidopsis thaliana* it is shown that WRKY TFs are placing in chloroplast envelope and nucleolus. In molecular function of *Arabidopsis thaliana* it is shown that as an activity ADP, transcription factor, calmodulin, identical protein, zinc ion and RNA polymerase II proximal promoter sequence-specific DNA binding activity and DNA-binding transcription factor activity (Figure 4.27).

In biological process of *Brachypodium distachyon* biological regulation, metabolic, cellular and regulation of biological process and response to stimulus are known. In cellular component of *Brachypodium distachyon* it is shown that WRKY TFs are placing in nucleolus. In molecular function of *Brachypodium distachyon* it is shown that as an activity binding and transcription regulator activity (Figure 4.28).

In biological process of *Oryza sativa* biological regulation, metabolic, cellular and regulation of biological process and response to stimulus are known. In cellular component of *Oryza sativa* it is shown that WRKY TFs are placing in nucleolus. In molecular function of *Oryza sativa* it is shown that as an activity protein, transcription regulatory region DNA binding, sequence specific DNA binding, DNA binding transcription factor activity (Figure 4.29).

In biological process of *Zea mays* biological regulation, metabolic, cellular and regulation of biological and multi organism process and response to stimulus are known. In cellular component of *Zea mays* it is shown that WRKY TFs are placing in nucleolus. In molecular function of *Oryza sativa* it is shown that as an activity binding and transcription regulatory activity (Figure 4.30).

If a comparison is made by looking at the biological processes of plants, biological processes are fully known because all studies are performed in *Arabidopsis thaliana*, since *Brachypodium distachyon* is not studied as much as *Arabidopsis thaliana*, its biological processes are not fully known. Although *Oryza sativa* is known as a model for the cereals and many studies have been done for it, it is clearly seen in Figure 4.29 that biological processes are not known as *Arabidopsis thaliana*. Similarly, although many studies have been done with *Zea mays*, its biological process like *Oryza sativa* have not been determined exactly.

In this study, unlike *Arabidopsis thaliana* WRKY TFs of all plants were located in the nucleolus. *Arabidopsis thaliana* WRKY TFs are located in the chloroplast and nucleolus. Molecular function of the WRKY transcription factors as the general properties of the binding protein formation, the formation of transcription regulator activities are shown in Figure 4.27, Figure 4.28, Figure 4.29 and Figure 4.30.

