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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

COMPARISON OF DROUGHT STRESS RESPONSE OF POTATO VARIETIES AT THE TRANSCRIPTOMIC LEVEL

MOHAMMAD HUSSAIN AZIMI

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M. Hussein AZIMI tarafından **Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE** danışmanlığında hazırlanan "**Comparison of Drought Stress Response of Potato Varieties at the Transcriptomic Level**" adlı bu çalışma jürimiz tarafından Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü **Tarımsal Genetik Mühendisliği (İngilizce)** Ana Bilim Dalı'nda Yüksek Lisans tezi olarak kabul edilmiştir.

Başkan : Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE, Ömer Halisdemir Üniversitesi

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ONAY:

Bu tez, Fen Bilimleri Enstitüsü Yönetim Kurulunca belirlenmiş olan yukarıdaki jüri üyeleri tarafından …./…./20.... tarihinde uygun görülmüş ve Enstitü Yönetim Kurulu'nun …./…./20.... tarih ve ..... sayılı kararıyla kabul edilmiştir.

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

Doç. Dr. Murat BARUT
MÜDÜR V.

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.

Mohammad Hussain AZIMI

SUMMARY

COMPARISON OF DROUGHT STRESS RESPONSE OF POTATO VARIETIES AT THE TRANSCRIPTOMIC LEVEL

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Solanum tuberosum L. is sensitive to drought mainly due to its fibrous root system. High temperature and drought episodes may cause yield decrease to almost 30%. Data on drought stress response of potato when compared to other crops, especially wheat, and model organisms, on the other hand is quite limited. There are a few works limited to microarrays that include only 12,000 potato cDNA clones. Therefore the aim of thesis is to differentiate physiological and transcriptomic changes in response to drought in tolerant and sensitive potato varieties. Physiological effects of drought stress were evaluated in two potato varieties and the changes in transcripts and metabolic pathways in response to drought stress were investigated via bioinformatics analysis of leaf transcriptome generated by next generation sequencing. Leaf transcriptomes were used to compare drought tolerant and sensitive potato varieties in response to water stress to identify metabolic differences between the varieties. Next generation sequencing approach was performed to identify leaf transcriptomes of drought stressed and control samples of both cultivars.

Keywords: Drought, potato, transcriptomics, next generation sequencing, bioinformatics

ÖZET

FARKLI PATATES ÇEŞİTLERİNİN KURAKLIĞA TEPKİLERİNİN TRANSKRİPTOM SEVİYESİNDE KARŞILAŞTIRILMASI

AZIMI, Mohammad Hussain
Niğde Ömer Halisdemir Üniversitesi
Fen Bilimleri Enstitüsü
Tarımsal Genetik Mühendisliği Anabilim Dalı

Danışman : Doç. Dr. Zahide Neslihan ÖZTÜRK GÖKÇE

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S.tuberosum L. esas olarak lifli kök sistemi nedeniyle kuraklığa duyarlıdır. Yüksek sıcaklık ve kuraklık dönemLeri verimin yaklaşık % 30 oranında düşürebilmektedir. Öte yandan patatesin kuraklık tepkimesi hakkında diğer bitkilerden, özellikle buğday ve model organzimalara kıyasladığımızda daha az bilgi bulunmaktadır. Yalnızca 12,000 patates cDNA klonu içeren mikrodizileme ile sınırlı sayıda çalışma vardır. Bu tezin amacı kuraklığa toleranslı ve kuraklığa hassas iki çeşit patates bitkisinde fizyolojik ve transkriptomdeki değişikliklerin kuraklık uygulamasının sonunda kuraklığa tepkimelerini karşılaştırılmaktadır. Kuraklik stresinin fizyolojik etkileri iki patates çeşidinde değerlendirilmiş ve kuraklık stresine tepki olarak transkriptlerdeki ve metabolik yolaklardaki değişiklikler yüksek verimLi dizileme ile elde edilen yaprak transkriptomunun biyoinformatik analizleri kullanarak araştırıldı. Kuraklığa toleranslı ve kuraklığa hassas iki patates çeşidinin yaprak transkriptomLarı kullanılarak metabolik farklılıklar belirlenmiş ve karşılaştırılmıştır. Kuraklık toleranslı ve hassas patates çeşitleri su eksikliğine maruz bırakılmış ve fizyolojik ve transkriptomik düzeyde değerlendirilmiştir. Her iki çeşitteki örneklerinin yaprak transkriptomLarını belirlemek için yeni nesil dizileme yaklaşımı uygulanmıştır.

Anahtar Sözcükler: Kuraklık, patates, transkriptomik, yeni nesil dizileme , biyoinformatik

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYMBOLS AND ABBREVIATION

Symbols	**Description**
%	Percentage
µ	Micro
µL	Microliter
M	Molarity
mg	Milligram
ng	Nanogram
^{o} C	Degree celsius
α	Alpha
Abbreviation	**Description**
ABA	Abscisic Acid
bZIP	Basic Region Leucine Zipper
cDNA	Complementary DNA
CIP	International Potato Center
CT	Control
DEG	Differentially Expressed Genes
DR	Drought
DREB	Dehydration Responsive Element Binding Protein
EF	Elongation Factor
ERF	Ethylene-responsive Element Binding Factor
EST	Expressed Sequence Tag
FAOSTAT	FAO Corporate Statistical Database
FPKM	Fragment per Kilo Base Pair of Exon Model per Million
GO	Gene Ontology
HSP	Heat-shock Protein
IRT	Infrared Thermometer
JA	Jasmonic Acid
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	KEGG Orthology Database
KOG/COG	Clusters of Orthologous Groups of Proteins

LEA	Late Embryogenesis Abundant
NCBI	National Center for Biotechnological Information
NGS	Next-generation Sequencing
Nr	NCBI non-redundant Protein Sequences
Nt	NCBI non-redundant Nucleotide Sequences
PCR	Polymerase Chain Reaction
PFAM	Protein Families Database
PGSC	Potato Genome Sequencing Consortium
qRT-PCR	Quantitative real-time PCR
RB	Russet Burbank
RNA	Ribonucleic Acid
RNase	Ribonuclease
RNA-Seq	RNA Sequencing
RWC	Relative Water Content
TBE	Tris/Borate/EDTA
UN	Unica
UV	Ultraviolet
Xg	G-force
MYC	Myelocytomatosis Oncogene
MYB	Myeloblastosis Oncogene

CHAPTER I

INTRODUCTION

Potato (*S.tuberosum* L.), frontier in non-cereal crops, is the most important food crop in the world. It has occupied fourth position regarding production among food crops after rice (*Oryza sativa*), wheat (*Triticum aesitvum*) and maize (*Zea mays*) in the whole world (CIP, 2013; Monneveux et al., 2013). The crop is primarily developed in cool temperature climates with adequate sunlight, mild day temperature and cold nights. Decrease in daylight generally lead to tuber formation in potato (Tarn et al., 1992). The whole world potato production was reported as 381.682.144 tones in 2014 (FAOSTAT, March 2017). Potato is a plant with high water use efficiency but because of the fibrous root system, it is less tolerant to the drought stress (Hassanpanah, 2010; Lahlou et al., 2005; Monneveux et al., 2013).

Plant respond to drought stress at different levels, including physiological, cellular and molecular (Figure 1.1). The responses are related to various factors, like as species and genotypes (Rampino et al., 2006), the period and severity of water loss (Bartels and Souer, 2004), the maturity and phase of development (Zhu et al., 2005), the organ and cell type (Wang and Jiao, 2006), and the sub-cellular compartment (Battaglia et al., 2007). Because plants are immobile organisms, they have developed adaptive mechanisms to maintain their lives and reproduction under drought conditions. Molecular and physiological changes of plants against drought stress start by various transcription factors and regulators, and by the activation of signal transmission cascades which enable re-programming of the transcription. The changes in transcript profile, induces molecular and cellular mechanisms responsible for repairing damages due to water loss of the plant and to ensure the continuity of growth and reproduction (Barnabas et al., 2008).

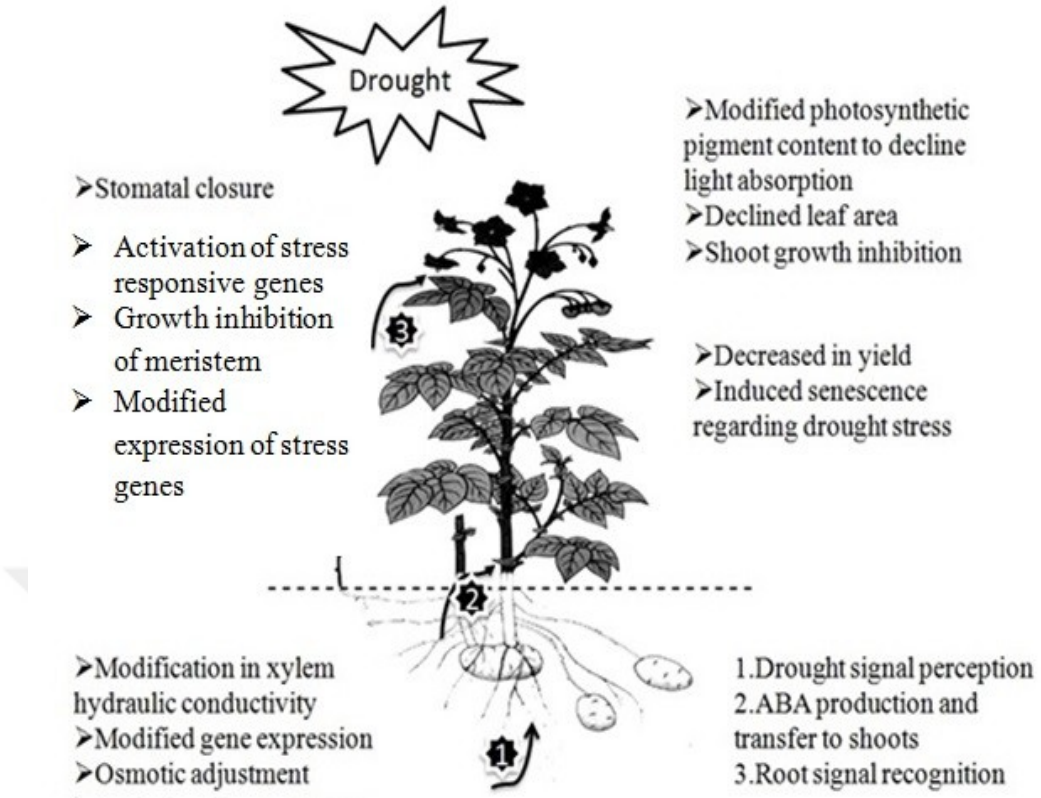


Figure 1.1. Summary of adaptive responses of plant under drought stress (altered from Chaves et al., 2003)

Drought stress is the most destructive and complex among different abiotic stresses (Pennisi, 2008). Plants have mainly three strategies to respond drought stress involving escape, avoidance and tolerance. Plants escape drought season by adapting to the environment which is characterized by rapid development, early maturation i.e. flowering, fruiting and senescence, which enables them to reproduce before the environment becomes more unfavorable. This pattern of growth keeps the tissues away from excessive exposure to drought (Price et al., 2002).

Reduction in water loss and improvement in uptake of water are the main tools of avoiding drought stress in plants. Reduced epidermal conductance, thickening in the wax region of cuticle, leaf rolling or folding which results in decreased absorption of radiation and reduction in evaporation surfaces are the key factors of reduction in water loss. Deeper and thicker root system with larger surface area results in efficient uptake of water. A balance between turgor maintenance and reduced water loss are the key factors of plants' survival in drought conditions (Mitra, 2001).

Drought tolerance can be defined as the capability of plant to develop, flower and give economic produce in water scarcity condition (Farooq et al., 2009). Plants can endure water scarce condition but up to a limit, however that moderate abridged interruption of plant water balance do not instantly affect yield (Schafleitner, 2009). Cellular stability and maintenance of turgor pressure via osmotic modification, harmonious solutes, antioxidants and a scavenging defense mechanism are the key drivers of the mechanism which enable plants to withstand drought stress (Madhava et al., 2006).

Agronomic practices and plant breeding programs can be improved with increase in knowledge of the impact of drought on plants. Many changes in physiological, metabolic and defense systems are activated by plant for survival and sustainable growth.

Any alteration in the ideal growing conditions is considered by plants as stress and they respond via chemical signals which involve protein phosphorylation and/or dephosphorylation, protein degradation, and calcium sensing. These chemical signaling results in over-expression of related genes which play role in repairing mechanisms or switch various transcription factors, thus resulting in regulation of stress response genes (Bartels and Sunkar, 2005). Control and activation of various drought stress-linked transcripts and proteins have been identified by transcriptomics, proteomics and gene expression studies, which are usually kept in two major groups. The first group is functional proteins consists of proteins which function in tolerance of any kind of stress. Basically, they are protection factors like late embryogenesis abundant (LEA) proteins, chaperones, and lipid transfer proteins. Proteins playing role in repair against damage, such as detoxification enzymes, ferritin, proteinases, protease inhibitors, and plant defense-related proteins and proteins involved in synthesis of osmoprotectants (proline, glycine betaine, sugars), also contains proteins associated in cellular metabolic pathways like carbohydrate metabolism, fatty acid metabolism, secondary metabolism, plant hormones biosynthesis abscisic acid (ABA), jasmonic acid (JA), ethylene and etc. Regulatory proteins are the second group associated in regulation of signal transduction and transcription as a component of the drought response. These are various gene families of transcription factors like dehydration responsive element binding protein (DREB), Ethylene-responsive element binding factor (ERF), WRKY, MYB, MYC,

basic region leucine zipper Zinc finger (bZIP), and NAC families. (Shinozaki et al., 2003a; Shinozaki et al., 2007b).

Conventional breeding approaches have introduced very few crop varieties with developed stress tolerance characteristics (Flowers, 2004). Compared to the traditional breeding approaches like marker assisted selection, direct use of genetic engineering methods to introduce genes to improve stress tolerance seems more promising and rapid solution (Dunwell, 2000; Wang et al., 2003).

Drought avoidance indicates plant's capability to maintain a high level relative hydration under water stress regarding soil or atmosphere (Blum, 1988). Drought avoidance has two characteristics involving reduced water loss and enhanced water uptake (Price et al., 2002b).

Drought stress causes direct reduction in tuber yield in the production of potato plantation areas where rainfall is insufficient; therefore potato should be watered frequently (Lahlou and Ledent, 2005; Levey et al., 2013; Liu et al., 2005). Drought below the level of 30-50% of field capacity reduce the number of leaves and leaf size of potatoes, inhibits the plant height elongation, reduces the photosynthesis activity, affects tuber mineral composition and in the start of tuber formation stage it can lead to very serious yield loss (Cabello et al., 2013; Lefevre et al., 2012; Onder et al., 2005; Schafleitner et al., 2007a; Shin et al., 2011; Watkinson et al., 2006).

Due to global climate change high temperatures that occur during production, dry season, the amount of rainfall falling in the ground and declines in groundwater sources using for irrigation in the next 30 years will lead to the potato yield loss up to 18-32% (Monneveux et al., 2013). This necessitates the development of resistant varieties of potatoes to the drought stress. Researchers based on information obtained from genomic and molecular approaches provided by phenotyping and breeding methods are important to develop high drought tolerance in agricultural crop varieties (Mir et al., 2012).

Potato is a plant regularly developed by breeding. It is propagated vegetatively by using tubers, therefore due to absence of genetic modification between generations breeding is

easily provided and varieties can be improved. However, at the moment the aim of developing potato varieties are to improve industrial quality, providing much higher yield and increasing edible quality. The investigation for increasing drought tolerance in potato breeding is a novel approach. As known, breeding approaches takes a long time like 10 years to achieve positive results. If the aim is to increase the tolerance to drought, to achieve reliable results needs enough replicates, obtaining from each cross line sufficient number of heterogenic sample and considering the interaction of genotype X environment (G X E) is required (Cominelli et al., 2013; Deikman et al., 2012; Lawlor, 2013; Mir et al., 2012; Tuberosa, 2012).

The aim of this thesis is to differentiate physiological and transcriptomic changes in response to drought in tolerant and sensitive potato varieties. The thesis was evaluated physiological effects of drought stress in two varieties to analyze the changes in transcripts and metabolic pathways in response to drought via bioinformatic analyses of leaf transcriptome generated by next generation sequencing. *S.tuberosum* L. is sensitive to drought mainly due to its fibrous root system. High temperature and drought episodes may cause yield decrease to almost 30%. Data on drought stress response of potato when compared to other crops, especially wheat, and model organisms, on the other hand is quite limited. There are a few works limited to microarrays that include only 12.000 potato cDNA clones (Potato Oligo Chip Initiative, early 2000).

Leaf transcriptome were used to compare drought tolerant and sensitive potato varieties in response to drought stress to identify metabolic differences between the varieties. Drought tolerant and sensitive potato varieties will be exposed to water deficiency and be evaluated at physiological and transcriptomic levels. NGS was performed to identify leaf transcriptomes of drought stressed and control samples of both cultivars. Bioinformatics tools were used to investigate the gene expression differences in drought tolerant and sensitive varieties before and after water deficit.

CHAPTER II

LITERATURE REVIEW

2.1 Transcriptome analysis upon stress conditions

It's possible to understand stress response of an organism in detail by transcriptomic studies (Jogaiah et al., 2012). Identification of plant adaptation and stress mechanisms to various environmental stresses has been discovered using effective genomics methods like transcriptome analysis (Urano et al., 2010).

Xu et al. (2013) determined early transcriptome response of remarkably tolerant *Gossypium aridum* related to salt tolerant stress. Analysis of digital gene expression was operated to recognize the genes regarding in control and salt stressed plant. Response to hormone stimulus, transport and signaling pathways were precisely discovered under salt stress conditions. GO (Gene Ontology) analysis has revealed that the most potential enriched GO terms were transporter activities and protein kinase.

Gong et al. (2014) performed transcriptome profiling of the potato plant under drought stress and water-stimulus conditions on the basis of NGS strategy. In this study, a total number of 3189, 1797 and 4230 differentially expressed genes (DEGs) including 1630, 1527 and 1596 transcriptional factor-encoding DEGs were discovered in comparison with control, drought and re-watering samples, accordingly.

2.2 Transcriptome *de novo* assemblies

NGS is a recent approach to study genome and transcriptome analysis of any organism (Wang et al., 2010). The recent NGS technologies are divided into three according to their characteristics and cost value. These are; reference-based, *de novo*, and a combination of reference-based and *de novo* (combined strategy) (Martin and Wang, 2011).

Reference-based assembly consists of three steps with high sensitivity when a reference genome is accessible. Alignment of RNA sequencing (RNA-Seq) reads is the first step of this process using a splice aware aligner. Constructing a graph is the second step to represent alternative splicing. Overlapping and assembled isoform extraction is the last step. Unicellular organisms such as bacteria, protozoa, and simple-eukaryotic organisms with shorter introns and less alternative splicing are used to study by reference-based assembly. Martin and Wang (2011) have mentioned the difficulty and inapplicability of this strategy to the mammalian and plants which have complex alternative splicing mechanism. Reference genome of organisms that are known letting us to use this approach (Trapnell et al., 2010).

De novo assembly of RNA-Seq was explored as an interesting approach to high-throughput gene detection in non-model organisms with a genome wide-scale. *De novo* assembly of RNA-Seq is providing to investigate transcriptomes of organisms with non-reference genome (Grabherr et al., 2011; Robertson et al., 2010). Trinity is the final version of assembly program among various assemblers (Grabherr et al., 2011). In order to perform *de novo* assembly analysis, Trinity-short reads assembly program is used. At first Trinity incorporates reads with assured length of overlap to provide longer fragments (called contigs). Trinity is powerful assembly software and the vigorous method in favor of constructing de novo assembly analysis (Grabherr et al., 2011). It's called Trinity considering it engages three major steps that have been created in the three software programs separately. The program first starts with *inchworm* which assembles RNA-Seq data into linear context, and then it is *chrysalis* (Figure 2.1) making group of contacts which are related to alternative splicing or (gene duplication) and creates *de-Bruijn* graphs. At the final *butterfly* explore reads in the form of various graphs and broadcasts final full-length transcripts and isoforms of transcripts (Brian et al., 2013).

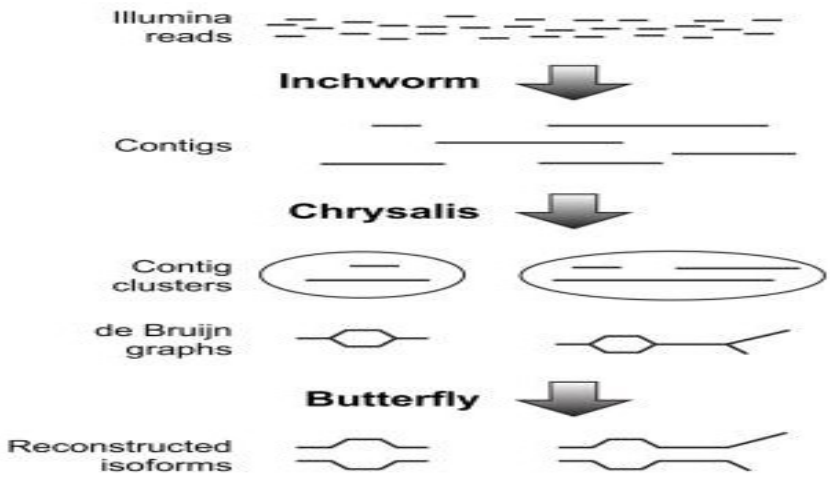


Figure 2.1. Overview of Trinity assembly (Brian et al., 2013)

Trinity attaches the contigs to those sequences that possibly are not extended on either end. These kinds of sequences are called as Unigenes. When various samples belong to identical species are sequenced, individual sample's assembled Unigenes are introduced to supplementary sequence splicing process and sequence clustering software are used to eliminate redundancy and to obtain non-redundant Unigenes. Clustered Unigenes are classified into two branches, the first "clusters" is shown with CL prefix, and the second is "singletons" termed as Unigene. Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), pFAM (protein family), KO (KEGG Orthology database), Swiss-Prot (A manually annotated and reviewed protein sequence database), KEGG (Kyoto Encyclopedia of Genes and Genomes), KOG (Clusters of Orthologous Groups of proteins) databases were then used to perform blastx alignment between Unigenes and protein E-value <0.00001 (Finn et al., 2008; Kaneshia et al., 2008; Young et al., 2010).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical materials and kits

The chemicals and kits including their suppliers are listed in Appendix A

3.1.2 Plant materials

Two potato genotypes with contrasting response to water stress were used. Unica with N^{o}392797.22 CIP code number is used as tolerant cultivar to drought (Cabello et al. 2012). Russet Burbank is used as sensitive cultivar to drought (Anonymous, 2016; Stark et al., 2013).

3.2 Methods

3.2.1 Growth of plants

To ensure the similar physiological development stage of plants, tuber pieces with a single eye were planted in the 5-liter volume pots having 2:1 ratio of torf and perlite, and were watered to soil saturation capacity. All plants were grown at 22 °C / 16 °C (day / night) temperature, 16-h light / 8-h dark, 60 % moisture content in greenhouse condition. During growth, insecticide treatment was done to control pests and disease carriers.

3.3 Application of drought stress

Drought treatment was started 45 days after leaf emergence. During this period control plants were given water regularly so that water level should not be less than soil saturation level. Drought treated plants were not irrigated for 23 days. The leaf samples of control and drought stress treated plants were collected in mid-day hours, were frozen directly in the liquid nitrogen and stored at – 80 °C to be used for NGS studies.

3.4 Physiological assessment

To approve of drought stress and to determine the changes caused by water stress, several physiological measurements were taken from both control and stress plants.

3.4.1 Stomatal conductance

The measurements in control and stress conditions were done in the mid-day hours. Apical leaflet of 3rd fully developed leaf of main stem on 4 individual plants per cultivar was measured. The measurement was done under constant light intensity and air flow conditions with LICOR-6400 portable photosynthesis device.

3.4.2 Photosynthesis rate

It was measured by LICOR-6400 portable photosynthesis machine having characteristics of 1000 μmol/m^2/s constant light intensity, 400 μmol/ mol CO_2 and 500 μmol s^{-1} air flow condition. Apical leaflet of 3rd fully developed leaf of main stem, on 4 individual plants per cultivar was measured and the mean value was calculated to find photosynthesis rate.

3.4.3 Transpiration rate

It has been measured under constant light intensity and air flow condition by using LICOR-6400 portable photosynthesis machine. Apical leaflet of 3rd fully developed leaf of main stem, on 4 individual plants per cultivar was measured and the mean value of them was calculated as transpiration rate.

3.4.4 Leaf relative water content (RWC)

Procedures to measure RWC is given at the below;

1. Three leaves from the apical of 3rd fully developed leaf of main stem was collected.
2. Fresh leaf samples weighted by precision scale and kept in pure water during night.
3. Leaf samples were dried by microwave with 500 W powers for 10 min then samples were transferred to oven and kept at 95 ^{o}C for 1 hour to dry the samples.
4. The dried leaf samples were measured by precision scale and the RWC were calculated using equation 3.1.

$$RWC\ (\%) = [(W - DW) / (TW-DW) \times 100) \quad (3.1)$$

Where,
W- Fresh weight of sample
TW– Turgid weight of sample
DW – Dry weight of sample

3.4.5 Chlorophyll index

Chlorophyll index was measured after starting the drought stress treatment every mid-day regularly. The measurement was taken from the top of the plant leaflet of both stressed and control plants. Five different plants were measured by Chlorophyll-Meter (Konica Minolta SPAD-502 Plus) and the mean value is counted as chlorophyll index.

3.4.6 Leaf temperature (°C)

Leaf temperature was measured every mid-day during stress treatment using Infrared Thermometer (IRT) device (MASTECH BM380). The measurement was taken from the top of the plant leaflet of both stressed and control plant plants. In each measurement 4 different plants from the same cultivar were used, each plant was measured two times and the mean value is given the leaf temperature.