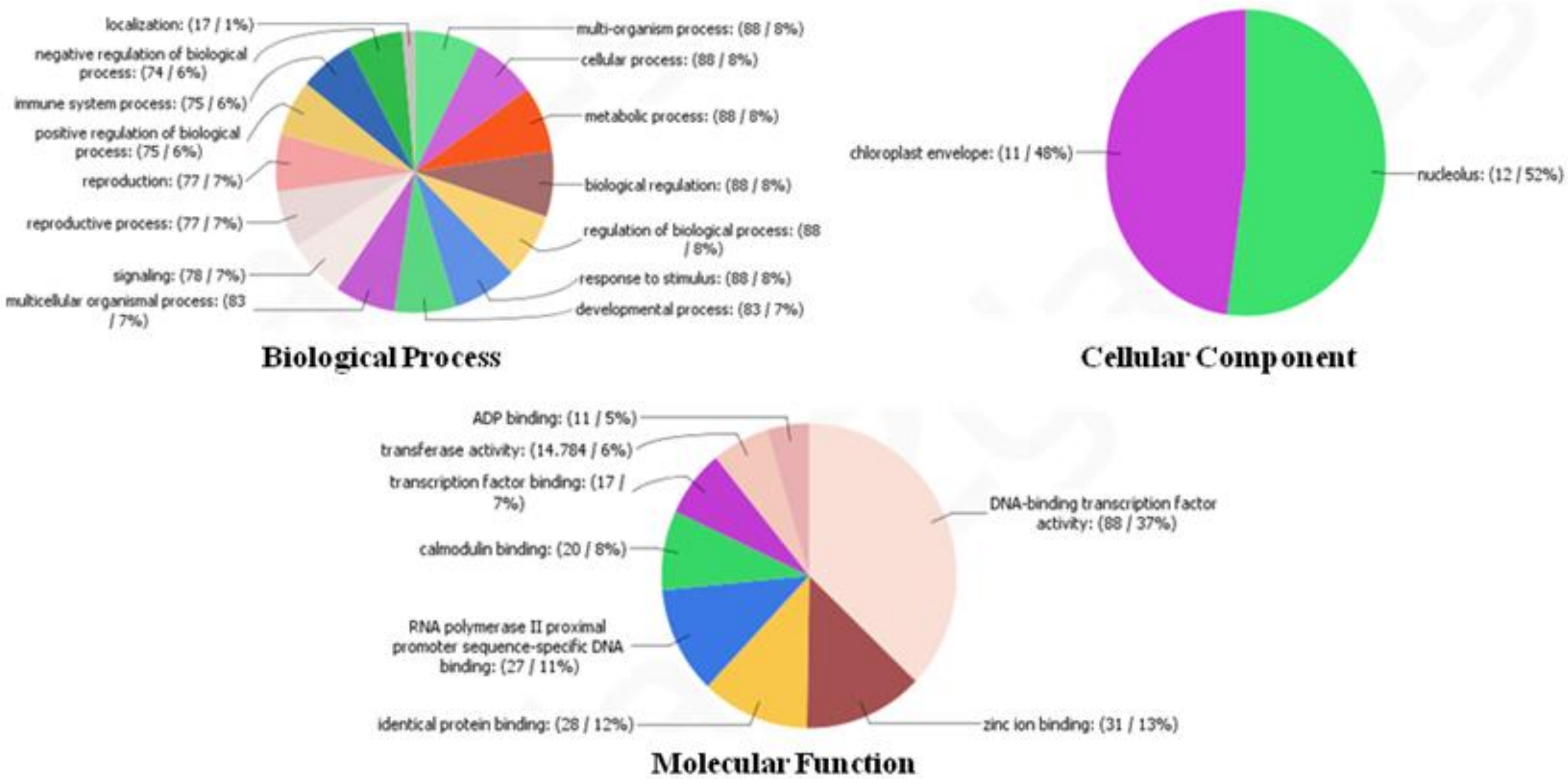

Figure 4.27. AtWRKY family gene ontology classification

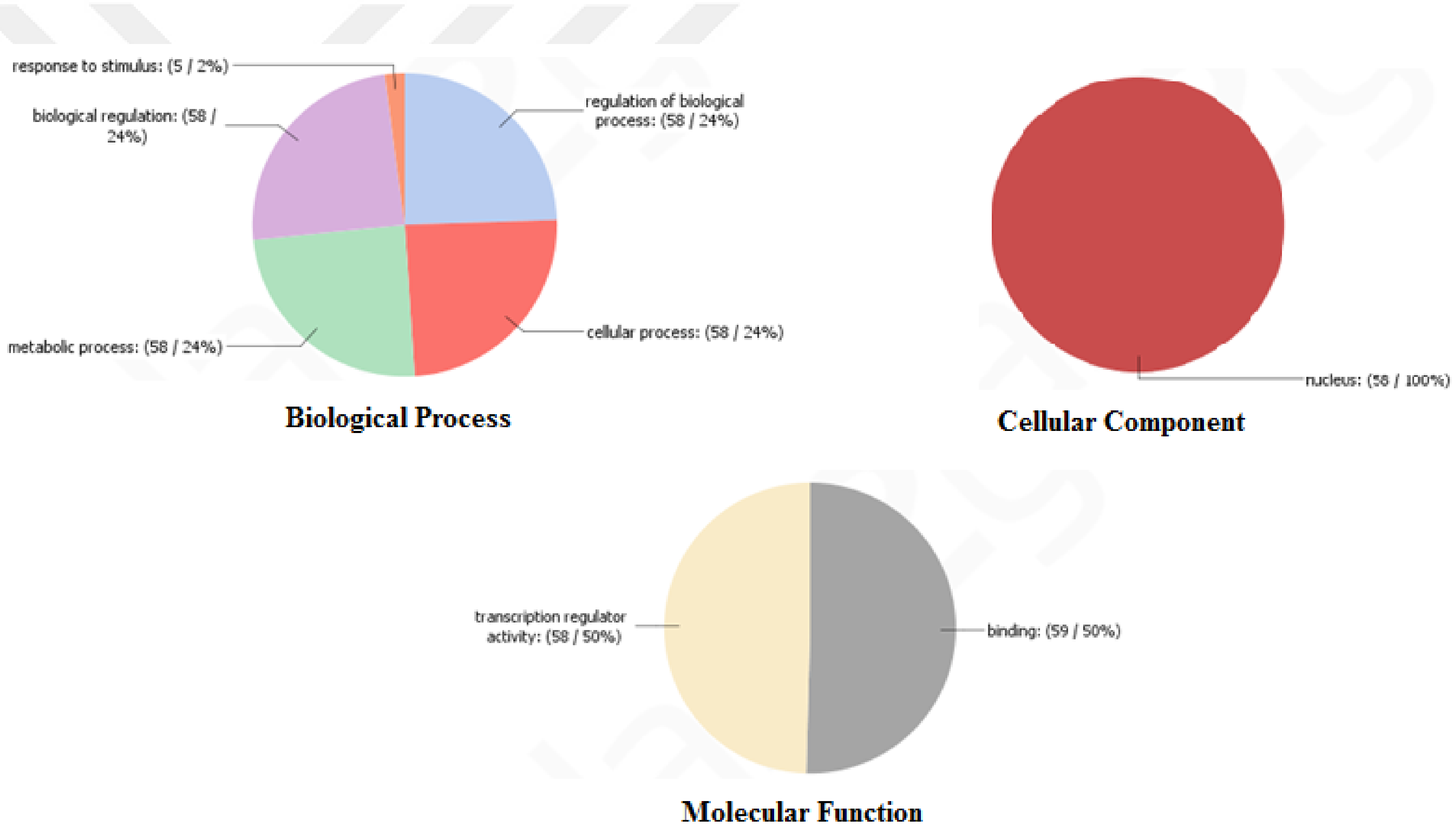

Figure 4.28. BdWRKY family gene ontology classification

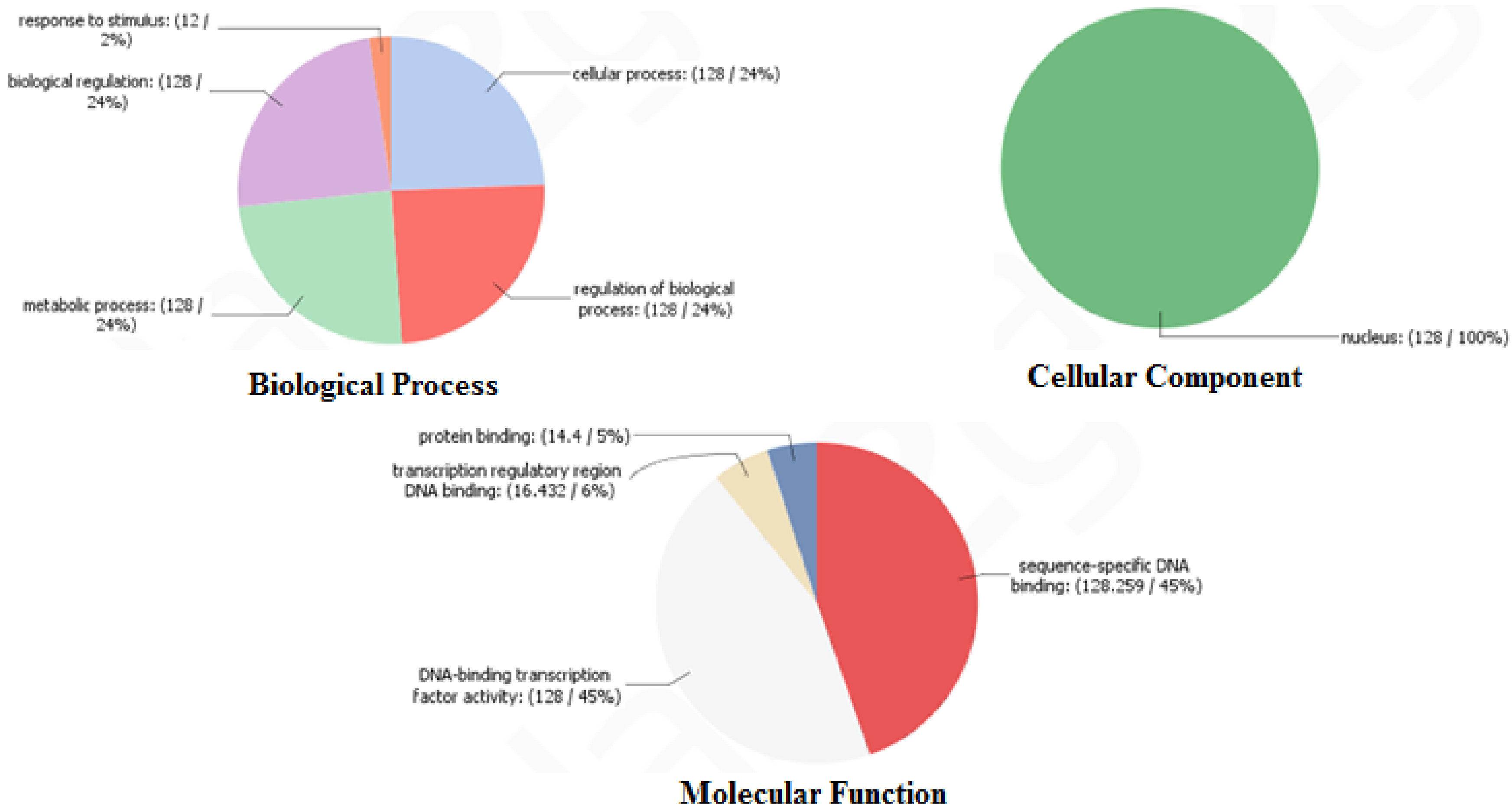

Figure 4.29. OsWRKY family gene ontology classification

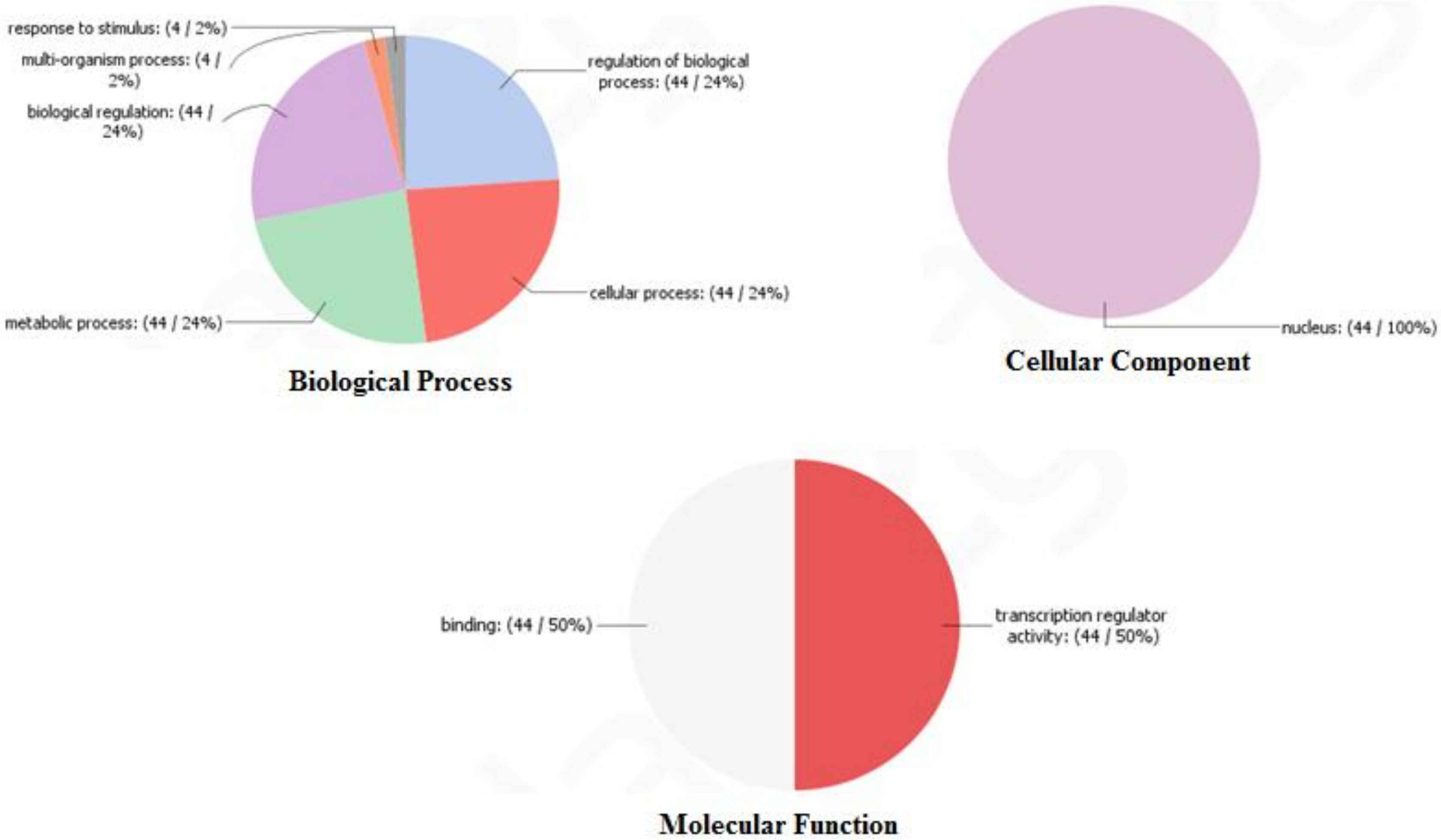

Figure 4.30. ZmWRKY family gene ontology classification

4.4 Comparison of Data For Evaluation Of The Tissue, Growth and Stress Responses

A gene expression database and service tool called Genevestigator was used to determine the WRKY TFs role under drought, salinity and heat stress conditions separately in the development of the *Arabidopsis thaliana*, *Oryza sativa subsp. japonica* and *Zea mays*. Program allowed us to see the results as a heat map. Detailed information about heat maps are shown in Appendix A, Appendix B, Appendix C, Appendix D, Appendix E, Appendix F and Appendix G. Program could not allow to check for *Brachypodium distachyon*. Therefore the expression of *Brachypodium distachyon* WRKYs were searched by litareture screening. As a result of the program, a heat map showing the percentages of WRKY transcription factors in the growth regions of plants under drought, temperature and salinity stresses separately was performed.

As a result of the process, it was found that 63 WRKY was expressed in the drought, heat and salinity stresses in *Arabidopsis thaliana*. Based on the result of table server in the drought stress at seedling stage, young rosette stage, developed rosette stage and developed flower stage the highly expressed WRKYs are like; WRKY1, WRRKY11, WRKY15, WRKY20, WRKY21, WRKY32, WRKY39, WRKY69 and WRKY70 (Figure 3.30). In salinity stress at seedling stage, young rosette stage, developed rosette stage and developed flower stage most expressed WRKYs are like; WRKY1, WRKY4, WRKY6, WRKY7, WRKY11, WRKY15, WRKY17, WRKY20, WRKY21, WRKY32, WRKY33, WRKY39, WRKY69 and WRKY70 (Figure 4.31). In heat stress at seedling stage, young rosette stage, developed rosette stage, developed flower stage and flowers and siliques stage most expressed WRKYs are like; WRKY1, WRKY3, WRKY4, WRKY7, WRKY11, WRKY15, WRKY18, WRKY20, WRKY21, WRKY32, WRKY33, WRKY39, WRKY40 and WRKY70 (Figure 4.32).

Consequently WRKY1, WRKY11, WRKY15, WRKY20, WRKY21, WRKY32, WRKY39, WRKY70 are highly expressed at all developmental stages for all drought, salinity and heat stress in *Arabidopsis thaliana*.

As a result of the process, it was found that 79 WRKY was expressed in the drought, heat and salinity stresses in *Oryza sativa*. Based on the result of table server in the drought stress at seedling stage, tillering stage, stem elongation stage, booting stage, heading stage and flowering stage most expressed WRKYs are like; WRKY3, WRKY5, WRKY13, WRKY16, WRKY21, WRKY24, WRKY25, WRKY30, WRKY39, WRKY45, WRKY46, WRKY47, WRKY51, WRKY53, WRKY55, WRKY67, WRKY68, WRKY69, WRKY71, WRKY74, WRKY76, WRKY87, WRKY88, WRKY89, WRKY90, WRKY94, WRKY95, WRKY96 and WRKY121 (Figure 3. 33). In salinity stress at seedling stage and tillering stage most expressed WRKYs are like; WRKY3, WRKY5, WRKY13, WRKY14, WRKY24, WRKY25, WRKY28, WRKY39, WRKY45, WRKY46, WRKY47, WRKY51, WRKY53, WRKY55, WRKY67, WRKY69, WRKY70, WRKY71, WRKY74, WRKY76, WRKY87, WRKY88, WRKY89, WRKY90, WRKY94, WRKY95, WRKY96, WRKY107, WRKY118, WRKY121 (Figure 4.34). In heat stress at seedling stage and tillering stage most expressed WRKYs are like; WRKY13, WRKY25, WRKY30, WRKY46, WRKY53, WRKY55, WRKY68, WRKY69, WRKY71, WRKY74, WRKY76, WRKY87, WRKY88, WRKY90, WRKY94, WRKY95 and WRKY121 (Figure 4.35).

Consequently WRKY13, WRKY25, WRKY46, WRKY53, WRKY55, WRKY69, WRKY71, WRKY74, WRKY76, WRKY87, WRKY88, WRKY90, WRKY94, WRKY95 and WRKY121 are highly expressed at all developmental stages for all drought, salinity and heat stress in *Oryza sativa*.