3.4.7 Proline determination

Proline content was determined applying a modified method developed by Bates et al. (1973). Proline content was measured as the following steps:

1. 100 mg leaf sample was grounded in 2 mL of 3 % sulfosalicylic acid, and then the sample was mixed by vortex.
2. The mix was precipitated by centrifugation in 10,000 xg for 10 min at 4 ^{o}C.
3. 0.2 mL of the supernatant was transferred to a new 1.5 mL centrifuge tube.
4. Freshly prepared 0.2 mL ninhydrin solution (1.25 ninhydrin, 30 mL glacial acetic acid, 20 mL 6 M orthophosphoric acid) was added.
5. The mixture was inverted 3-4 times for 15 seconds.
6. The mixture was incubated at 90 ^{o}C for 1 hour, and then the reaction was terminated on ice.
7. One mL of toluene was added to each reaction mixture, and then mixed for 15 second by vortex.
8. The mixture was incubated at room temperature keeping in the dark place for 20 min.
9. 330 µL of pinkish supernatant mixture was added to the spectrophotometer cuvette; later 670 µL toluene was added on it.
10. The sample was measured at 520 nm wavelength in the spectrophotometer.
11. The spectrophotometer was blanked by adding 1 mL toluene.

The proline concentration was calculated from the obtained standard curve prepared by pure proline with 0.375 µg/µL, 0.750 µg/µL, 1.500µg/µL, 3.00 µg/µL, 3.750 µg/µL, 5.625 µg/µL, 7.500 µg/µL, 11.250 µg/µL concentration values were used to draw the standard graphic.

3.5 Identification of modified gene expression and bioinformatics analysis

3.5.1 Total RNA isolation

RNA samples were isolated from leaves with Trizol reagent (Invitrogen™, Catalog number: 155926) according to the guidelines of the provider. Total isolated RNA was quantified by nanodrop (BioSpec UV-vis Spectrophotometer, SHIMADZU) and quality was assessed by agarose gel electrophoresis. Isolations with enough quality and quantity were sent to NGS via Illumina HiSeq2500.

RNA isolation was performed with following procedure:

1. Plant tissue leaves were crushed inside mortar using liquid nitrogen to obtain powder status, 100 mg powder were weighed by precision balance scale and transferred to appropriate tube.
2. 1 mL Trizol was added to the tube and homogenized.
3. Samples were kept 10 min at 4 ^{o}C
4. Samples centrifuged for 10 min at 4 ^{o}C maximum.
5. 450 µL supernatant were taken from upper phase and transferred to 1.5 mL RNAse-free centrifuge tubes.
6. 200 µL chloroform was added to the tubes and were shaken by hand for 15 seconds.
7. Samples were incubated at room temperature for 5 min.
8. Samples were centrifuged at 4 ^{o}C at maximum for 15 min.
9. Supernatant was transformed to a new RNAse-free tube and 500 µL cold isopropylalcohol were added to the tubes.
10. Samples were kept at room temperature for 10 min and then centrifuged at 11.000 rpm for 10 min (4 ^{o}C).
11. One mL of 75 % EtOH was added and centrifuged at 9.000 rpm for 5 min (4 ^{o}C).
12. The pellet dried at room temperature then dissolved in enough sterile RNAse-free water to measure the RNA concentration by nanodrop machine.
13. Samples were stored at -20 ^{o} C.

RNAse-free water was used to blank the machine, and the samples were verified by agarose gel analysis. In this method 0.5 X Tris/Borate/EDTA (TBE) with 1.2 % concentration agarose prepared, then samples and 3 µL marker (Thermo Scientific, 100 bp Gene ruler) were loaded to the gel and run for 50 min at (7 V/ cm), later the gel checked under UV machine to integrity of total RNAs isolated.

3.5.2 Identification of modified gene expression and bioinformatics analysis

First part of bioinformatics analyses included checking quality reads where error rate of single base should be lower than 1 %, and for special case, the maximum error rate for single base lower than 6 % is acceptable and transcriptome assembly was performed by Trinity program according to the specialty of Illumina HiSeq2500 facility. The unique gene sequences were then annotated by GO (Ashburner et al., 2000; Gene Ontology Consortium, 2015) and KEGG databases to identify their intracellular functions both in metabolic and transcriptomic levels. The two different transcriptome sets were compared in order to see what is different in tolerant potato variety that gives it an advantage over water limiting conditions, and to observe metabolic pathways uniquely activated only in the tolerant variety. The identification of metabolic pathways that are uniquely down-regulated in sensitive variety was also studied to observe the reasons behind its sensitivity.

3.6 Verifying gene expression with real – time PCR (qRT - PCR)

Selected transcripts were used to verify gene expression level by qRT-PCR and primers designed according to Primer3 program. This part of the thesis provides verification of sequencing results only.

3.6.1 cDNA synthesis

In this step cDNA was constructed from mRNA using Omniscript Reverse Transcription (Omniscript RT Kit, Catalog No: 201511) with the following procedure;

1. RNA samples were incubated at 65 ^{o}C for 5 min
2. Total mix was prepared by adding chemicals (RT buffer, dNTP mix, RNAse inhibitor, Omniscript RT, Oligo dT primer, dH_2O). (Table 3.1.) Total mix was distributed to tubes equally, and then RNA samples were added.
3. Samples were incubated at 37 ^{o}C for one hour.

4. Then inactivated at 70 ^{o}C for 10 min.

Table 3.1. Chemicals used in constructing cDNA synthesized from mRNA (given amounts are for one cDNA synthesis)

Chemicals	Amount(µL)	Concentration
RT Buffer	2	10X
dNTP mix	2	5 µM
Oligo dT primer	2	10 µM
RNAse inhibitor	0.25	40 µM
OmniScript RT	1	
RNA	5	
dH_2O	7.75	100 ng/µL
Total volume	20	

3.6.2. qRT-PCR

The cDNA synthesized from mRNA using chemicals in (Table 3.2.) and PCR conditions (Table 3.3.) and primers (Table 3.4)

Table 3.2. PCR content

Chemicals	Amount (µL)
Total mix (QIAGEN)	5.0
F Primer (2 µM)	0.4
R Primer (2 µM)	0.4
dH_2O	1.7
cDNA	2.5
Total volume	20

Table 3.3. PCR conditions

Step	Function	Temperature (°C)	Duration (H:m:s)	Cycle
1	Initial Denaturation	94	0:2:00	No
2	Denature	94	0:01:00	30
3	Anneal	60	0:00:15	30
4	Extend	72	0:00:20	30
5	Final extension	4	∞	No

Table 3.4. List of primers used in PCR verification

Primer name	Left primer	Right primer
BAG primer	CGGAGATGGGAGCCTCTGAA	CGCCGTGCATGTATCCTCAC
Super primer	CGCCCACTCAATCTTCACCA	CCCATGAAGTCCAGGAGCAA
Plastidal primer	CATGCAGGTCCCAGGGGTAG	TGCCCAAAGGATTTGGCATT
Diphospate primer	CAGAGGCGCTATGGTGGACA	AAGACATGCCCGGGAACTTG
DOPA primer	TGCGCCATATAAACCTGAACCA	TCCCAAGCCAATCCATGACA
POZ primer	GCAAGGGGATTCAAGCGGTA	CAGGTCAAGCCTGCAAGCAA
bHLH primer	TGGGTGGAAGCCCTAACTGG	TCAGGCTGGTTCAGGAACGTC
MYB primer	GATTGATTGCCGGATGTCAGC	GCGGCGACGATTTTTCACTT
Homebox primer	TTTGCCCCTGCCTGTTCTTC	GCGAGGCTGCAAACCAAGTT
Ascorbate primer	ACCTTGGGAAGGGCACACAA	TCCCAGCTCTTCCGATCACC
Heat primer	ACCGTCGCCGTTTAAAGCAA	GCTTATCACCAGGCCCAGGA
Early primer	TTGGACGAGCCAGCATCAAG	TTGGCTTTGGCATGCTCAGT
LHY primer	TGCAAAACCCAGCAGCACAT	GGTTCCTGAGCATGGGGAGA
GAST primer	CGTGATGAGCAGCAGCAACA	TTGGGCCACCTCTCTTGGTC

Table 3.4. (continue) List of primers used in PCR verification

Rubisco	TGCTGCAGCTGCTGAGGAAT	GTTGCCATCCAGGCAAAACC
Auxin primer	GCCGACGGTACGGAAAGAGA	GCCCACAACTCGCAGAGAAAA
Lea primer	GGAATTGGGTCTCGCCATTG	GCGCGCAGCACAAAATAAGA
Ethylene primer	GACAGCTCCGCCGGTTCTAA	GCACCAGATTTTCCGGCATC
Cryptochrome primer	CAGGGGTGGAACTCGGAATG	GCAAGGCCCCTTTCCCTTTA
RF2a primer	CGAAGAATGAGCCCGGAGAA	TGAGCCAAAGCTGCAATTCG
AP2 primer	CGGATGGGGAGTGGAACAAG	TCAACAATCTCCGCCTTGGA
EF primer	GGACCCAACTGGTGCCAAAG	CTCGCCACCGCCTATCAAGT

CHAPTER IV

RESULTS

4.1 Plant growth and drought treatment

Two potato varieties were used to perform drought treatment, Unica (CIP code: N^{o} 392797.22) as a drought tolerant variety were chosen for its adaptation to warm and dry environment in comparison to other *S.tuberosum* L. cultivars (Cabello et al., 2012; Ramirez et al., 2015). Russet Burbank is a cultivar having late maturing characteristic and is considered as profoundly sensitive to water deficit (Stark et al., 2013).

4.2 Drought treatment and sample collection

Russet Burbank and Unica potato varieties with single eye were planted to perform the experiment. Each treatment had 4 repeats, and each repeats had 6 pots (volume: 12 L). The pots were daily watered at the soil saturation level. Tuber initiation and tuber bulking starting at 30-50 days after germination depending on the genotypes are the most sensitive period of drought stress in potato (Lahlou et al., 2003). Based on this information, tuber initiation and tuber bulking periods were regularly controlled and drought treatment was started 45 days after germination (Figure 4.1).

Figure 4.1. Tuber initiation stages after 45 days of emergence

Based on daily observations of physiological characters, 23 days of drought treatment was performed. Figure 4.2 shows the plant status after drought treatment compared to control ones.

Unica (Control: left, Stress: right)

Russet Burbank (Control: left, Stress: right)

a b

Figure 4.2. Unica (a) and Russet Burbank (b) status after stress compared to control

4.2.1 Stomatal conductance

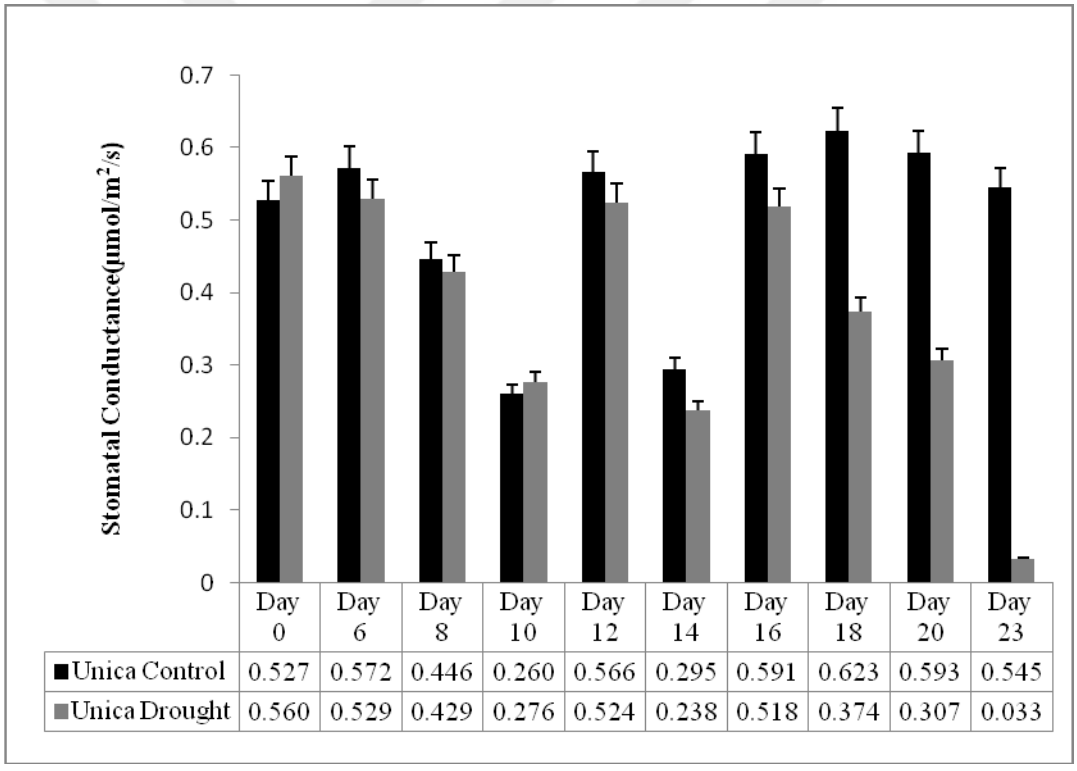
Stomatal conductance was measured 45 days after plant seedling in Russet Burbank and Unica potato varieties on days 0, 6, 8, 10, 12, 14, 16, 18, 20 and 23 both in control and drought conditions. The stomatal conductance measurements are given at Figures 4.3. and 4.4.