As a result of the process, it was found that 43 WRKYs was expressed in the drought and heat stresses in *Zea mays*. The program couldn't allow to reach information about the expression in the salinity stress. According to the result of the table server in the drought stress at seedling stage, stem elongation stage, inflorescence formation stage and anthesis stage the highly expressed *Zea mays* WRKYs are like; WRKY1, WRKY11, WRKY34, WRKY46, WRKY50, WRKY55, WRKY65, WRKY71 and WRKY74 (Figure 4.36). In the heat stress only at seedling stage the highly expressed *Zea mays* WRKYs are like; WRKY2, WRKY7, WRKY11, WRKY25, WRKY34, WRKY46, WRKY48, WRKY50, WRKY55, WRKY65, WRKY71 and WRKY74 (Figure 4.36).

Consequently WRKY11, WRKY34, WRKY46, WRKY55, WRKY65 and WRKY71 are highly expressed in both drought and heat stress (Figure 4.37).

When we look at the expression level of WRKYs which are placed at Table 4.1; AtWRKY2, AtWRKY12, AtWRKY22, AtWRKY27, AtWRKY31, AtWRKY47, AtWRKY50, AtWRKY50, AtWRKY54, AtWRKY65, AtWRKY70 and AtWRKY71; OsWRKY2, OsWRKY12, OsWRKY22, OsWRKY27, OsWRKY31, OsWRKY47, OsWRKY50, OsWRKY50, OsWRKY54, OsWRKY65, OsWRKY70 and OsWRKY71 has almost the same expression level under drought, salinity and heat stress; ZmWRKY2, ZmWRKY12, ZmWRKY22, ZmWRKY27, ZmWRKY31, ZmWRKY47, ZmWRKY50, ZmWRKY50, ZmWRKY54, ZmWRKY65, ZmWRKY70 and ZmWRKY71 has almost the same expression level under drought and heat stress.

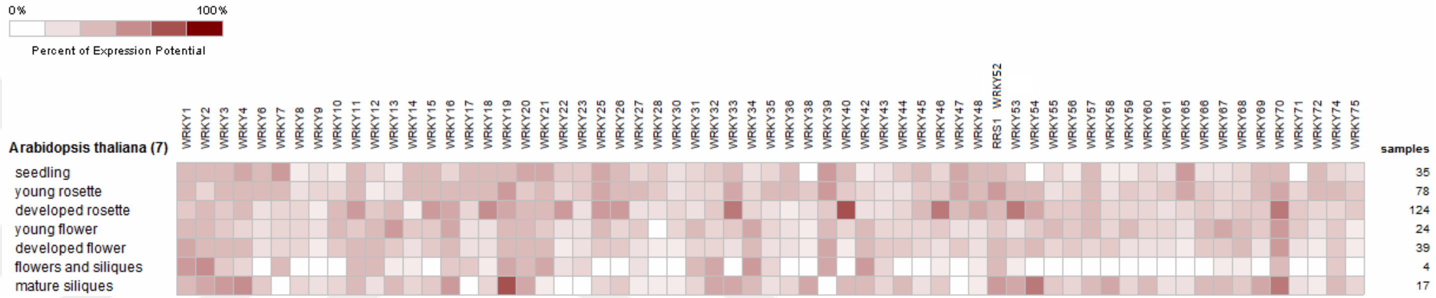

Figure 4.31. Heat map of 63 WRKY TFs at the developmental stage in *Arabidopsis thaliana* at drought stress

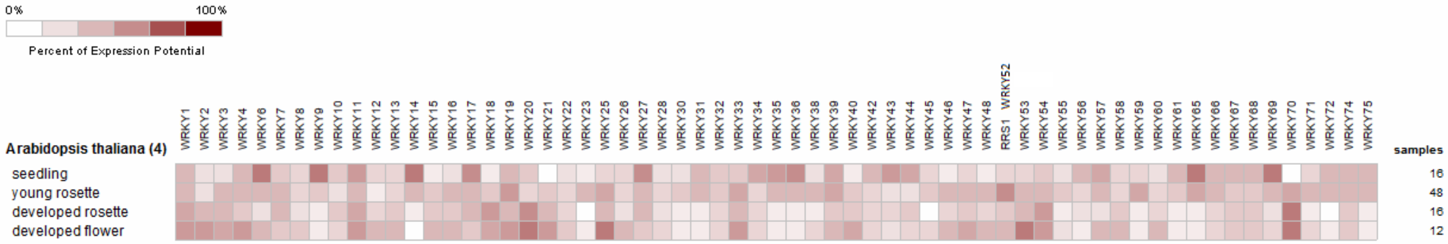

Figure 4.32. Heat map of 63 WRKY TFs at the developmental stage in *Arabidopsis thaliana* at salinity stress

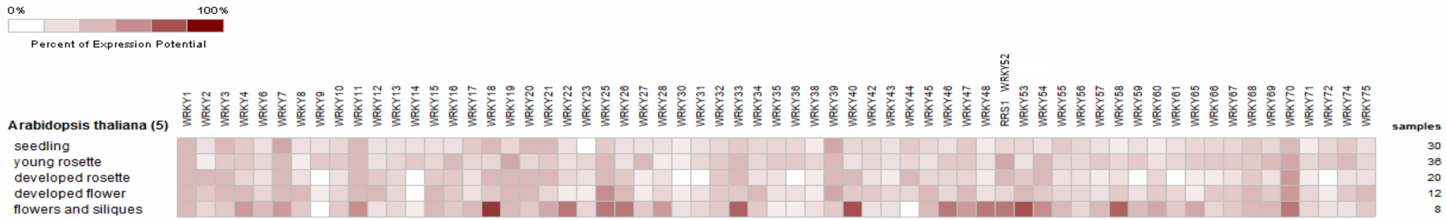

Figure 4.33. Heat map of 63 WRKY TFs at the developmental stage in *Arabidopsis thaliana* at heat stress

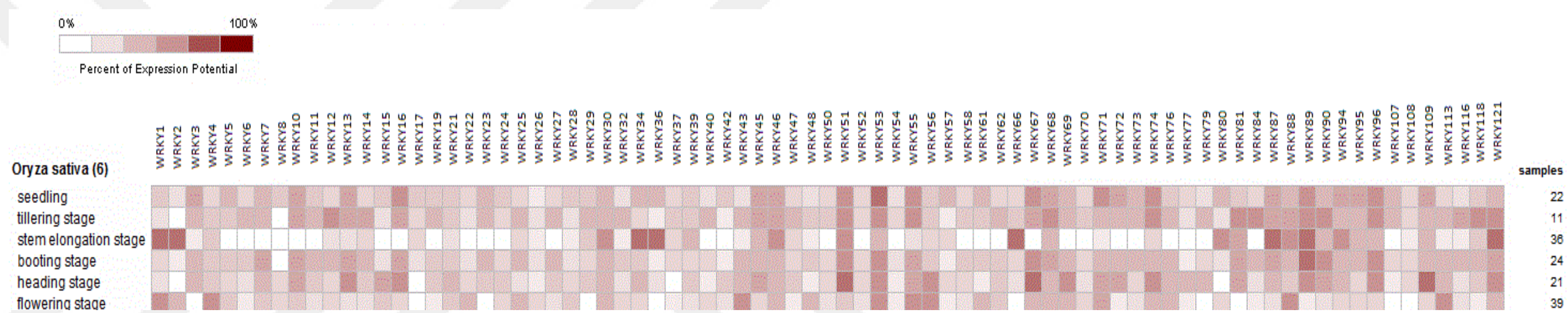

Figure 4.34. Heat map of 79 WRKY TFs at the developmental stage in *Oryza sativa subsp. Japonica* for drought stress

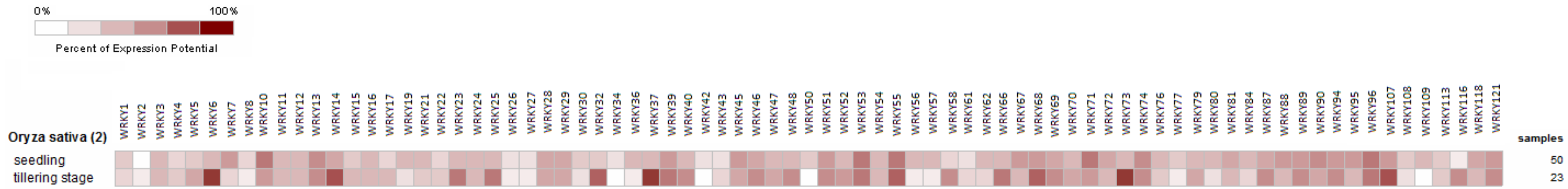

Figure 4.35. Heat map of 79 WRKY TFs at the developmental stage in *Oryza sativa subsp. Japonica* for salinity stress

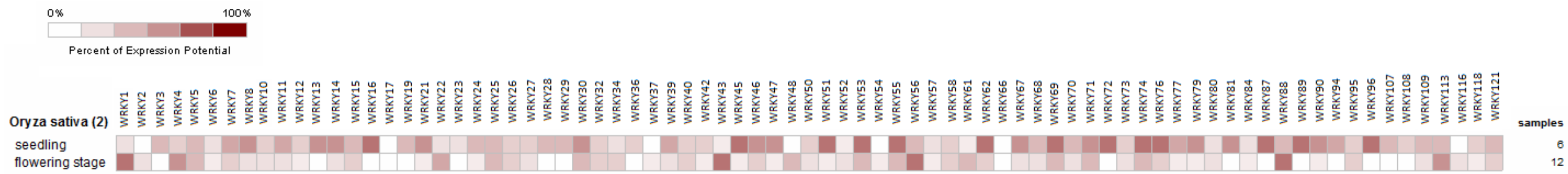

Figure 4.36. Heat map of 79 WRKY TFs at the developmental stage in *Oryza sativa subsp. Japonica* for heat stress

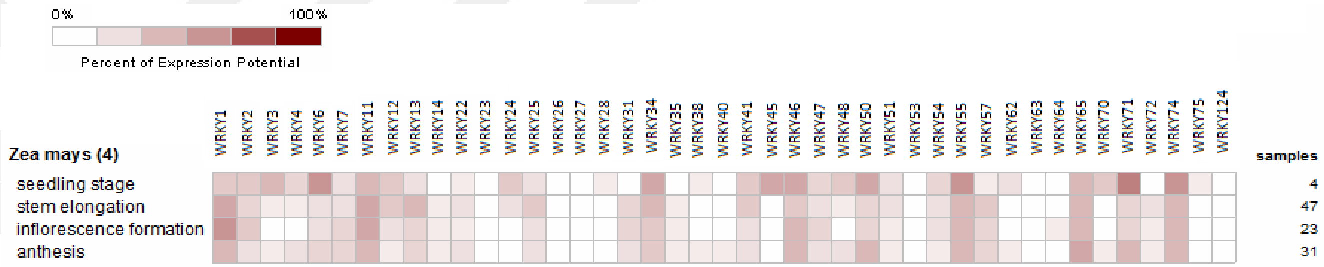

Figure 4.37. Heat map of 43 WRKY TFs at the developmental stage in *Zea mays* for drought stress

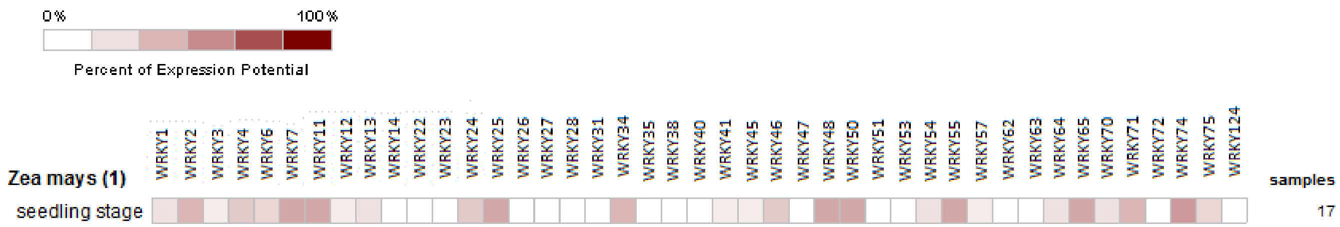

Figure 4.38. Heat map of 43 WRKY TFs at the developmental stage in *Zea mays* for heat stress

4.4.1 *Brachypodium distachyon*

Genevestigator tool didn't allow to get information about expression level of *Brachypodium distachyon* WRKY proteins under stress conditions. Therefore literature screening was used to get information about *Brachypodium distachyon* WRKY expression levels.

Most important plant species on the earth includes wheat, grasses (Poaceae), sorghum and rice are major source of sustainable energy and nutrition. Recently, Poaceae grass considers *Brachypodium distachyon* as a new model organism, as it is closely related to many economically important Poaceae species such as turf grasses, wheat, rice and sorghum. While data indicated that almost 50% of the BdWRKY genes were downregulated under three or more stress conditions, which is consistent with the results of most BdWRKY gene down-regulated by ABA treatment. For example, by applying PEG (drought stress) BdWRKY19, BdWRKY222, BdWRKY251, and BdWRKY252 were negatively regulated and similarly, their expression levels were very low after ABA treatment. These correlations genes expression levels of BdWRKY between phytohormone treatment and abiotic stress suggest that BdWRKY regulation of downstream gene expression may be linked to stress-induced phytohormone alteration. Wen et al., (2014) were confirmed *Brachypodium distachyon* transcription levels in three different tissues, leaves, stems, and roots. According to result BdWRKY7, BdWRKY224, BdWRKY231, BdWRKY238, BdWRKY261, and BdWRKY264, showed higher expression levels than other members of the WRKY family in all the tissues tested. BdWRKY78 was highly induced in the root while its expression level was relatively low in the leaf and stem. BdWRKY10, BdWRKY233, BdWRKY259, and BdWRKY265 were up-regulated in both heat and cold treatments, while BdWRKY81 showed a high up-regulation under heat and salt stresses (Wen et. al. 2014).

The entire genome of wheat has not yet been fully sequenced. For this reason, Brachypodium distachyon was selected as a model plant. In the literature review, there is not enough study to see the expression of *Brachypodium distachyon* WRKY proteins under abiotic stress factors such as drought, temperature and salinity.

4.5 *in silico* Identification of miRNAs Targeting WRKY Genes

Out of 72 *Arabidopsis thaliana* WRKY transcription factors, 13 WRKY TF's (WRKY2, WRKY3, WRKY4, WRKY7, WRKY8, WRKY25, WRKY31, WRKY36, WRKY47, WRKY48,WRKY50, WRKY60 and WRKY71) miRNAs were determine by using mirBase database. Target miRNAs of these WRKY genes were determined using the psRNATarget (a Plant Small RNA Target Analysis Server) online tool. Among these target miRNAs, ath-miR5021 was found to be the target miRNA for the 4 WRKY genes (AtWRKY2, AtWRKY3, AtWRKY7 and AtWRKY8). ath-miR5658 was also found to be the target miRNA for the 3 WRKY genes (AtWRKY2, AtWRKY25 and AtWRKY48) (Table 4.2).

Out of 60 *Brachypodium distachyon* WRKY transcription factors, 10 WRKY TF's (WRKY7, WRKY8, WRKY9, WRKY16, WRKY27, WRKY33, WRKY35,WRKY41, WRKY64 and WRKY71) miRNAs were determine by using mirBase database. bdi-miR5174e-3p.2 was found to be the target miRNA for the 3 WRKY genes (BdWRKY35, and BdWRKY41) (Table 4.3).

Out of 44 *Zea mays* WRKY transcription factors only 1 WRKY TF (WRKY45) miRNA were determine by using mirbase database. zma-miR166k-5p, zma-miR166n-5p and zma-miR166a-5p are the target miRNA of ZmWRKY45 (Table 4.4).

Out of 101 *Oryza sativa* WRKY transcription factors 23 WRKY TF's (WRKY2, WRKY14, WRKY15, WRKY19, WRKY21, WRKY26, WRKY27, WRKY29, WRKY31, WRKY36, WRKY40, WRKY49,WRKY56, WRKY57, WRKY62, WRKY64, WRKY71, WRKY72, WRKY76, WRKY82, WRKY88, WRKY115 and WRKY119) miRNAs were determine by using mirbase database. osa-miR5501 was found to be the target miRNA for the 2 WRKY genes (OsWRKY56 and OsWRKY119) (Table 4.5).

It was estimated that each WRKY would have a large amount of target miRNA At the end of the study.

Table 4.2. Target miRNA of *Arabidopsis thaliana*

miRNA Name	Target AtWRKYs	Expectation	miRNA start	miRNA end	Target start	Target end	miRNA_aligned_fragment	Target_aligned_fragment
ath-miR5021	AtWRKY2-3-7-8	3,5	1	20	2754	2773	UGAGAAGAAGAAGAAGAAAA	UCUUCUUCUUCUUCUUCUGC
ath-miR5658	AtWRKY2-25-48	3	1	21	67	87	AUGAUGAUGAUGAUGAUGAAA	CCCCAUCAUCGUCAUCAUCUU
ath-miR838	AtWRKY2-7	3,5	1	21	1438	1458	UUUUCUUCUACUUCUUGCACA	UCAUCAAGAGGAAGAAGAAGA
ath-miR414	AtWRKY2-25	2	1	21	1933	1953	UCAUCUUCAUCAUCAUCGUCA	UAAUGAUGAAGAUGAAGAUGA
ath-miR8177	AtWRKY47-60	4,5	1	22	740	761	GUGUGAUGAUGUGUCAUUUAUA	ACGAAGGGACACAUAACCACAC

Table 4.3. Target miRNA of *Brachypodium distachyon*

miRNA Name	Target BdWRKYs	Expectation	miRNA start	miRNA end	Target start	Target end	miRNA_aligned_fragment	Target_aligned_fragment
bdi-miR5174e-3p.2	wrky35-41	4,5	1	21	213	234	UUUAUGGAACGGAG-GGAGUAG	CUUCUCCGUUCCGUUUCAUAAG

Table 4.4. Target miRNA of *Zea mays*

	Target ZmWRKY	Expectation	miRNA start	miRNA end	Target start	Target end	miRNA aligned_fragment	Target_aligned_fragment
zma-miR166k-5p	ZmWRKY45	4.0	1	21	282	302	GGAUUGUUGUCUGGCUCGGGG	GGCCGAGGCGGACGACAGGCC

Table 4.5. Target miRNA of *Oryza sativa*

miRNA name	Target OsWRKYs	Expectation	miRNA start	miRNA end	Target start	Target end	miRNA_aligned_fragment	Target_aligned_fragment
osa-miR5501	OsWRKY56-119	5.0	1	21	59	79	UCUUGUGGCUAGAAGGGUGAG	GACACCGAUCUAGUCCCAGGG

CHAPTER V

CONCLUSION

The increasing impact of global warming is known by everyone, so it is necessary to make some strategic plans to minimize the level of damage and reduce the threat on crop plants. Abiotic stress factors are increasing day by day and having WRKY transcription factors contributes to the smooth functioning of the physiological and molecular mechanisms of plants.

In the first part of our study, the members of the WRKY transcription factors family model organisms *Arabidopsis thaliana and Brachypodium distachyon*, and *Oryza sativa* and *Zea mays* which are important food stuffs were determined. As a result of the analyzes, 72 WRKY TFs in *Arabidopsis thaliana*, 60 WRKY TFs in *Brachypodium distachyon*, 101 WRKY TFs in *Oryza sativa* and 44 WRKY TF in *Zea mays* were determined.

Then chromosomal locations, exon-intron regions, phylogenetic trees, motifs, gene ontology analysis, in silico identification of miRNAs targeting WRKY genes and comparison of data for evaluation of the tissue, growth and stress responses at three different abiotic stresses (drought, salinity and heat) has been determined. By determining gene expression levels, it was determined which specific WRKY played a role in the growth stages of plants. At the end of these processes monocot and dicot plants has compared and neighbour joining analysis of 277 WRKY proteins from *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa and Zea mays* suggested that BdWRKY and ZmWRKY domains are evolutionarily more closely related to *O. sativa* WRKY domains than those of *A. thaliana.*

Our study is important to understand the structure, organization and expression profiles of the WRKY protein families. It has also revealed important informations in terms of the use of omix approaches.

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APPENDIX

Appendix-A Average expression table of *Arabidopsis thaliana* at drought stress

	Average Expression											
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY6	WRKY7	WRKY8	WRKY9	WRKY10	WRKY11	WRKY12	WRKY13
Seedling	11,98	9,9	10,75	11,17	10,91	11,3	8,2	8,06	8,03	11,87	8,22	7,93
Young Rosette	11,78	9,15	10,48	10,94	10,94	10,85	8,34	8,55	8,38	11,82	7,88	8,19
Developed Rosette	11,39	10,14	10,24	10,49	9,6	9,7	9,12	7,79	8,99	12,53	8,67	8,9
Young Flower	11,57	9,93	10,04	9,97	9,52	9,87	9,01	8,04	8,99	10,99	8,88	9,53
Developed Flower	12,16	10,22	10,5	10,53	9,68	9,77	9,03	7,77	8,62	11,57	8,86	8,53
Flowers and Siliques	12,41	11,1	10,14	9,87	8,18	10,28	7,72	6,48	7,95	11,21	9,14	7,77
Mature Siliques	11,84	10,33	11,47	11,65	10,27	8,88	8,75	7,88	8,46	11,22	8,03	8,7

	Average Expression										
	WRKY14	WRKY15	WRKY16	WRKY17	WRKY18	WRKY19	WRKY20	WRKY21	WRKY22	WRKY23	WRKY25
Seedling	9,28	12,06	9,87	11,07	11,65	9,51	11,65	11,75	10,22	10,09	10,91
Young Rosette	9,24	12,16	10,3	10,93	11,4	10,29	11,2	11,34	9,91	10,34	10,92
Developed Rosette	7,98	13,17	10,73	10,13	13,28	10,02	11,27	11,49	12,05	9,48	11,32
Young Flower	8,94	11,64	10,71	9,59	9,77	9,77	11,58	10,9	9,18	9,41	9,9
Developed Flower	8,25	11,69	10,17	9,61	9,55	9,76	11,5	11,59	8,64	9,32	9,76
Flowers and Siliques	8,09	10,45	9,94	10,55	9,19	9,85	11,5	11,82	9,29	9,97	6,87
Mature Siliques	8,59	11,67	10,83	8,15	10,31	11,65	11,72	10,31	8,16	8,78	9,25