The stomatal conductance in Russet Burbank potato variety in control condition was measured as follow:

0.482 µmol/m^2/s on day 0, 0.377 µmol/m^2/s on day 6, 0,332 µmol/m^2/s on day 8, 0.212 µmol/m^2/s on day 10, 0.425 µmol/m^2/s on day 12, 0.241 µmol/m^2/s on day 14, 0.415 on µmol/m^2/s on day 16, 0.371 µmol/m^2/s on day 18, 0.342 µmol/m^2/s on day 20, 0.464 µmol/m^2/s on day 23, whereas the measured amounts in Russet Burbank potato variety in drought condition were:

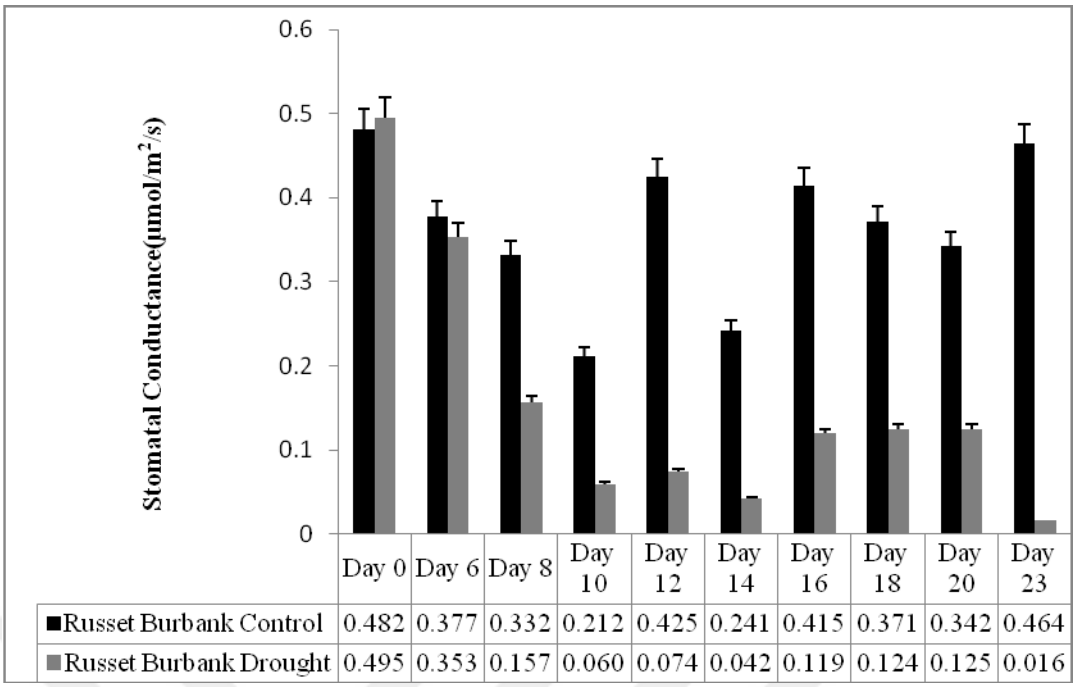
0.495 µmol/m2/s on day 0, 0.353 µmol/m2/s on day 6, 0.157 µmol/m2/s on day 8, 0.060 µmol/m2/s on day 10, 0.074 µmol/m2/s on day 12, 0.042 µmol/m2/s on day 14, 0.119 µmol/m2/s on day 16, 0.124 µmol/m2/s on day 18, 0.125 µmol/m2/s on day 20, 0.016 µmol/m2/s on day 23. Stomatal conductance has been started decreasing on day 6 in

drought condition in Russet Burbank potato variety whereas; stomatal conductance was decreased on day 8 in drought condition in Unica potato variety. As the intensity of stress increasing in the Russet Burbank potato variety under drought condition, stomatal conductance decreasing gradually to the lowest level whereas; stomatal conductance in Unica potato variety under drought treatment maintain to average level except on 23rd day. According to the obtained information in this experiment, decreasing of stomatal conductance under drought stress may lead the plants to control the water loss by stomatal closure mechanism. Therefore; under drought stomata close in proportion to scales of stress, continuously lessen CO_2 accessibility in chloroplast. CO_2 assimilation is reduced and the CO_2/O_2 proportion drops, leads to the increase of photorespiration (Merdano et al., 2002).



	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 23
Unica Control	0.527	0.572	0.446	0.260	0.566	0.295	0.591	0.623	0.593	0.545
Unica Drought	0.560	0.529	0.429	0.276	0.524	0.238	0.518	0.374	0.307	0.033

Figure 4.3. Unica's stomatal conductance during 23 days of drought treatment



	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 23
Russet Burbank Control	0.482	0.377	0.332	0.212	0.425	0.241	0.415	0.371	0.342	0.464
Russet Burbank Drought	0.495	0.353	0.157	0.060	0.074	0.042	0.119	0.124	0.125	0.016

Figure 4.4. Russet Burbank's stomatal conductance during 23 days of drought treatment

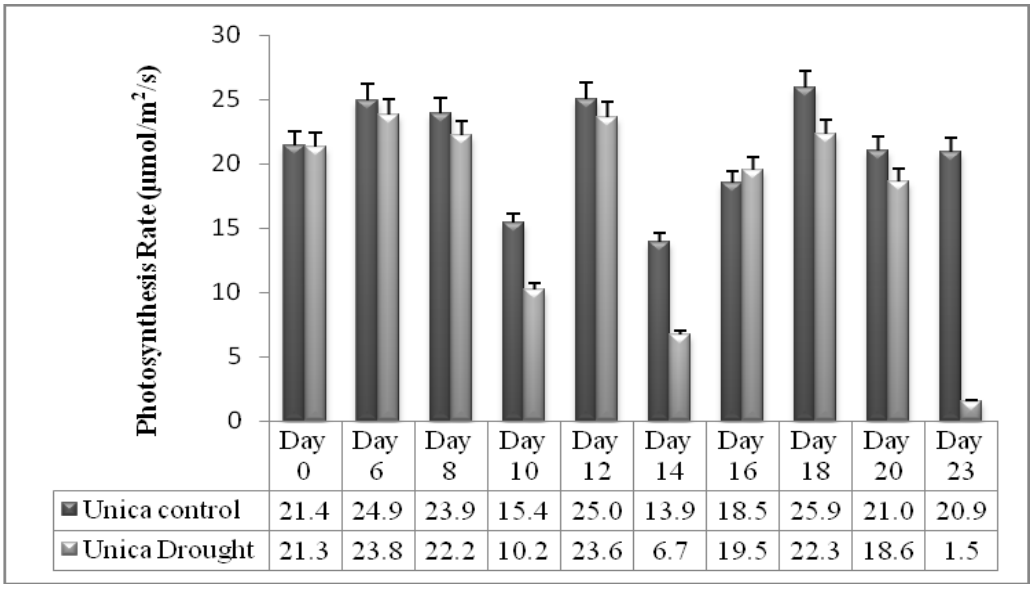
4.2.2 Photosynthesis rate

Photosynthesis rates were measured 45 days after plant seedling in both control and drought stress of Russet Burbank and Unica potato varieties on days 0, 6, 8, 10, 12, 14, 16, 18, 20 and 23. The measurements are given at figures 4.5. and 4.6.

The photosynthesis measurements in Russet Burbank control variety were as below: 18.3 µmol/m^2/s on 0 day, 19.9 µmol/m^2/s on day 6, 20.7 µmol/m^2/s on day 8, 16.6 µmol/m^2/s on day 10, 20.5 µmol/m^2/s on day 12, 15.5 µmol/m^2/s on day 14, 15.5 µmol/m^2/s on day 16, 21.4 µmol/m^2/s on day 18, 17.9 µmol/m^2/s on day 20, 21.0 µmol/m^2/s on day 23, whereas in Russet Burbank drought potato variety were as follow: 19.4 µmol/m^2/s on day 0, 19.5 µmol/m^2/s on day 6, 15.4 µmol/m^2/s on day 8, 7.9 µmol/m2/s on day 10, 10.2 µmol/m^2/s on day 12, 4.2 µmol/m^2/s on day 14, 9.5 µmol/m^2/s on day 16, 16.2 µmol/m^2/s on day 18, 13.1 µmol/m^2/s on day 20, 0.9 µmol/m^2/s on day 23.

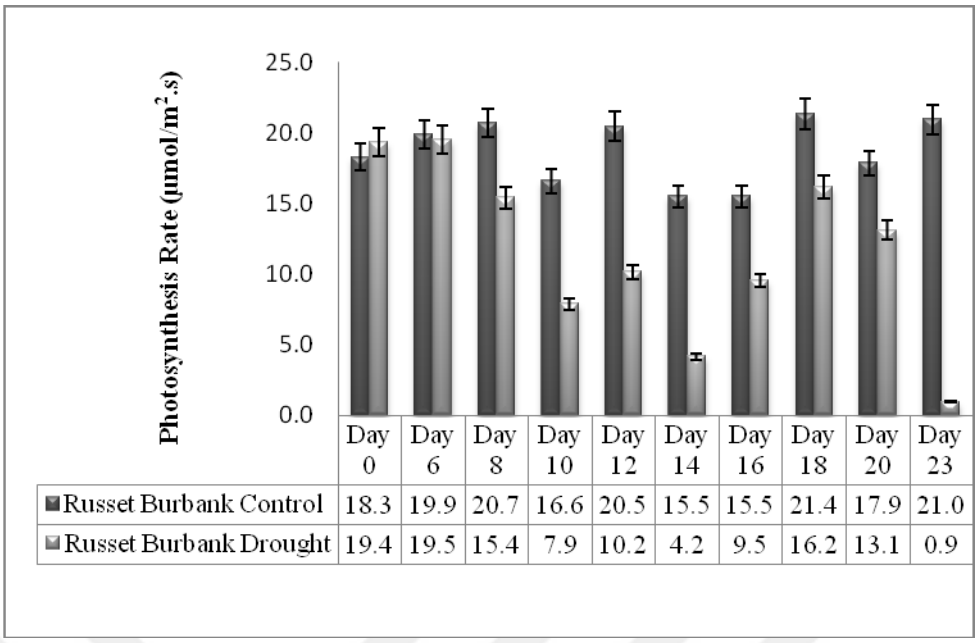
The photosynthesis rates in Unica control potato variety were measured as below: 21.4 µmol/m^2/s on day 0, 24.9 µmol/m^2/s on day 6, 23.9 µmol/m^2/s on day 8, 15.4 µmol/m^2/s on day 10, 25.0 µmol/m^2/s on day 12, 13.9 µmol/m^2/s on day 14, 18.5 µmol/m^2/s on day 16, 25.9 µmol/m^2/s on day 18, 21.0 µmol/m^2/s on day 20, 20.9 µmol/m2/s on day 23,

whereas the amounts in Unica drought potato variety were as follow: 21.3 µmol/m^2/s on day 0, 23.8 µmol/m^2/s on day 6, 22.2 µmol/m^2/s on day 8, 10.2 µmol/m^2/s on day 10, 23.6 µmol/m^2/s on day 12. 6.7 µmol/m^2/s on day 14, 19.5 µmol/m^2/s on day 16, 22.3 µmol/m^2/s on day 18, 18.6 µmol/m^2/s on day 20, 1.5 µmol/m^2/s on day 23. Figure 4.4 shows in Russet Burbank drought potato variety on day 8 photosynthesis rate decreased by 20 %, on day 12 in control condition photosynthesis rate has increased 12 % , while on the same day in drought condition photosynthesis rate decreased 48 %. In Unica potato variety photosynthesis rate has been started decreasing on day 10 in drought condition. Among abiotic stresses, photosynthesis is the fundamental case to be affected by drought (Chaves, 1991). The consequence can be direct, as the decreased CO_2 accessibility originated by diffusion limitation via the stomata and the mesophyll (Flexas et al., 2004, 2007) or changes in photosynthetic metabolism (Lawlor and Cornic, 2002) or they can motivate increase of secondary effects, known as oxidative stress. Finally, drought can actively affect leaf photosynthetic machinery (Ort, 2001). Photosynthetic response to drought is one of the most complex processes. It includes the interaction of limitations occurring at different parts of the cell/leaf and at various time scales regarding to plant development. Data obtained in this experiment indicates that Unica potato variety under drought stress after 14th day of drought treatment behaved more different from Russet Burbank under drought variety.