	Average Expression										
	WRKY26	WRKY27	WRKY28	WRKY30	WRKY31	WRKY32	WRKY33	WRKY34	WRKY35	WRKY36	WRKY38
Seedling	9,92	8,62	9,2	8,1	8,42	10,32	11,81	7,05	8,44	9,23	7,43
Young Rosette	9,51	9,39	9,28	8,5	8,74	10,28	12,16	7,25	9,01	9,24	9,21
Developed Rosette	10,76	8,44	8,43	8,73	8,09	10,5	14,36	7,42	7,88	7,78	8,22
Young Flower	8,52	8,94	7,69	8,67	9,19	10,05	10,58	8,15	8,28	8,19	7,78
Developed Flower	8,83	8,36	8	7,89	8,57	10,1	9,88	8,07	8,15	7,95	7,86
Flowers and Siliques	6,55	8,45	6,95	6,81	9,5	11,25	8,37	8,43	8,34	6,93	6,64
Mature Siliques	8,84	8,13	8,15	7,84	8,42	11,41	12,31	7,7	7,86	8,52	10,78

	Average Expression										
	WRKY39	WRKY40	WRKY42	WRKY43	WRKY44	WRKY45	WRKY46	WRKY47	WRKY48	WRKY52	WRKY53
Seedling	12,35	10,85	7,59	7,33	8,29	10,71	9,91	10,63	10,57	9,47	9,57
Young Rosette	12,25	10,59	7,93	7,34	7,72	9,65	10,33	10,44	10,49	10,31	9,97
Developed Rosette	11,22	15,26	7,74	7,26	8,03	10,52	13,81	10,02	11,13	9,49	13,15
Young Flower	11,42	9,79	8,76	7,78	7,86	9,27	10,09	8,88	9,05	9,27	8,66
Developed Flower	12,03	8,61	8,49	7,9	8,16	9,37	9,34	9,3	8,95	9,47	9,51
Flowers and Siliques	12,02	7,74	9,21	7,67	7,06	7,57	8,65	7,48	8,49	9,73	8,2
Mature Siliques	9,57	11,69	8,43	7,57	8,57	8,9	9,95	8,14	8,45	10,31	11,23

	Average Expression										
	WRKY54	WRKY55	WRKY56	WRKY57	WRKY58	WRKY59	WRKY60	WRKY61	WRKY65	WRKY66	WRKY67
Seedling	8,08	9,01	7,18	10,03	7,84	7,64	9,47	7,88	11,88	7,64	7,5
Young Rosette	10,59	8,94	7,51	9,78	8,09	8,68	9,3	8,36	11,36	8,15	7,64
Developed Rosette	11,52	9,53	7,71	9,8	8,22	8,21	9,29	7,66	9,73	8,14	7,69
Young Flower	10,15	9,65	7,83	10,15	8,41	9	9,55	8,15	9,48	8,55	8,57
Developed Flower	11	9,1	7,68	9,25	8,67	7,98	8,99	7,83	8,94	8,06	7,97
Flowers and Siliques	7,75	8,36	6,76	8,04	7,32	6,96	8,18	6,61	7,9	7,13	7,13
Mature Siliques	13,14	9,28	7,48	9,93	9,69	8,46	10,4	8	9,39	8,58	8,31

	Average Expression						
	WRKY68	WRKY69	WRKY70	WRKY71	WRKY72	WRKY74	WRKY75
Seedling	7,61	10,64	11,03	7,86	9,37	9,18	8,35
Young Rosette	7,62	11,22	11,92	8,6	9,83	9,76	9,3
Developed Rosette	7,86	10,56	13,53	8,86	8,61	9,22	9,04
Young Flower	8,04	10,32	12,62	8,97	8,08	9,51	7,71
Developed Flower	8,07	9,72	12,7	8,48	7,82	9,51	7,79
Flowers and Siliques	7,4	9,15	11,09	7,46	6,81	9,35	6,62
Mature Siliques	7,88	11,36	13,57	8,37	7,66	9,71	8,68

Appendix-B Average expression table of *Arabidopsis thaliana* at salinity stress

	Average Expression											
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY6	WRKY7	WRKY8	WRKY9	WRKY10	WRKY11	WRKY12	WRKY13
Seedling	11,79	8,96	9,58	10,75	13,13	10,03	8,93	11,59	8,71	12,4	8,35	8,42
Young Rosette	11,82	8,86	10,62	10,83	11,22	10,83	8,93	8,93	8,26	11,71	7,6	8,24
Developed Rosette	12,05	9,94	10,42	10,32	9,54	10,66	8,68	7,5	8,68	12,04	8,41	8,55
Developed Flower	12,45	10,76	11,17	11,51	11,04	10,23	9,21	7,47	8,38	12,5	8,93	8,95

	Average Expression										
	WRKY14	WRKY15	WRKY16	WRKY17	WRKY18	WRKY19	WRKY20	WRKY21	WRKY22	WRKY23	WRKY25
Seedling	11,21	10,84	9,52	12,28	9,31	9,47	11,14	10	9,24	8,9	8,93
Young Rosette	9,41	11,76	10,37	10,85	10,81	10,21	10,99	11,04	9,54	10,7	10,85
Developed Rosette	7,95	11,79	9,95	10,75	12,57	9,55	12,18	11,37	9,57	8,45	10,48
Developed Flower	7,86	12,38	10,36	10,6	12,25	10,12	12,23	11,93	10,15	9,13	12,36

	Average Expression										
	WRKY26	WRKY27	WRKY28	WRKY30	WRKY31	WRKY32	WRKY33	WRKY34	WRKY35	WRKY36	WRKY38
Seedling	8,5	10,74	8,43	8,45	9,66	10,54	10,88	8,16	10,24	11,11	8,14
Young Rosette	8,55	9,78	8,96	9,06	9,21	10,31	12,38	7,3	9,08	9,23	10,04
Developed Rosette	8,15	9,24	8,56	7,73	7,97	10,23	11,31	7,37	7,75	7,84	7,88
Developed Flower	9,66	9,04	8,59	7,94	7,88	10,1	13,07	7,29	7,66	7,86	8,59

	Average Expression										
	WRKY39	WRKY40	WRKY42	WRKY43	WRKY44	WRKY45	WRKY46	WRKY47	WRKY48	WRKY52	WRKY53
Seedling	11,99	8,79	8,43	9,09	8,86	9,22	8,74	9,27	8,44	9,05	9,41
Young Rosette	12,11	10,35	7,99	7,43	7,68	9,57	10,99	9,98	10,33	10,55	10,19
Developed Rosette	11,19	10,5	8,11	7,55	8,09	8,18	9,9	9,36	9,46	9,29	9,94
Developed Flower	11,85	11,92	8,08	7,37	8,05	9,38	10,73	10,59	10,23	9,79	12,59

	Average Expression										
	WRKY54	WRKY55	WRKY56	WRKY57	WRKY58	WRKY59	WRKY60	WRKY61	WRKY65	WRKY66	WRKY67
Seedling	9,87	8,93	8,17	10,42	8,31	8,34	9,04	8,87	13,04	8,55	8,32
Young Rosette	11,09	9,51	7,56	10,15	8,45	9,78	9,45	8,7	11,27	8,51	7,96
Developed Rosette	12,11	8,59	7,32	8,85	8,71	7,69	9,23	7,49	8,86	8	7,85
Developed Flower	11,72	8,82	7,15	9,94	9,29	7,74	10,24	7,48	8,81	7,79	7,87

	Average Expression						
	WRKY68	WRKY69	WRKY70	WRKY71	WRKY72	WRKY74	WRKY75
Seedling	8,13	12,32	8,44	8,68	9,89	9,59	9,74
Young Rosette	7,8	11,16	12,07	9,37	10,02	9,77	9,84
Developed Rosette	7,92	9,67	13,84	8,17	7,43	9,4	7,73
Developed Flower	7,85	10,19	13,94	8,52	7,7	9,42	7,84

Appendix-C Average expression table of *arabidopsis Thaliana* at heat stress

	Average Expression											
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY6	WRKY7	WRKY8	WRKY9	WRKY10	WRKY11	WRKY12	WRKY13
Seedling	11,86	9,04	10,42	10,28	9,37	11	8,35	8,06	8,43	11,23	8,17	7,98
Young Rosette	11,67	8,6	10,15	10,38	9,81	10,35	8,29	8,69	8,6	11,25	8,05	8,28
Developed Rosette	11,79	9,81	10,36	10,03	9,32	10,43	8,55	7,23	8,16	11,89	8,67	8,33
Developed Flower	11,79	9,59	10,46	10,49	9,97	10,4	9,79	7,66	8,46	11,72	8,85	8,32
Flowers and Siliques	11,6	9,37	10,18	11,43	11,61	11,4	9,38	7,29	8,57	12,98	7,98	8,39

	Average Expression										
	WRKY14	WRKY15	WRKY16	WRKY17	WRKY18	WRKY19	WRKY20	WRKY21	WRKY22	WRKY23	WRKY25
Seedling	8,41	11,18	9,52	10,52	11,17	8,75	11,56	11,28	9,29	8,79	9,51
Young Rosette	9,02	11,47	10,29	10,06	10,33	9,94	10,98	10,85	9,08	9,67	10,32
Developed Rosette	7,69	11,85	9,98	9,79	11,47	9,79	11,58	11,14	9,92	9,61	10,37
Developed Flower	7,85	12,18	10,02	9,77	10,91	9,47	11,46	10,62	8,66	8,84	12,05
Flowers and Siliques	7,92	12,3	10,03	11,1	15,32	9,58	11,16	11,71	13,18	9,98	12,91

	Average Expression										
	WRKY26	WRKY27	WRKY28	WRKY30	WRKY31	WRKY32	WRKY33	WRKY34	WRKY35	WRKY36	WRKY38
Seedling	8,23	8,31	8,29	7,64	8,23	10,05	10,54	7,42	8,33	8,41	7,86
Young Rosette	8,31	9,34	8,05	7,93	8,73	10,48	11,16	7,39	8,6	8,83	8,67
Developed Rosette	9	8,12	8,42	7,61	7,65	10,43	11,96	7,13	8,16	7,42	7,8
Developed Flower	9,85	8,23	8,69	8,02	8,17	10,31	10,71	7,67	7,99	8,11	7,86
Flowers and Siliques	12,01	9,1	10,63	8,13	8,69	10,47	15	7,64	7,57	7,69	8

	Average Expression										
	WRKY39	WRKY40	WRKY42	WRKY43	WRKY44	WRKY45	WRKY46	WRKY47	WRKY48	WRKY52	WRKY53
Seedling	12	10	7,92	7,43	8,3	9,1	9,43	9,29	8,81	8,93	9,08
Young Rosette	11,79	9,48	8,03	7,41	7,83	9,06	9,96	9,29	9,24	9,9	8,88
Developed Rosette	11,55	10,8	7,71	7,19	8,74	9,16	9,88	9,17	8,7	9,83	9,6
Developed Flower	12,2	10,28	8,28	7,78	8,03	10,8	9,74	9,91	9,23	8,99	10,54
Flowers and Siliques	11,3	15,6	7,8	7,22	7,23	10,31	13,8	11,01	12,43	10,68	13,79

	Average Expression										
	WRKY54	WRKY55	WRKY56	WRKY57	WRKY58	WRKY59	WRKY60	WRKY61	WRKY65	WRKY66	WRKY67
Seedling	8,79	9,45	7,5	9,01	7,88	8,06	8,67	7,77	9,86	7,94	7,72
Young Rosette	10,3	9,18	7,58	9,5	8,42	8,81	9,07	8,28	10,25	8,23	7,79
Developed Rosette	10,16	8,96	7,29	8,69	7,96	7,52	9,24	7,23	8,95	7,39	7,3
Developed Flower	9,67	9,02	7,57	8,9	8,82	7,92	9,59	8,05	9,01	8,12	7,94
Flowers and Siliques	12,27	9,85	7,64	9,81	11,1	9,04	10,71	8,53	11,54	7,85	7,76

	Average Expression						
	WRKY68	WRKY69	WRKY70	WRKY71	WRKY72	WRKY74	WRKY75
Seedling	7,97	9,93	11,7	7,98	8,39	9,33	8,29
Young Rosette	7,89	10,68	11,87	8,71	9,07	9,67	8,89
Developed Rosette	7,87	9,85	12,6	7,96	7,2	8,96	8,01
Developed Flower	8,15	10,29	12,46	8,51	7,84	9,45	9,63
Flowers and Siliques	8	10,93	14,18	8,74	8,17	8,81	9,42

Appendix-D Average expression table of *Oryza sativa subsp. japonica* at drought stress

	Average Expression											
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY5	WRKY6	WRKY7	WRKY8	WRKY10	WRKY11	WRKY12	WRKY13
Seedling	9,95	7,94	11,82	9,21	11,09	8,25	10,94	9,81	11,18	9,82	8,76	13,2
Tillering Stage	8,74	7,23	11,52	9,45	10,58	9,18	11,36	7,83	11,22	10,63	10,37	13,36
Stem elongation Stage	12,95	11,35	9,98	9,51	9,43	7,16	7,75	7,04	7,41	7,42	8,61	11,49
Booting Stage	9,22	7,39	11,26	9,42	10,67	8,67	11,72	7,81	11,4	10,29	9,63	13,43
Heading Stage	8,94	7,22	11,38	9,57	10,3	7,95	11,54	9,43	11,14	9,68	8,84	13,81
Flowering Stage	12,19	9,33	9,67	10,18	10,57	7,78	10,6	9,22	9,88	9,14	8,86	11,63

	Average Expression											
	WRKY14	WRKY15	WRKY16	WRKY17	WRKY19	WRKY21	WRKY22	WRKY23	WRKY24	WRKY25	WRKY26	WRKY27
Seedling	9,68	9,97	11,45	8,05	10,09	10,25	8,6	9,26	11,33	10,92	8,85	9,02
Tillering Stage	11,5	9,19	10,96	7,82	10,32	10,2	8,43	9,68	10,56	11,73	9,32	9,78
Stem elongation Stage	9,59	8,87	7,57	7,93	8,55	9,22	7,83	6,87	8,65	10,84	8,83	8,25
Booting Stage	10,5	9,24	11,33	7,65	9,82	9,86	8,48	9,77	10,45	11,77	9,32	9,68
Heading Stage	10,21	10,81	11,67	6,86	10,22	10,79	8,77	8,73	11,23	10,57	9,09	9,22
Flowering Stage	9,31	10,11	9,62	7,01	8,79	9,97	9,4	7,33	10,62	11,42	9,7	8,83

	Average Expression										
	WRKY28	WRKY29	WRKY30	WRKY32	WRKY34	WRKY36	WRKY37	WRKY39	WRKY40	WRKY42	WRKY43
Seedling	10,63	8,92	10,65	8,9	9,65	9,55	8,47	10,84	8,85	9,49	8,79
Tillering Stage	9,7	9,3	10,79	9,9	9	7,81	8,92	10,85	10,21	8,18	9,27
Stem Elongation Stage	8,81	9,18	11,92	8,62	12,43	12,81	8,48	10,99	8,01	7,39	8,5
Booting Stage	9,54	8,82	10,73	9,64	9,26	8	8,26	10,13	9,35	8,42	9,41
Heading Stage	9,77	9,21	11,22	9,13	9,68	8,38	7,18	10,53	8,42	9,47	9,33
Flowering Stage	10,42	7,27	11,15	9,28	9,06	7,24	7,55	9,92	9,36	8,59	10,28

	Average Expression											
	WRKY45	WRKY46	WRKY47	WRKY48	WRKY50	WRKY51	WRKY52	WRKY53	WRKY54	WRKY55	WRKY56	WRKY57
Seedling	12,99	13,15	10,45	8,48	9,77	12,19	8,54	15,4	7,99	12,76	8,78	9,85
Tillering Stage	13,04	13,09	11,28	9,15	10,43	12,14	8,67	14,76	8,21	12,97	8,73	9,06
Stem Elongation Stage	12,05	13,29	10,64	8,22	8,3	12,21	7,32	12,85	7,04	10,18	8,69	9,48
Booting Stage	11,82	12,59	10,53	9,03	9,95	12,31	8,73	14,31	7,99	12,58	8,87	9,09
Heading Stage	13,02	12,76	10,82	8,47	10,06	13,09	8,61	14,89	7,51	12,23	9,69	9,55
Flowering Stage	10,3	12,3	9,76	9,5	9,59	10,85	8,41	14,62	7,47	12,71	9,82	9,06

	Average Expression											
	WRKY58	WRKY61	WRKY62	WRKY66	WRKY67	WRKY68	WRKY69	WRKY70	WRKY71	WRKY72	WRKY73	WRKY74
Seedling	8,5	10,15	9,42	8,86	12,4	13,41	11,4	10,22	14,12	10,72	9,04	13,39
Tillering Stage	8,65	9,48	11,02	9,13	11,84	13,89	11,66	10,4	12,69	9,58	9,39	13,35
Stem Elongation Stage	7,73	7,72	7,71	11,39	8,13	13,02	9,04	8,23	9,79	6,76	7,64	11,11
Booting Stage	8,68	9,45	10,15	9,01	12,11	13,39	11,45	9,86	12,3	9,64	9,27	12,68
Heading Stage	8,51	8,32	9,92	8,58	13,27	12,59	12,34	9,51	13,16	10,98	8,77	13,57
Flowering Stage	8,39	9,98	9,91	8,01	10,92	12,66	11,68	10,07	13,1	7,81	8,47	12,57

	Average Expression											
	WRKY76	WRKY77	WRKY79	WRKY80	WRKY81	WRKY84	WRKY87	WRKY88	WRKY89	WRKY90	WRKY94	WRKY95
Seedling	10,86	8,87	8,34	8,85	9,64	8,14	13,49	12,48	12,46	13,02	14,39	13,09
Tillering Stage	11,79	8,37	9,75	7,98	10,59	10,33	13,52	12,97	12,49	13,75	14,18	12,65
Stem Elongation Stage	7,73	7,27	6,92	9,79	10,37	6,86	14,89	13,73	13,27	12,72	14,8	12,43
Booting Stage	10,97	8,21	8,82	8,04	10,38	8,94	13,58	12,88	13,02	13,45	13,97	12,27
Heading Stage	11,86	9,91	10,22	7,42	10,5	7,78	13,42	12,34	12,4	13,21	13,91	11,05
Flowering Stage	11,03	8,13	7,62	8,11	8,14	7,25	12,08	13,64	9,96	11,92	13,28	11,13

	Average Expression							
	WRKY96	WRKY107	WRKY108	WRKY109	WRKY113	WRKY116	WRKY118	WRKY121
Seedling	13,61	10,2	9,88	11,81	9,64	8,44	10,8	12,34
Tillering Stage	13,31	10,21	10,17	10,86	10,11	9,73	12,68	14,01
Stem Elongation Stage	11,99	7,24	9,16	11,32	9,09	8,26	10,69	15,16
Booting Stage	13,12	9,15	10,14	11,28	10,28	9,25	10,98	13,35
Heading Stage	13,29	8,06	9,94	12,93	10,16	8,59	10,85	13,82
Flowering Stage	10,9	7,88	8,76	9,93	10,7	8,43	9,36	12,35

Appendix-E Average expression table of WRKYs of *Oryza sativa subsp. japonica* at salinity stress

	Average Expression												
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY5	WRKY6	WRKY7	WRKY8	WRKY10	WRKY11	WRKY12	WRKY13	WRKY14
Seedling	9,61	7,31	11,45	9,37	10,55	9,03	12,22	9,02	12,8	10,62	9,71	14	11,46
Tillering Stage	9,39	7,64	11,5	9,4	11,36	11,73	10,04	8,18	11,77	10,11	9,81	14,03	13,8

	Average Expression											
	WRKY1 5	WRKY16	WRKY17	WRKY19	WRKY21	WRKY22	WRKY23	WRKY24	WRKY25	WRKY26	WRKY27	WRKY28
Seedling	10,09	10,73	7,72	10,92	10,98	8,75	9,83	12,05	11,51	9,17	8,78	12,68
Tillering Stage	10,28	10,72	8,81	9,37	10,09	8,98	11,86	11,96	13,06	8,91	8,65	12,6

	Average Expression											
	WRKY29	WRKY30	WRKY32	WRKY34	WRKY36	WRKY37	WRKY39	WRKY40	WRKY42	WRKY43	WRKY45	WRKY46
Seedling	10,34	10,55	9,59	8,65	9,89	9,22	12,53	9,84	8,92	8,72	13,31	13,18
Tillering Stage	10,3	9,42	11,66	7,87	7,59	12,57	13,71	11,47	8,08	8,9	12,6	13,72

	Average Expression											
	WRKY47	WRKY48	WRKY50	WRKY51	WRKY52	WRKY53	WRKY54	WRKY55	WRKY56	WRKY57	WRKY58	WRKY61
Seedling	11,93	9,41	10,12	12,12	9,21	15,42	8,85	13,64	9,03	9,86	8,5	9,02
Tillering Stage	12,22	10,29	8,28	12,53	10,15	15,43	8,73	15,03	8,22	8,98	9,83	9,13

	Average Expression											
	WRKY62	WRKY66	WRKY67	WRKY68	WRKY69	WRKY70	WRKY71	WRKY72	WRKY73	WRKY74	WRKY76	WRKY77
Seedling	10,9	9,76	12,56	14	12,26	11,64	15,09	10,81	9,14	14,01	12,07	9,58
Tillering Stage	9,59	11,59	11,53	15,5	12,75	11,92	14,06	9,96	11,28	14,05	10,81	8,17