	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 23
Unica control	21.4	24.9	23.9	15.4	25.0	13.9	18.5	25.9	21.0	20.9
Unica Drought	21.3	23.8	22.2	10.2	23.6	6.7	19.5	22.3	18.6	1.5

Figure 4.5. Unica's photosynthesis rate during 23 days of drought treatment

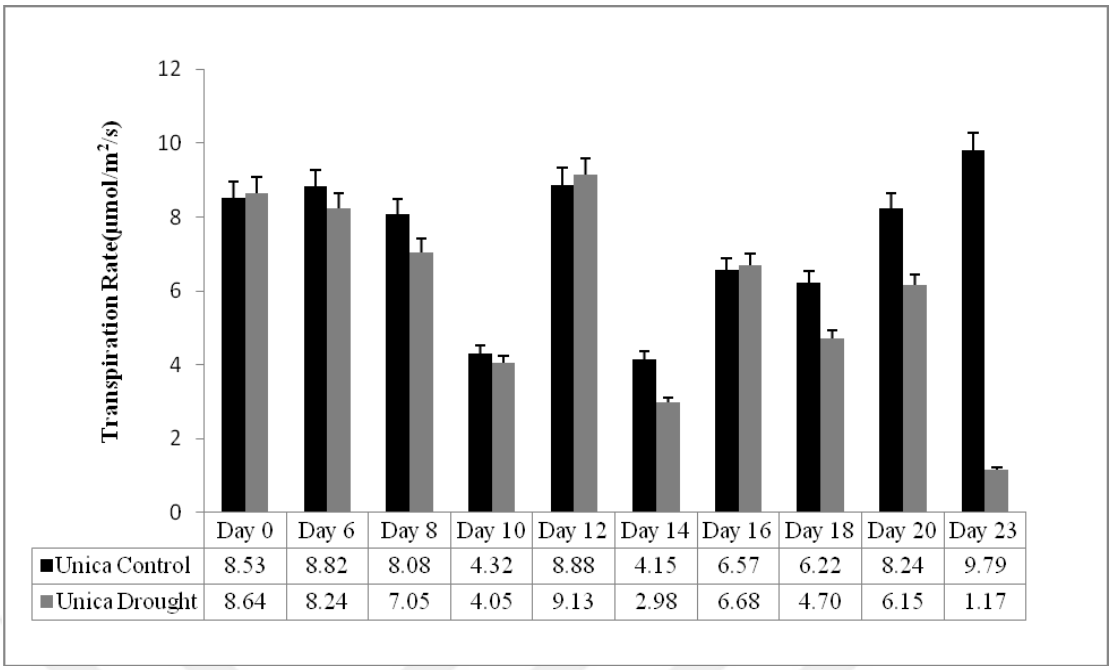


	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 23
Russet Burbank Control	18.3	19.9	20.7	16.6	20.5	15.5	15.5	21.4	17.9	21.0
Russet Burbank Drought	19.4	19.5	15.4	7.9	10.2	4.2	9.5	16.2	13.1	0.9

Figure 4.6. Russet Burbank's photosynthesis rate during 23 days of drought treatment

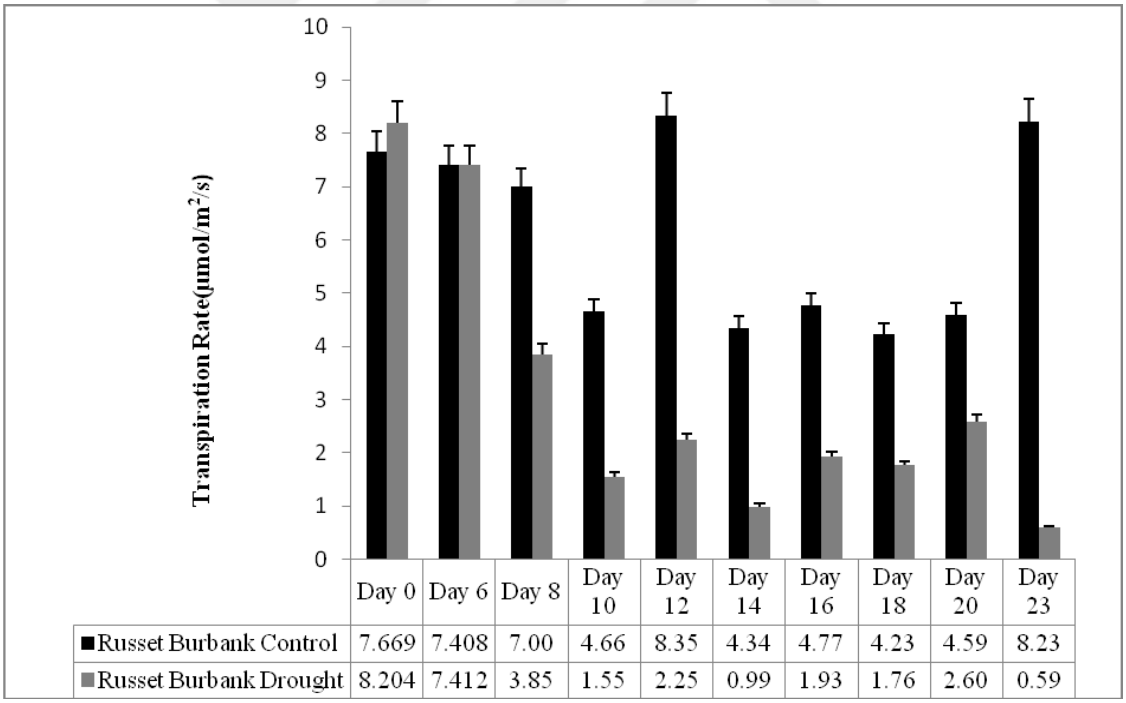
4.2.3 Transpiration rate

Transpiration rates were measured 45 days after plant seedling from 3rd completely developed apical leaflet of main stem on 4 individual plants from Russet Burbank and Unica potato varieties on days 0, 6, 8, 10, 12, 14, 16, 18 ,20 and 23 in both control and drought conditions. The measurements are given at Figures 4.7. and 4.8. Transpiration rates have been started decreasing on day 6 in Russet Burbank potato variety in drought condition. It has increased in Russet Burbank control by 9 %, whereas it has decreased on same day in Russet Burbank potato variety under drought condition by 73 %. It has increased in Unica potato variety under control condition by 3%, whereas it has decreased in Unica potato variety under drought condition by 5 %. Normally, by decreasing water availability, the root:shoot ratio of plants increases due to less sensitivity of roots than shoots to growth inhibition by low water potentials (Wu and Cosgrove, 2000). Under drought stress treatment roots induce a signal cascade to the shoots through xylem leading physiological alterations finally determining the level of tolerance to the stress. Abscisic acid, ethylene, and other unidentified factors have been involved in the root-shoot signaling. This drought induced root to leaf signaling through the transpiration steam results in stomatal closure, which is an important adjustment to limited water supply in the field.



	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 23
Unica Control	8.53	8.82	8.08	4.32	8.88	4.15	6.57	6.22	8.24	9.79
Unica Drought	8.64	8.24	7.05	4.05	9.13	2.98	6.68	4.70	6.15	1.17

Figure 4.7. Unica's transpiration rate during 23 days of drought treatment



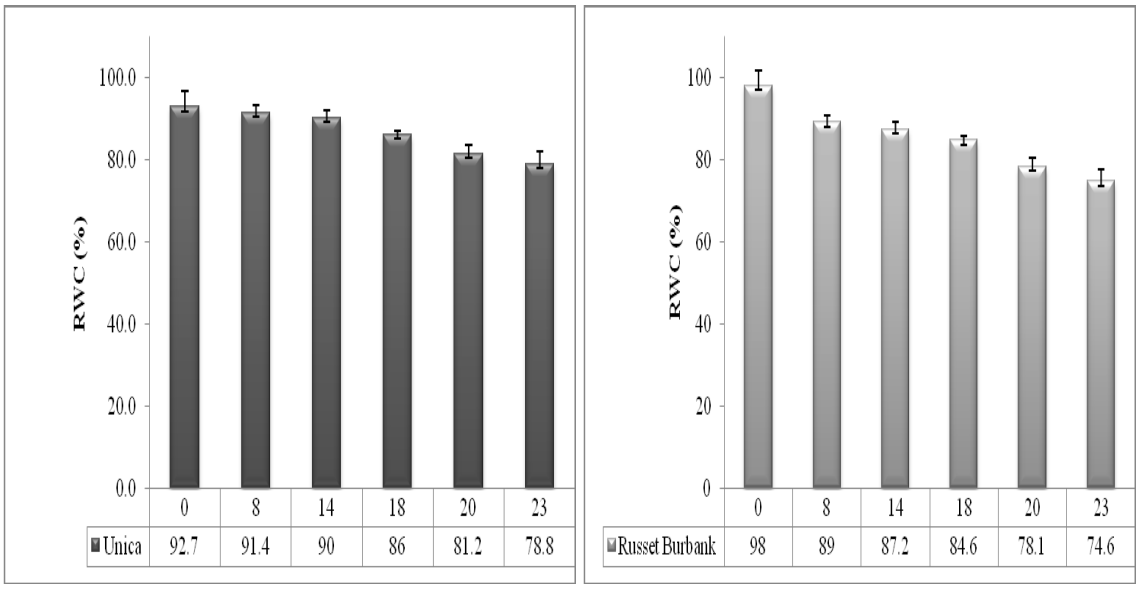
	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 23
Russet Burbank Control	7.669	7.408	7.00	4.66	8.35	4.34	4.77	4.23	4.59	8.23
Russet Burbank Drought	8.204	7.412	3.85	1.55	2.25	0.99	1.93	1.76	2.60	0.59

Figure 4.8. Russet Burbank's transpiration rate during 23 days of drought treatment

4.2.4 Relative water content

Relative water content was measured in drought stress implemented samples during 23 days of drought treatment. For RWC measurement, leave discs were taken from the apical leaflet of the 3rd completely expanded leaf on days 0, 8, 14, 18, 20, 23 of drought treatment. RWC measurements are given in Figures 4.9.a and 4.9.b.

RWC measurements in Russet Burbank potato variety were as follow: 98 % on day 0, 89 % on day 8, 87.2 % on day 14, 84.6 % on day 18, 78.1 % on day 20, 74.6 % on ay 23. RWC measurements in Unica potato variety were as follow: 92.7 % on day 0, 91.4 % on day 8, 90 % on day 14, 86 % on day 18, 81.2 % on day 20, 78.8 % on day 23. On day 8 in Russet Burbank RWC decreased 9 % while in Unica it decreased by 1 %. On day 18 in Russet Burbank RWC decreased 14 %, whereas the amount in Unica decreased to 7 %. On day 23 RWC decreased 24 %, while the amount in Unica decreased to 15 %. RWC depends on water uptake by the roots as well as water loss by transpiration. It has been reported by (Nayyar and Gupta, 2006) that decrease in RWC occurring in a broad range of plants in response to drought stress.



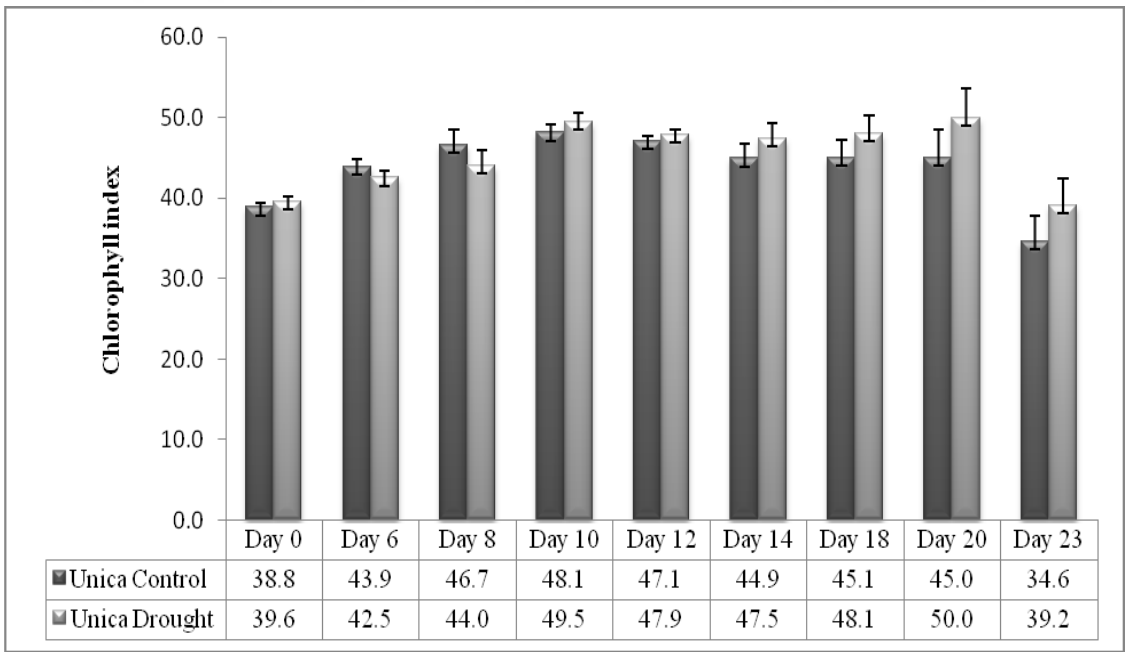
a b

Figure 4.9. RWC measurements Unica (a) of Russet Burbank (b) and varieties during drought treatment

4.2.5 Chlorophyll index

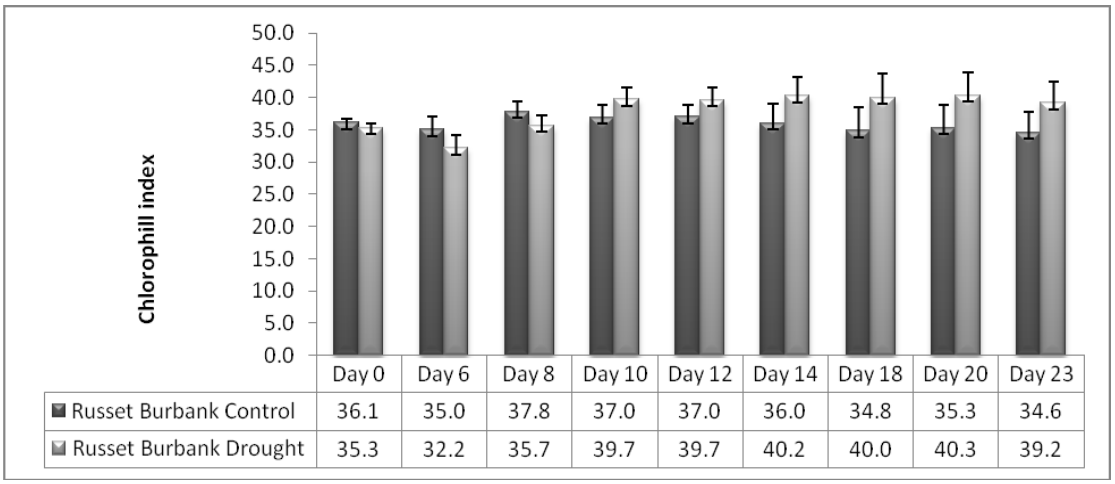
Chlorophyll indexes (SPAD values) were measured in both Unica and Russet Burbank potato varieties on days 0, 6, 8, 10, 12, 14, 16, 18, 20 and 23rd day. The measurements are given at figures 4.10 and 4.11.