	Average Expression											
	WRKY79	WRKY80	WRKY81	WRKY84	WRKY87	WRKY88	WRKY89	WRKY90	WRKY94	WRKY95	WRKY96	WRKY107
Seedling	10,15	8,88	10,41	9,26	13,45	13,24	12,19	13,98	14,76	13,49	13,8	11,86
Tillering Stage	10,83	7,87	9,87	8,34	14,03	12,58	12,62	13,42	15,14	12,28	14,28	14,64

	Average Expression					
	WRKY108	WRKY109	WRKY113	WRKY116	WRKY118	WRKY121
Seedling	10,6	11,02	9,79	8,18	11,93	13,57
Tillering Stage	9,37	8,73	9,75	10,3	11,62	14,05

Appendix-F Average expression table of WRKYs of *Oryza sativa subsp. japonica* at heat stress

	Average Expression												
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY5	WRKY6	WRKY7	WRKY8	WRKY10	WRKY11	WRKY12	WRKY13	WRKY14
Seedling	8,63	7,22	11,7	9,22	10,73	8,19	11,99	12,33	9,98	11,21	9,27	13,98	12,15
Flowering Stage	13,06	7,76	9,22	10,53	10,75	7,87	9,68	8,51	9,11	9,01	8,25	10,97	9,22

	Average Expression											
	WRKY15	WRKY16	WRKY17	WRKY19	WRKY21	WRKY22	WRKY23	WRKY24	WRKY25	WRKY26	WRKY27	WRKY28
Seedling	10,3	12,53	6,98	10,41	12,13	8,54	8,13	11,57	11,47	9,6	9,35	12,12
Flowering Stage	9,84	8,32	6,88	8,08	8,74	9,56	7,28	9,44	11,45	9,77	8,88	8,34

	Average Expression											
	WRKY29	WRKY30	WRKY32	WRKY34	WRKY36	WRKY37	WRKY39	WRKY40	WRKY42	WRKY43	WRKY45	WRKY46
Seedling	9,56	11,92	9,55	8,4	8,71	7,02	11,89	9,6	9,52	8,53	15,19	13,49
Flowering Stage	7,13	11,26	9,38	8,92	6,93	7,57	9,9	9,67	8,26	10,81	9,24	11,82

	Average Expression											
	WRKY47	WRKY48	WRKY50	WRKY51	WRKY52	WRKY53	WRKY54	WRKY55	WRKY56	WRKY57	WRKY58	WRKY61
Seedling	12,43	7,52	10,14	13,41	7,81	16,11	7,24	13,77	9,1	9,23	8,81	8,37
Flowering Stage	9,56	9,74	9,54	9,73	8,32	13,61	7,64	11,98	10,03	9,01	8,59	9,99

	Average Expression											
	WRKY62	WRKY66	WRKY67	WRKY68	WRKY69	WRKY70	WRKY71	WRKY72	WRKY73	WRKY74	WRKY76	WRKY77
Seedling	13,33	7,96	12,87	12,89	13,53	10,89	13,86	12,99	9	15,06	14,74	10,8
Flowering Stage	10,07	7,64	9,2	11,45	11,03	9,61	12,11	7,46	8,47	11,97	10,19	7,93

	Average Expression											
	WRKY79	WRKY80	WRKY81	WRKY84	WRKY87	WRKY88	WRKY89	WRKY90	WRKY94	WRKY95	WRKY96	WRKY107
Seedling	10,86	7,88	11,01	7,7	14,33	12,69	13,36	13,74	14,6	11,61	13,9	10,33
Flowering Stage	7,69	8,08	7,62	7,37	11,18	14,54	9,26	11,66	12,64	11,09	9,77	7,89

	Average Expression					
	WRKY108	WRKY109	WRKY113	WRKY116	WRKY118	WRKY121
Seedling	10,01	11,48	10,19	8,09	10,5	13,02
Flowering Stage	8,18	9,42	10,92	8,45	9,33	12,07

Appendix-F Average expression table of *Zea mays* at drought stress

	Average Expression											
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY6	WRKY7	WRKY11	WRKY12	WRKY13	WRKY14	WRKY22	WRKY23
seedling stage	2,2	1,89	2,09	1,39	3,32	1,41	2,98	1,55	1,11	0,12	0,37	0,17
stem elongation	3,23	1,28	0,64	0,54	1,23	1,4	3,86	1,54	2,18	0,77	0,55	0,08
inflorescence formation	3,56	1,7	0,4	0,47	1,28	1,68	3,77	1,05	1,5	0,67	0,45	0,05
anthesis	2,71	1,13	0,64	0,96	1,33	1,83	3,42	0,62	1,33	0,54	0,54	0,07

	Average Expression										
	WRKY24	WRKY25	WRKY26	WRKY27	WRKY28	WRKY31	WRKY34	WRKY35	WRKY38	WRKY40	WRKY41
seedling stage	1,59	1,23	0	0	0,4	0,22	3,21	0,08	0,62	0	1,65
stem elongation	1,06	1,84	0,02	0,06	0,21	0,84	2,44	0,31	0,32	0,02	1,69
inflorescence formation	0,09	1,16	0,01	0,05	0,22	0,91	1,92	0,44	0,21	0,03	1,25
anthesis	0,2	1,28	0,02	0,16	0,19	0,64	2,02	0,41	0,73	0,45	1,07

	Average Expression										
	WRKY45	WRKY46	WRKY47	WRKY48	WRKY50	WRKY51	WRKY53	WRKY54	WRKY55	WRKY57	WRKY62
seedling stage	2,81	3,14	1,03	1,3	3,64	0,8	0	1,24	4,22	0,58	1,22
stem elongation	0,12	2,29	0,87	0,62	1,75	0,66	0,11	0,99	2,81	1,42	0,36
inflorescence formation	0,22	2,43	1	0,32	1,91	0,49	0,05	0,94	2,82	1,02	0,14
anthesis	0,52	2,75	0,69	0,83	2,5	0,4	0,1	0,65	3,1	1,05	0,75

	Average Expression								
	WRKY63	WRKY64	WRKY65	WRKY70	WRKY71	WRKY72	WRKY74	WRKY75	WRKY124
seedling stage	0	0,16	3,13	1,67	6,05	0,16	3,49	0,84	0,26
stem elongation	0,02	0,24	2,79	0,36	2,2	0,92	2,79	0,1	0,05
inflorescence formation	0	0,34	3,14	0,32	2,15	0,73	2,26	0,09	0,03
anthesis	0,01	0,24	3,41	0,57	3,09	0,76	2,68	0,11	0,25

Appendix-G Average expression table of *Zea mays* at heat stress

	Average Expression												
	WRKY1	WRKY2	WRKY3	WRKY4	WRKY6	WRKY7	WRKY11	WRKY12	WRKY13	WRKY14	WRKY22	WRKY23	WRKY24
Seedling Stage	1,52	2,51	0,68	1,78	1,31	3,81	3,8	0,59	0,96	0,09	0,17	0,08	1,84

	Average Expression											
	WRKY25	WRKY26	WRKY27	WRKY28	WRKY31	WRKY34	WRKY35	WRKY38	WRKY40	WRKY41	WRKY45	WRKY46
Seedling Stage	2,84	0	0,15	0,1	0,08	2,46	0,01	0,28	0,12	0,68	0,65	2,19

	Average Expression											
	WRKY47	WRKY48	WRKY50	WRKY51	WRKY53	WRKY54	WRKY55	WRKY57	WRKY62	WRKY63	WRKY64	WRKY65
Seedling Stage	0,35	2,25	3,52	0,08	0,08	0,96	3,43	0,56	0,28	0	0,54	3,47

	Average Expression					
	WRKY70	WRKY71	WRKY72	WRKY74	WRKY75	WRKY124
Seedling Stage	0,98	4,13	0,13	3,95	1,36	0,12

Appendix-H Transcription factor identical definition and gene name of *Arabidopsis thaliana, Brachypodium distachyon, Oryza sativa* and *Zea mays*

Arabidopsis thaliana		*Brachypodium distachyon*		*Oryza sativa*		*Zea mays*	
TF ID	GENE NAME	TF ID	GENE NAMEI	TF ID	GENE NAME	TF ID	GENE NAME
AT1G13960.1	WRKY4	Bradi1g07970.1.p	WRKY2	LOC_Os01g08710.1	WRKY102	GRMZM2G018487_P01	ZmWRKY1
AT1G18860.1	WRKY61	Bradi1g09170.1.p	WRKY4	LOC_Os01g09080.1	WRKY107	GRMZM2G027972_P01	ZmWRKY2
AT1G29280.1	WRKY65	Bradi1g13207.1.p	WRKY62	LOC_Os01g09100.1	WRKY10	GRMZM2G020254_P01	ZmWRKY3
AT1G29860.1	WRKY71	Bradi1g14300.3.p	WRKY6	LOC_Os01g14440.1	WRKY1	GRMZM2G059562_P01	ZmWRKY4
AT1G30650.1	WRKY14	Bradi1g16120.2.p	WRKY7	LOC_Os01g18584.1	WRKY9	GRMZM5G871347_P01	ZmWRKY6
AT1G55600.1	WRKY10	Bradi1g17660.1.p	WRKY8	LOC_Os01g40260.1	WRKY77	GRMZM2G137802_P01	ZmWRKY7
AT1G62300.1	WRKY6	Bradi1g22680.2.p	WRKY9	LOC_Os01g40430.1	WRKY27	GRMZM2G071907_P01	ZmWRKY11
AT1G64000.1	WRKY56	Bradi1g23340.1.p	WRKY10	LOC_Os01g43550.1	WRKY12	GRMZM2G377217_P01	WRKY12
AT1G66550.1	WRKY67	Bradi1g30870.1.p	WRKY11	LOC_Os01g43650.1	WRKY11	GRMZM2G151444_P01	ZmWRKY13
AT1G66560.1	WRKY64	Bradi1g47690.1.p	WRKY12	LOC_Os01g46800.1	WRKY15	GRMZM2G024898_P01	ZmWRKY14
AT1G66600.1	WRKY63	Bradi1g51030.1.p	WRKY14	LOC_Os01g47560.1	WRKY16	GRMZM2G105140_P01	ZmWRKY22
AT1G68150.1	WRKY9	Bradi1g59180.2.p	WRKY15	LOC_Os01g51690.1	WRKY26	GRMZM2G461648_P01	ZmWRKY23
AT1G69310.1	WRKY57	Bradi1g63220.1.p	WRKY16	LOC_Os01g53040.1	WRKY14	GRMZM2G015433_P01	ZmWRKY24
AT1G69810.1	WRKY36	Bradi1g63910.1.p	WRKY17	LOC_Os01g53260.1	WRKY23	GRMZM2G148561_P01	ZmWRKY25
AT1G80590.1	WRKY66	Bradi2g00280.1.p	WRKY18	LOC_Os01g54600.1	WRKY13	GRMZM2G008029_P01	ZmWRKY26
AT1G80840.1	WRKY3	Bradi2g05234.2.p	WRKY13	LOC_Os01g60490.1	WRKY22	GRMZM2G156529_P01	ZmWRKY27
AT2G03340.1	WRKY40	Bradi2g05500.1.p	WRKY19	LOC_Os01g60520.1	WRKY116	GRMZM2G414315_P01	ZmWRKY28
AT2G04880.1	WRKY1	Bradi2g08620.1.p	WRKY21	LOC_Os01g60540.1	WRKY20	GRMZM2G092694_P01	ZmWRKY31
AT2G21900.1	WRKY59	Bradi2g11170.2.p	WRKY22	LOC_Os01g60600.1	WRKY108	GRMZM2G151407_P01	ZmWRKY34
AT2G23320.1	WRKY15	Bradi2g15360.1.p	WRKY23	LOC_Os01g60640.1	WRKY21	GRMZM2G383594_P01	ZmWRKY35
AT2G24570.1	WRKY17	Bradi2g16150.1.p	WRKY25	LOC_Os01g61080.1	WRKY24	GRMZM2G432583_P01	ZmWRKY38
AT2G25000.1	WRKY60	Bradi2g18530.1.p	WRKY26	LOC_Os01g62510.1	WRKY119	GRMZM2G057011_P01	ZmWRKY40
AT2G30250.1	WRKY25	Bradi2g19070.1.p	WRKY27	LOC_Os01g62514.1	WRKY56	GRMZM2G063880_P01	ZmWRKY41

Arabidopsis thaliana		*Brachypodium distachyon*		*Oryza sativa*		*Zea mays*	
TF ID	GENE NAME	TF ID	GENE NAME	TF ID	GENE NAME	TF ID	GENE NAME
AT2G30590.1	WRKY21	Bradi2g22440.1.p	WRKY28	LOC_Os01g74140.1	WRKY17	GRMZM2G106560_P01	ZmWRKY45
AT2G34830.1	WRKY35	Bradi2g30800.2.p	WRKY30	LOC_Os02g08440.1	WRKY71	GRMZM2G063216_P01	ZmWRKY46
AT2G37260.1	WRKY44	Bradi2g33540.1.p	WRKY31	LOC_Os02g16540.1	WRKY39	GRMZM2G327349_P01	ZmWRKY47
AT2G38470.1	WRKY33	Bradi2g44090.1.p	WRKY32	LOC_Os02g26430.1	WRKY42	GRMZM2G468056_P01	ZmWRKY48
AT2G40740.1	WRKY55	Bradi2g44270.1.p	WRKY33	LOC_Os02g43560.1	WRKY34	GRMZM5G863420_P01	ZmWRKY50
AT2G40750.1	WRKY54	Bradi2g45480.1.p	WRKY34	LOC_Os02g47060.1	WRKY66	GRMZM2G101405_P01	ZmWRKY51
AT2G44745.1	WRKY12	Bradi2g45900.1.p	WRKY35	LOC_Os02g53100.1	WRKY32	GRMZM2G003551_P01	ZmWRKY53
AT2G46130.1	WRKY43	Bradi2g48090.1.p	WRKY36	LOC_Os03g20550.1	WRKY55	GRMZM2G411766_P01	ZmWRKY54
AT2G46400.1	WRKY46	Bradi2g49020.1.p	WRKY38	LOC_Os03g21710.1	WRKY79	GRMZM2G030272_P01	ZmWRKY55
AT2G47260.1	WRKY23	Bradi2g53500.2.p	WRKY40	LOC_Os03g33012.1	WRKY81	GRMZM2G018721_P01	ZmWRKY57
AT3G01080.1	WRKY58	Bradi2g53510.2.p	WRKY41	LOC_Os03g45450.1	WRKY60	GRMZM2G169149_P01	WRKY62
AT3G01970.1	WRKY45	Bradi2g53520.1.p	WRKY42	LOC_Os03g53050.1	WRKY121	GRMZM2G400559_P01	ZmWRKY63
AT3G04670.1	WRKY39	Bradi2g53760.1.p	WRKY43	LOC_Os03g55080.1	WRKY3	GRMZM2G382350_P01	ZmWRKY64
AT3G56400.1	WRKY70	Bradi2g54720.2.p	WRKY44	LOC_Os03g55164.1	WRKY4	GRMZM2G141299_P01	ZmWRKY65
AT3G58710.1	WRKY69	Bradi3g06070.1.p	WRKY46	LOC_Os03g58420.1	WRKY6	GRMZM2G381378_P01	WRKY70
AT3G62340.1	WRKY68	Bradi3g09810.1.p	WRKY47	LOC_Os03g63810.1	WRKY80	GRMZM2G120320_P01	ZmWRKY71
AT4G01250.1	WRKY22	Bradi3g18580.1.p	WRKY48	LOC_Os04g21950.1	WRKY51	GRMZM2G176489_P01	ZmWRKY72
AT4G01720.1	WRKY47	Bradi3g19640.1.p	WRKY49	LOC_Os04g39570.1	WRKY35	GRMZM2G139815_P01	ZmWRKY74
AT4G04450.1	WRKY42	Bradi3g34850.1.p	WRKY50	LOC_Os04g46060.1	WRKY36	GRMZM2G111354_P01	ZmWRKY75
AT4G11070.1	WRKY41	Bradi3g39340.2.p	WRKY51	LOC_Os04g50920.1	WRKY37	GRMZM2G111711_P01	ZmWRKY124
AT4G12020.1	WRKY19	Bradi3g50360.1.p	WRKY52	LOC_Os04g51560.1	WRKY68	AC165171.2_FGP002	ZmWRKY49

Arabidopsis thaliana		*Brachypodium distachyon*		*Oryza sativa*	
TF ID	GENE NAME	TF ID	GENE NAME	TF ID	GENE NAME
AT4G18170.1	WRKY28	Bradi3g52420.1.p	WRKY53	LOC_Os05g03900.1	WRKY109
AT4G22070.1	WRKY31	Bradi3g57710.1.p	WRKY54	LOC_Os05g04640.1	WRKY5
AT4G23550.1	WRKY29	Bradi4g01950.1.p	WRKY55	LOC_Os05g09020.1	WRKY67
AT4G23810.1	WRKY53	Bradi4g02680.1.p	WRKY56	LOC_Os05g14370.1	WRKY82
AT4G24240.1	WRKY7	Bradi4g06690.1.p	WRKY57	LOC_Os05g25770.1	WRKY45
AT4G26440.1	WRKY34	Bradi4g19060.1.p	WRKY59	LOC_Os05g27730.1	WRKY53
AT4G26640.1	WRKY20	Bradi4g28280.1.p	WRKY60	LOC_Os05g39720.1	WRKY70
AT4G30935.1	WRKY32	Bradi4g30360.1.p	WRKY61	LOC_Os05g40060.1	WRKY48
AT4G31550.1	WRKY11	Bradi4g33370.1.p	WRKY63	LOC_Os05g40070.1	WRKY84
AT4G31800.1	WRKY18	Bradi4g44350.1.p	WRKY64	LOC_Os05g40080.1	WRKY54
AT4G39410.1	WRKY13	Bradi4g44360.1.p	WRKY65	LOC_Os05g45230.1	WRKY58
AT5G01900.1	WRKY62	Bradi4g44370.2.p	WRKY66	LOC_Os05g46020.1	WRKY7
AT5G07100.1	WRKY26	Bradi4g45290.1.p	WRKY67	LOC_Os05g49100.1	WRKY49
AT5G13080.1	WRKY75	Bradi5g13090.1.p	WRKY69	LOC_Os05g49210.1	WRKY43
AT5G15130.1	WRKY72	Bradi5g13090.2.p	WRKY70	LOC_Os05g49620.1	WRKY19
AT5G22570.1	WRKY38	Bradi5g20700.1.p	WRKY71	LOC_Os05g50610.1	WRKY8
AT5G24110.1	WRKY30			LOC_Os05g50700.1	WRKY111
AT5G26170.1	WRKY50			LOC_Os06g05380.1	WRKY73
AT5G28650.1	WRKY74			LOC_Os06g06360.1	WRKY113
AT5G41570.1	WRKY24			LOC_Os06g30860.1	WRKY31
AT5G43290.1	WRKY49			LOC_Os06g44010.1	WRKY28
AT5G45050.1	WRKY16			LOC_Os07g02060.1	WRKY29
AT5G45260.1	WRKY52			LOC_Os07g27670.1	WRKY115
AT5G46350.1	WRKY8			LOC_Os07g39480.1	WRKY87
AT5G49520.1	WRKY48			LOC_Os07g40570.1	WRKY88
AT5G52830.1	WRKY27			LOC_Os07g48260.1	WRKY47
AT5G56270.1	WRKY2			LOC_Os08g09800.1	WRKY105
AT5G64810.1	WRKY51			LOC_Os08g09810.1	WRKY106

Oryza sativa	
TF ID	GENE NAME
LOC_Os08g09900.1	WRKY118
LOC_Os08g13840.1	WRKY25
LOC_Os08g13840.1	WRKY25
LOC_Os08g17400.1	WRKY89
LOC_Os08g29660.1	WRKY69
LOC_Os08g38990.1	WRKY30
LOC_Os09g09630.1	WRKY112
LOC_Os09g16510.1	WRKY74
LOC_Os09g25060.1	WRKY76
LOC_Os09g25070.1	WRKY62
LOC_Os09g30400.1	WRKY90
LOC_Os10g18099.1	WRKY18
LOC_Os10g42850.1	WRKY2
LOC_Os11g02470.1	WRKY52
LOC_Os11g02480.1	WRKY46
LOC_Os11g02520.1	WRKY104
LOC_Os11g02530.1	WRKY40
LOC_Os11g02540.1	WRKY50
LOC_Os11g29870.1	WRKY72
LOC_Os11g45850.1	WRKY61
LOC_Os11g45920.1	WRKY63
LOC_Os12g01180.1	WRKY57
LOC_Os12g02400.1	WRKY114
LOC_Os12g02420.1	WRKY97
LOC_Os12g02440.1	WRKY95
LOC_Os12g02450.1	WRKY64
LOC_Os12g02470.1	WRKY65
LOC_Os12g32250.1	WRKY96
LOC_Os12g40570.1	WRKY94

CURRICULUM VITAE

Esra Karakaş was born on June 15, 1995 in Adana, Turkey. She completed her higher secondary education from Yavuz Sultan Selim Anatolian High School, Niğde in 2013. Afterwards she joined Necmettin Erbakan University, Konya Turkey in 2013 for her undergraduate studies. She went to Szczecin/Poland for one semester with Erasmus exchange program in 2015. She completed her B.Sc in Molecular Biology and Genetics, Faculty of Sciences in 2017. She enrolled in Graduate School of Natural and Applied Sciences, Department of Agricultural Genetic Engineering at Niğde Ömer Halisdemir University, Niğde, Turkey to pursue her master education under the guidance of Dr. Zahide Neslihan ÖZTÜRK GÖKÇE. During her master thesis research she worked on identification of tissue, developmental stage and stress response specificity of WRKY transcription factor family. She knows English and Turkish languages. During her master's degree, she did an internship about abiotic stress on Uniop Erasmus program for two and a half months at Max Planck Institute in Germany.