Chlorophyll indexes on day 6 in Russet Burbank control decreased by 3%, whereas it decreased in Russet Burbank in drought condition by 9 %. It was reached to the same level on day 14 in Russet Burbank control, whereas it decreased in Russet Burbank under drought condition by 14 %. On day 20, it decreased in Russet Burbank control by 2 %, whereas it increased in Russet Burbank under drought conditions by 14 %. Chlorophyll content increased on day 6 in Unica potato variety both in control and drought conditions. On day 20, it increased 16 % in Unica control and 26 % in Unica drought. The decrease in chlorophyll content under drought stress has been investigated a common symptom of oxidative stress and may be the consequence of pigment photo-oxidation and chlorophyll degradation. The importance of photosynthetic pigments for the plants is basically on harvesting light and production of reducing powers. Chlorophyll a and b are both leaning to soil dehydration (Farooq et al., 2009).



	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 18	Day 20	Day 23
Unica Control	38.8	43.9	46.7	48.1	47.1	44.9	45.1	45.0	34.6
Unica Drought	39.6	42.5	44.0	49.5	47.9	47.5	48.1	50.0	39.2

Figure 4.10. Unica's chlorophyll index during 23 days drought treatment



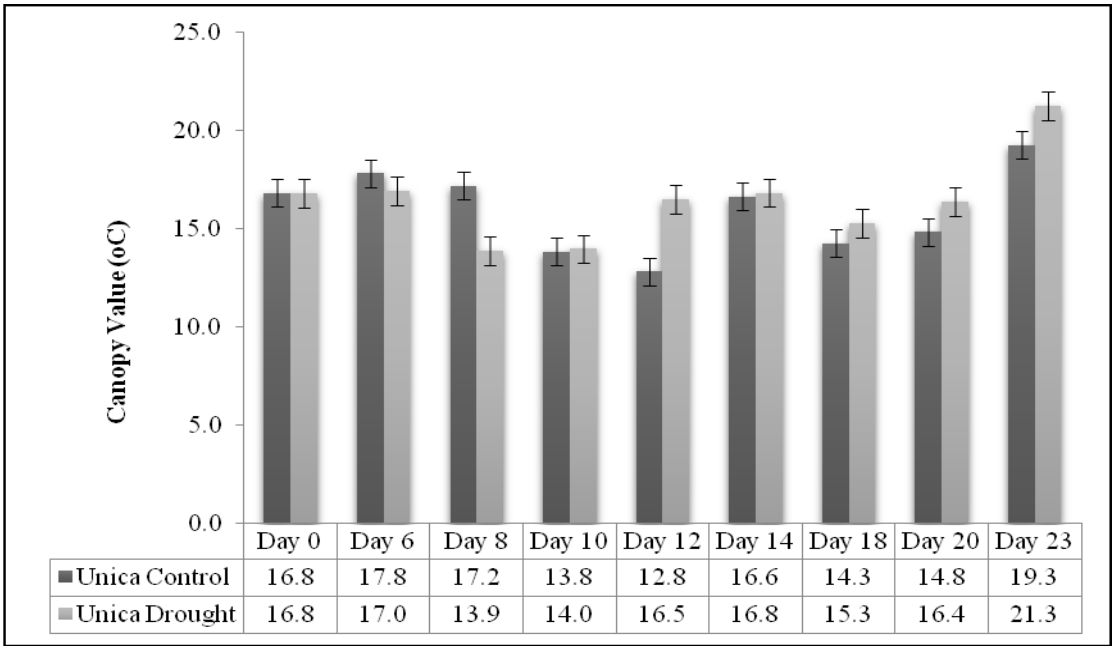
	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 18	Day 20	Day 23
Russet Burbank Control	36.1	35.0	37.8	37.0	37.0	36.0	34.8	35.3	34.6
Russet Burbank Drought	35.3	32.2	35.7	39.7	39.7	40.2	40.0	40.3	39.2

Figure 4.11. Russet Burbank's chlorophyll values during 23 days drought treatment

4.2.6 Leaf temperature

Leaf temperature (canopy) were measured in Russet Burbank and Unica potato varieties on days 0, 6, 8, 10, 12, 14, 16, 18, 20 and 23 in drought conditions. The measured leaf temperature is given at Figure 4.12 and 4.13.

Leaf temperature increased under drought stress that might have happened due to increased respiration and decreased transpiration as a sequence from stomatal closure (Siddique et al., 2000).



	Day 0	Day 6	Day 8	Day 10	Day 12	Day 14	Day 18	Day 20	Day 23
Unica Control	16.8	17.8	17.2	13.8	12.8	16.6	14.3	14.8	19.3
Unica Drought	16.8	17.0	13.9	14.0	16.5	16.8	15.3	16.4	21.3

Figure 4.12. Unica's canopy value during 23 day of drought treatment

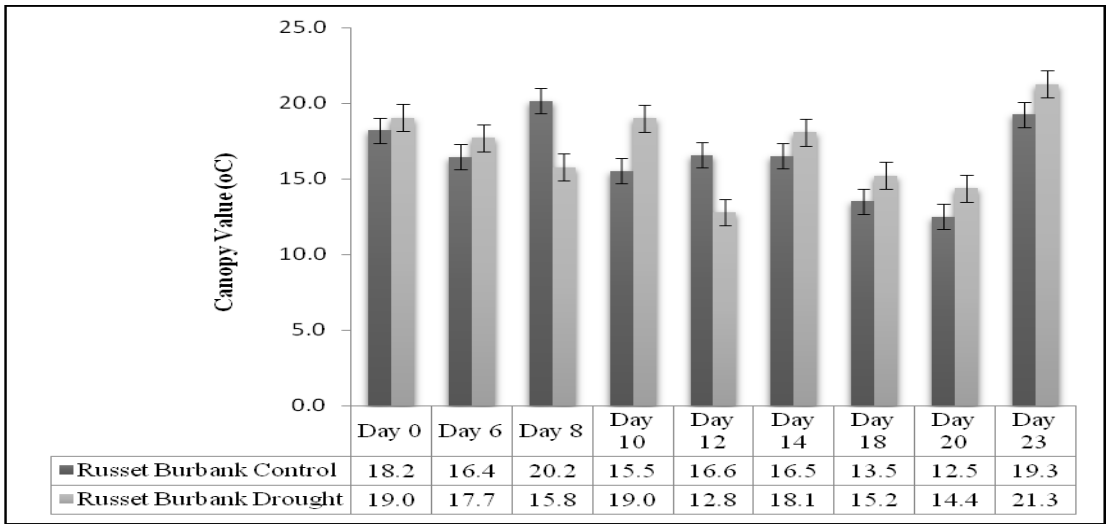


Figure 4.13. Russet Burbank's canopy value during 23 day of drought treatment

4.2.7 Proline content

Proline content measured in Russet Burbank and Unica potato varieties 45 days after plant seedling at 23rd day of drought treatment. The results are given at Figure4.14 and 4.15. Proline content in Russet Burbank increased 10.8% more than Unica under drought condition. It is known that plants accumulate various types of organic and inorganic solutes in the cytosol to minimize osmotic potential for the purpose of cell turgor (Rhodes and Samaras, 1994). Proline is one of the solutes that most extensively studied regarding its importance in stress tolerance. Proline accumulation is the primary response of plants for the purpose of decreasing injury to the cell. The proline content increase as the drought stress developed (Anjum et al., 2011). Proline can play as signaling molecules to modulate mitochondrial functions, influence cell proliferation and trigger specific gene expression, which can act a vital role for plant recovery from stress (Szabados and Savoure, 2009). Proline accumulation in many plant species has been associated to stress tolerance. It effects protein salvation and conserve membrane integrity under dehydration stress (Demiral and Turkan, 2004).

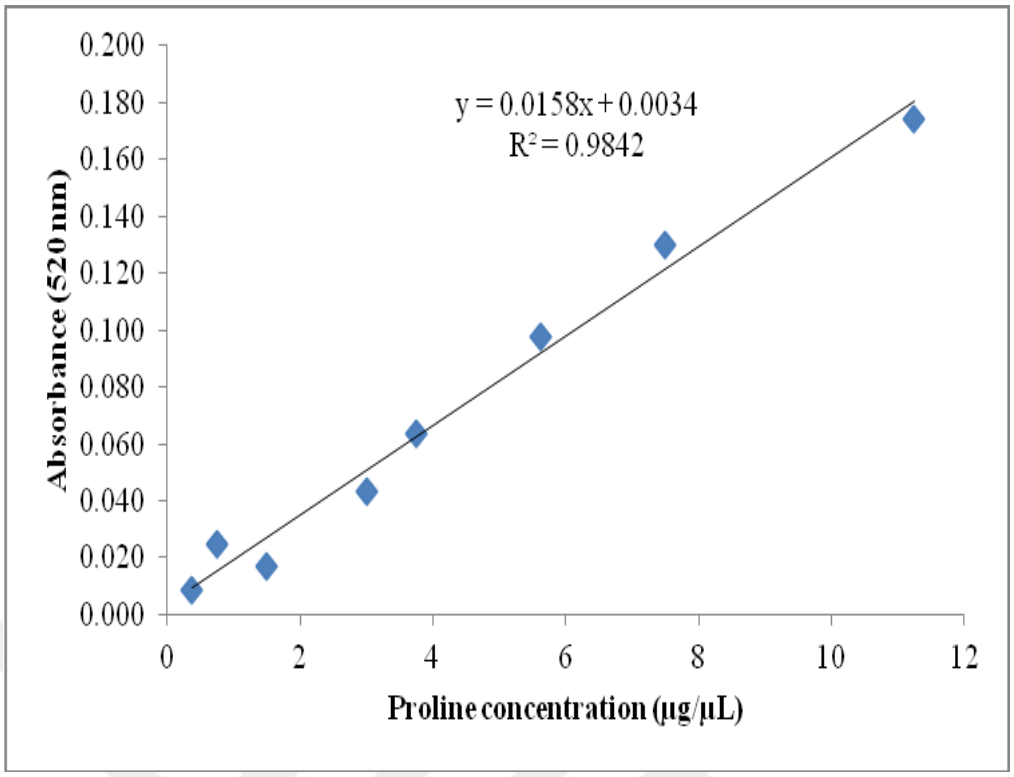


Figure 4.14. Standard curve used in calculation of accumulated proline after drought treatment

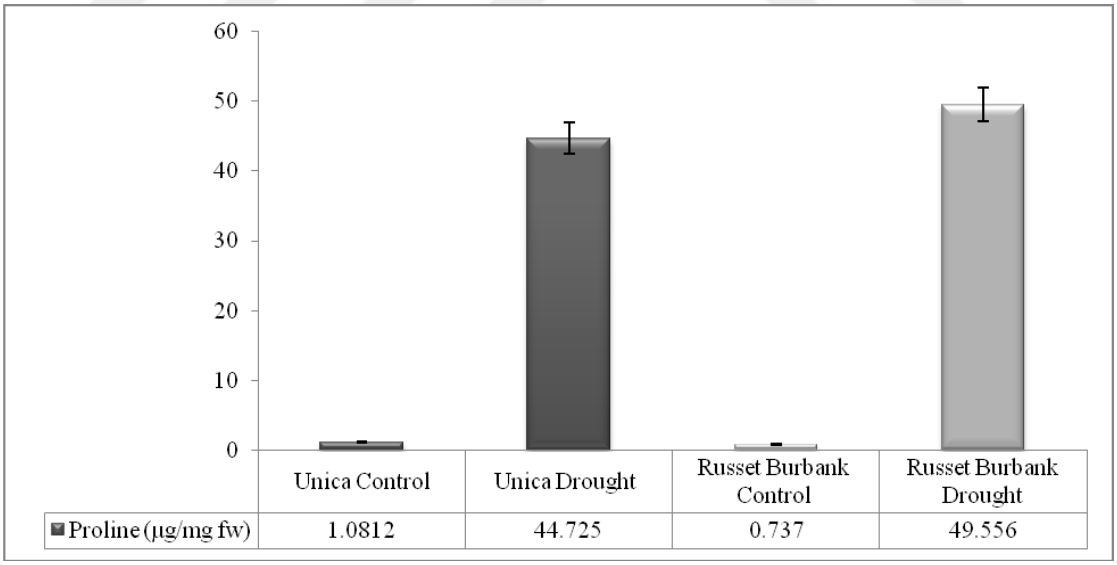


Figure 4.15. Accumulation of proline in Unica and Russet Burbank during 23 days of drought treatment

4.2.8 Results of t-test analysis

The samples were collected on 23rd day of drought treatment to investigate and compare their transcriptome level. Statistical analysis of data indicates that during drought treatment, Unica's photosynthesis rate was higher than Russet Burbank potato variety. The differences in photosynthesis rate between two varieties statistically were significant. The difference on 23rd day of drought treatment was considered insignificant Table 4.1.

During drought treatment, stomatal conductance in Unica was higher than Russet Burbank. The differences in stomatal conductance in two potato varieties were significant statistically Table 4.2.

According to statistical data obtained during drought treatment, transpiration rate was higher in Unica than Russet Burbank potato variety. The differences in transpiration rate between two potato varieties were statistically significant Table 4.3.

Table 4.1. Photosynthesis rate of Unica and Russet Burbank Varieties under drought treatment and t-test analysis results

		Photosynthesis rate ($\mu molm^{-2}s^{-1}$)	Number of observation	Standard deviation	Degrees of freedom	t value	0.05 table value
Day 0	Unica	**21.306**	4	0.54	6	**4.802***	2.447
	Russet Burbank	19.358	4	0.60			
Day 6	Unica	**23.831**	4	0.47	6	**8.760***	2.447
	Russet Burbank	19.549	4	0.86			
Day 8	Unica	**22.214**	4	2.01	6	**3.807***	2.447
	Russet Burbank	15.439	4	2.94			
Day 10	Unica	**13.550**	3	1.03	4	**6.902***	2.776
	Russet Burbank	7.882	3	0.98			
Day 12	Unica	**23.607**	4	1.58	6	**11.103***	2.447
	Russet Burbank	10.162	4	1.83			
Day 14	Unica	**8.896**	3	1.51	5	**4.022***	2.571
	Russet Burbank	4.161	4	1.56			
Day 16	Unica	**19.543**	4	0.19	6	**13.259***	2.447
	Russet Burbank	9.546	4	1.47			
Day 18	Unica	**22.308**	4	4.16	6	**2.740***	2.447
	Russet Burbank	16.181	4	1.64			
Day 20	Unica	**18.646**	4	1.20	6	**6.583***	2.447
	Russet Burbank	13.139	4	1.17			
Day 23	Unica	**1.502**	4	1.62	6	0.363^{N}	2.447
	Russet Burbank	0.943	4	2.61			

Note: * Statistical significance, ^{N} Not important

Table 4.2. Stomatal conductance of Unica and Russet Burbank varieties under drought treatment and t-test analysis results

		Stomatal conductance ($mmolm^{-2}s^{-1}$)	Number of observation	Standard deviation	Degree of freedom	t value	0.05 Table value
Day 0	Unica	**560.41**	4	0.030	6	**3.746***	2.447
	Russet Burbank	495.08	4	0.018			
Day 6	Unica	**528.65**	4	0.036	6	**7.578***	2.447
	Russet Burbank	352.64	4	0.029			
Day 8	Unica	**429.15**	4	0.048	6	**7.436***	2.447
	Russet Burbank	157.01	4	0.056			
Day 10	Unica	**275.95**	3	0.052	4	**6.956***	2.776
	Russet Burbank	59.52	3	0.013			
Day 12	Unica	**524.44**	4	0.082	6	**10.543***	2.447
	Russet Burbank	74.07	4	0.024			
Day 14	Unica	**237.93**	3	0.116	5	**3.475***	2.571
	Russet Burbank	42.18	4	0.009			
Day 16	Unica	**518.25**	4	0.048	6	**12.411***	2.447
	Russet Burbank	119.21	4	0.043			
Day 18	Unica	**374.38**	4	0.112	6	**4.075***	2.447
	Russet Burbank	123.81	4	0.051			
Day 20	Unica	**307.09**	4	0.090	6	**3.988***	2.447
	Russet Burbank	124.73	4	0.017			
Day 23	Unica	**33.39**	4	0.009	6	**2.972***	2.447
	Russet Burbank	16.13	4	0.007			

Note: * Statistical significance, ^{N} Not important

Table 4.3. Transpiration rates of Unica and Russet Burbank varieties under drought treatment and t-test analysis results

		Transpiration rate ($\mu molm^{-2}s^{-1}$)	Number of observation	Standartd deviation	Degree of freedom	t-value	0.05 table value
Day 0	Unica	**8.64**	4	0.06	6	**4.142***	2.447
	Russet Burbank	8.20	4	0.20			
Day 6	Unica	**8.24**	4	0.30	6	**2.508***	2.447
	Russet Burbank	7.41	4	0.58			
Day 8	Unica	**7.05**	4	0.47	6	**5.648***	2.447
	Russet Burbank	3.85	4	1.03			
Day 10	Unica	**4.05**	3	0.73	4	**5.426***	2.776
	Russet Burbank	1.55	3	0.33			
Day 12	Unica	**9.13**	4	0.76	6	**14.121***	2.447
	Russet Burbank	2.25	4	0.62			
Day 14	Unica	**2.98**	3	1.12	5	**3.579**	2.571
	Russet Burbank	0.99	4	0.19			
Day 16	Unica	**6.69**	4	0.42	6	**14.090***	2.447
	Russet Burbank	1.93	4	0.53			
Day 18	Unica	**4.71**	4	1.53	6	**3.664***	2.447
	Russet Burbank	1.76	4	0.49			
Day 20	Unica	**6.15**	4	1.01	6	**6.768***	2.447
	Russet Burbank	2.60	4	0.28			
Day 23	Unica	**1.17**	4	0.27	6	**2.992***	2.447
	Russet Burbank	0.59	4	0.28			

Note: * Statistical significance, ^{N} Not important

4.3 Identification of modified gene expression with NGS and bioinformatics analysis

4.3.1 Total RNA isolation

Total RNA isolation was done from the collected leaf samples of Unica and Russet Burbank both from control and drought treatment, and their quality and concentration were investigated. To evaluate plant's general gene expression profile using transcriptome sequencing, from the same variety and different plants many young leaves were collected and crushed to provide a pool. By this way, it doesn't need to perform biological and repeated sequencing, and the samples were created that are representing all groups from each variety and treatment. The obtained RNA sample's concentration was measured by spectrophotometer (nanodrop) with the mean calculation of two samples concentration (Table 4.4). One µg total RNA was loaded in each well to see the image under UV light and the gel was prepared by 0.5 X TBE, 1.2% agarose gel (Figure 4.16.). The results of isolated total RNA were suitable for NGS both for quality and quantity demands.

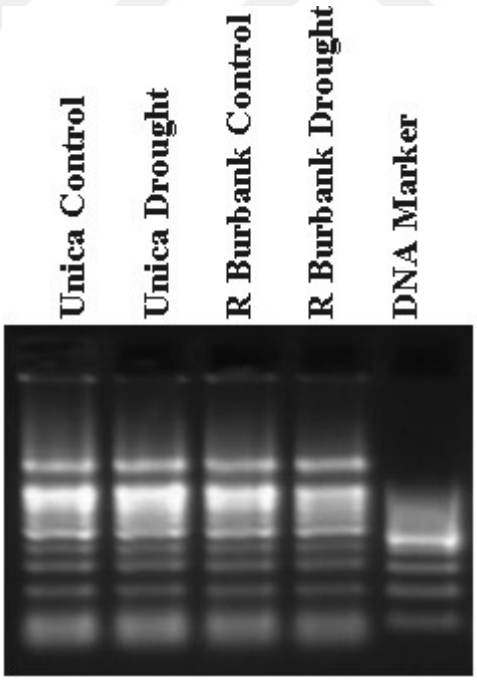


Figure 4.16. Gel images of isolated total RNA under UV, 1.2 % agarose, and 0.5 X TBE

Table 4.4. Total RNA concentration measured by spectrophotometer

Sample	Concentration (ng/µL)	OD260/280	OD260/230	Average Concentration (ng/µL)
Unica Control_1	388.74	2.1	1.27	389.175
Unica Control_2	389.61	2.1	1.29	
Unica Drought_1	212.11	2.07	1.49	212.355
Unica Drought_2	212.6	2.06	1.49	
Russet Burbank Control_1	272.73	2.09	1.31	272.98
Russet Burbank Control_2	273.23	2.09	1.18	
Russet Burbank Drought_1	101.36	2.02	1.09	102.755
Russet Burbank Drought_2	104.15	2.04	0.96	

4.4 Bioinformatics analysis of NGS results

The 23 day drought treated Unica control, Unica drought, Russet Burbank control and Russet Burbank drought leaves (4 libraries) were sequenced by Illumina® HiSeq 2500 system, from both directions, and in total 340,573,814 unique reads were achieved. The details regarding these libraries are in the Table 4.5 at the below. The number of reads in the libraries in total was persuading the demands of read in the same band for four samples in NGS.

Table 4.5. NGS results

Library	Cluster PF (%)	%≥Q30	Total reads
Unica Control (UN_CT)	100	94.07	86.595.313
Unica Drought (UN_DR)	100	93.74	71.038.591
Russet Burbank Control (RB_CT)	100	93.69	96.060.880
Russet Burbank Drought (RB_DR)	100	93.94	86.879.030

Note: * Q30 indicates base calling correct rate with 99.9 % accuracy

Results of NGS were compared to potato reference genome (Potato Genome Sequencing Consortium, 2011), and the obtained total number of the transcripts were classified by their transcript length (Figure 4.17.). The obtained results were in the predicted range and show the suitability of sequencing.

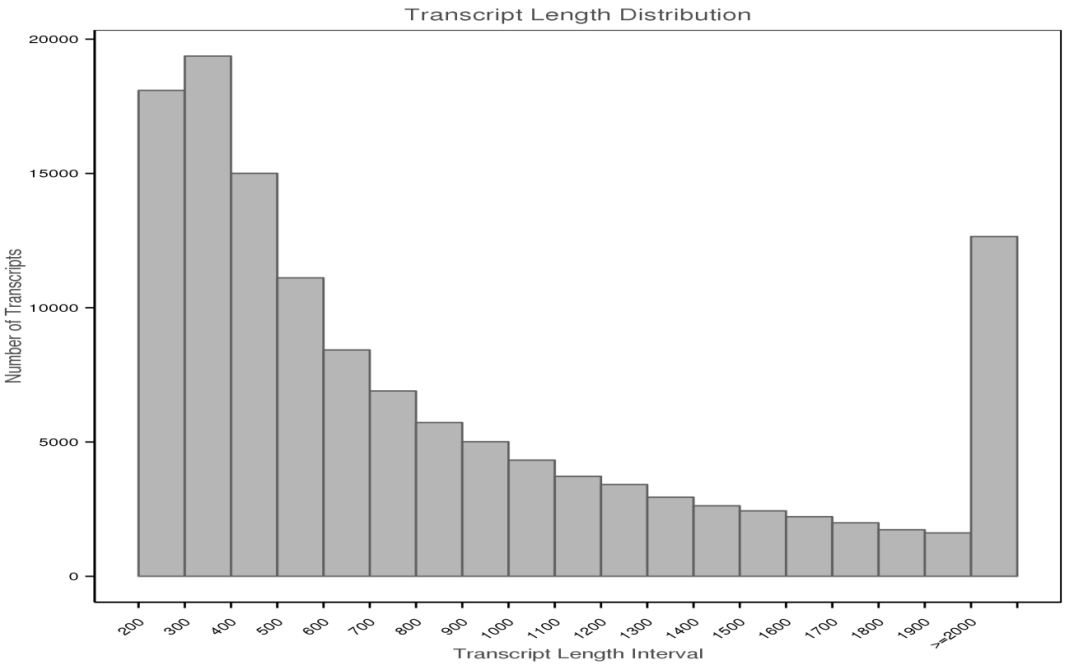


Figure 4.17. Transcripts length distribution

Seven different databases (GO, KO, KOG, NR, NT, PFAM, SwissProt) were used to annotate the obtained unique genes. Figure 4.18 shows annotation distribution regarding these databases. According to Figure 4.19., the highest transcripts annotation was achieved in NCBI_ Nt which is nucleotide database, and Figure 4.21. shows the name of plants with the highest sequence similarity. The topmost sequence similarity is found in *S.tuberosum* L., as the potato genome sequencing has been already completed.

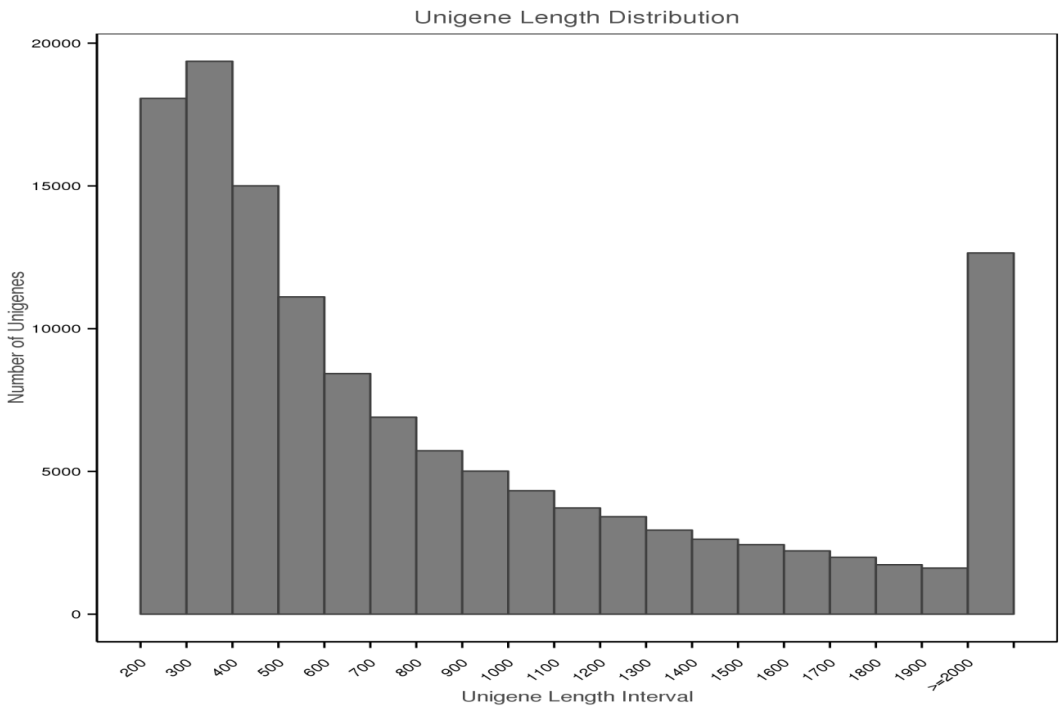


Figure 4.18. Unigene length distributions

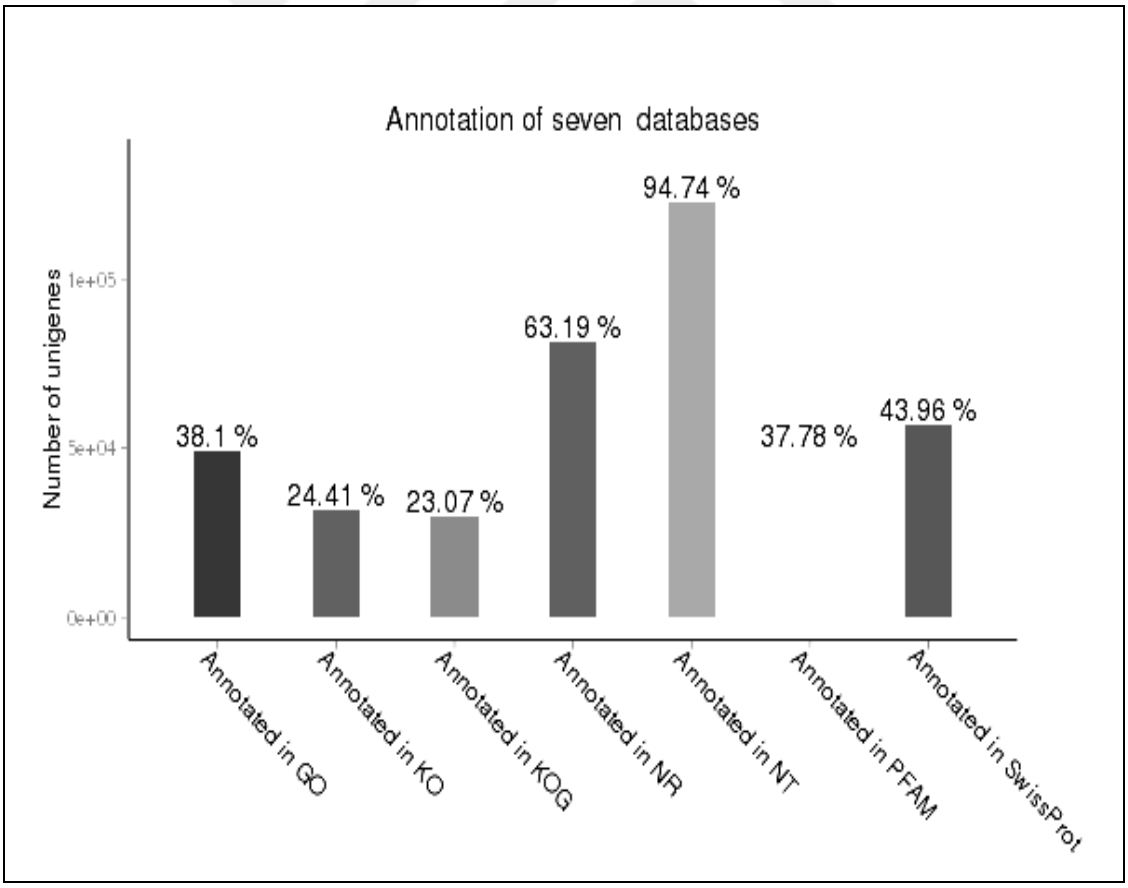


Figure 4.19. Unique transcripts explored by NGS in 7 different databases and their percentages

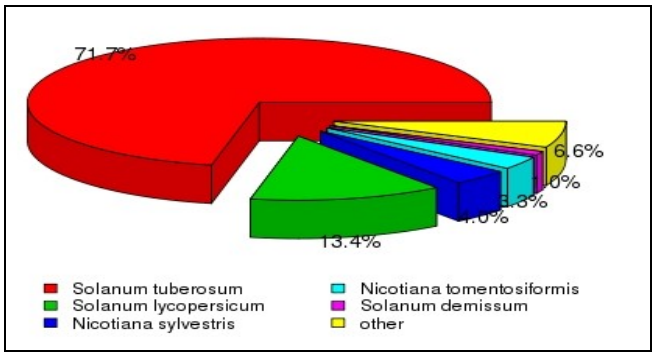


Figure 4.20. The highest annotated sequence similarity between plants species

Go database were used to classify the function of unigenes generated from assembly of all transcriptomes, according to the results of homology, all unigenes were categorized into 57 groups (Figure 4.21.) that consists of three main categories (biological process, cellular component, molecular function). In classification by GO database, most of the genes are involved in cellular functions and metabolic activities, but a significant portion of the transcripts appear to be located in the metabolic pathways of the "response to stimulus" and "signaling" as predicted after drought.

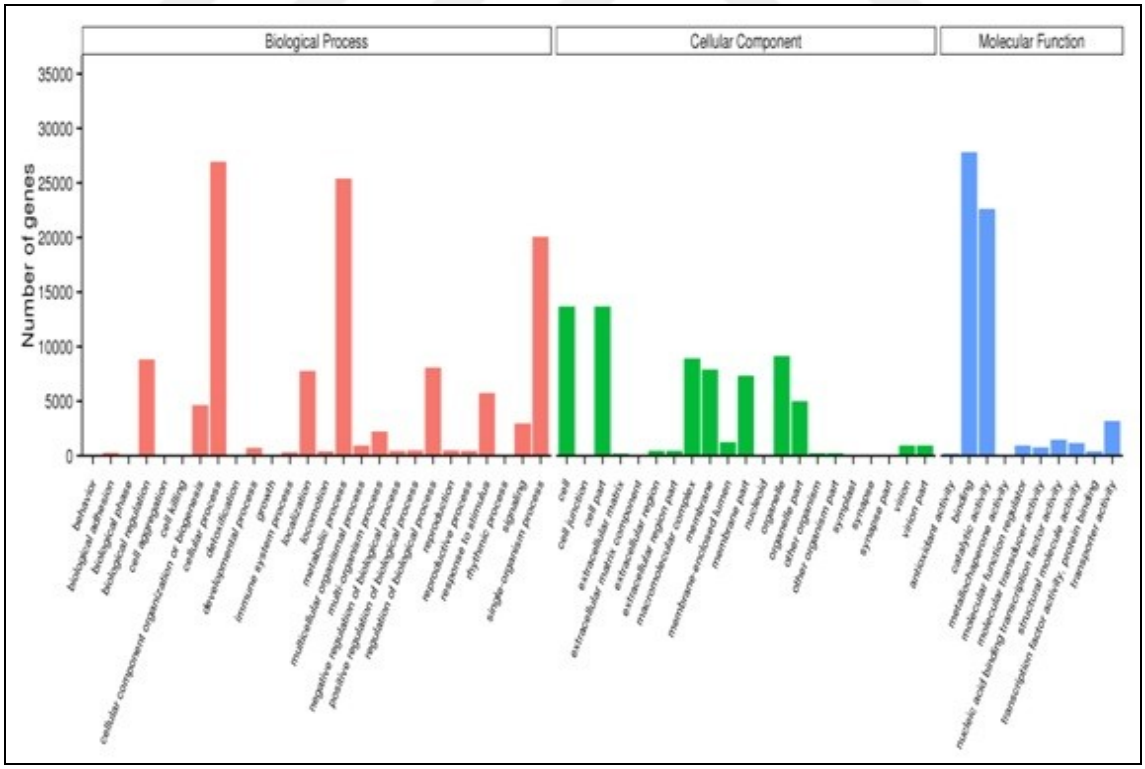


Figure 4.21. Annotated transcripts, their metabolic pathways, their localization inside the cell, and their molecular function based on GO database

To classify the orthologous gene products, unigenes were subdivided into 26 groups (Figure 4.22.), among this classification, the cluster of "signal transduction mechanism" and "defense mechanisms" represented the most important groups.

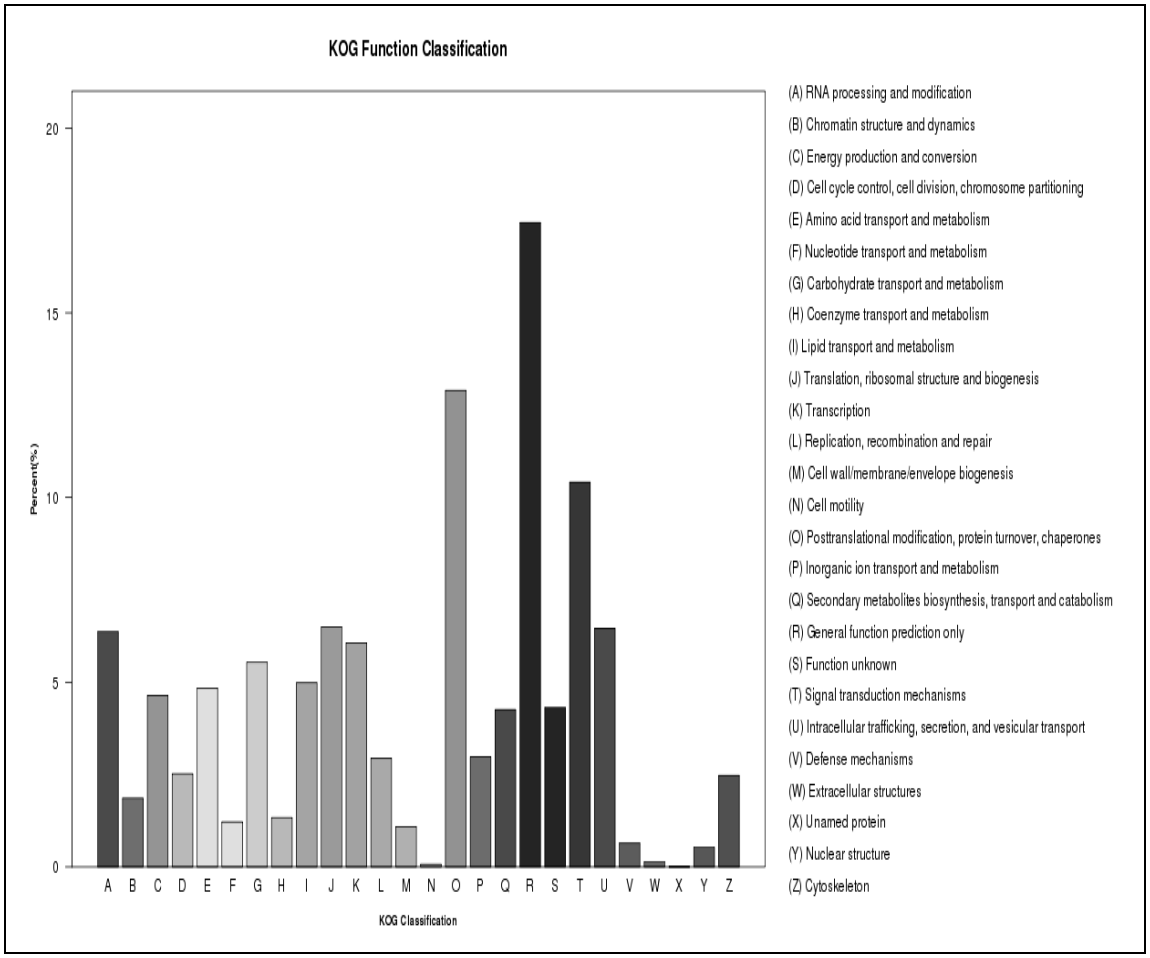


Figure 4.22. Functional classification of obtained transcripts according to KOG database

To identify the biological pathways in the annotated sequences using the Kyoto Encyclopedia of Genes and Genomes, the assembled unigenes were conducted into five specific pathways, involving Cellular Processes(A), Environmental Information Processing (B), Genetic Information Processing (C), Metabolism (D) and Organism Systems (E) (Figure 4.23.). According to the obtained results from the KEGG database, it indicates that high presence of transcripts that play a role especially in "environmental adaptation".

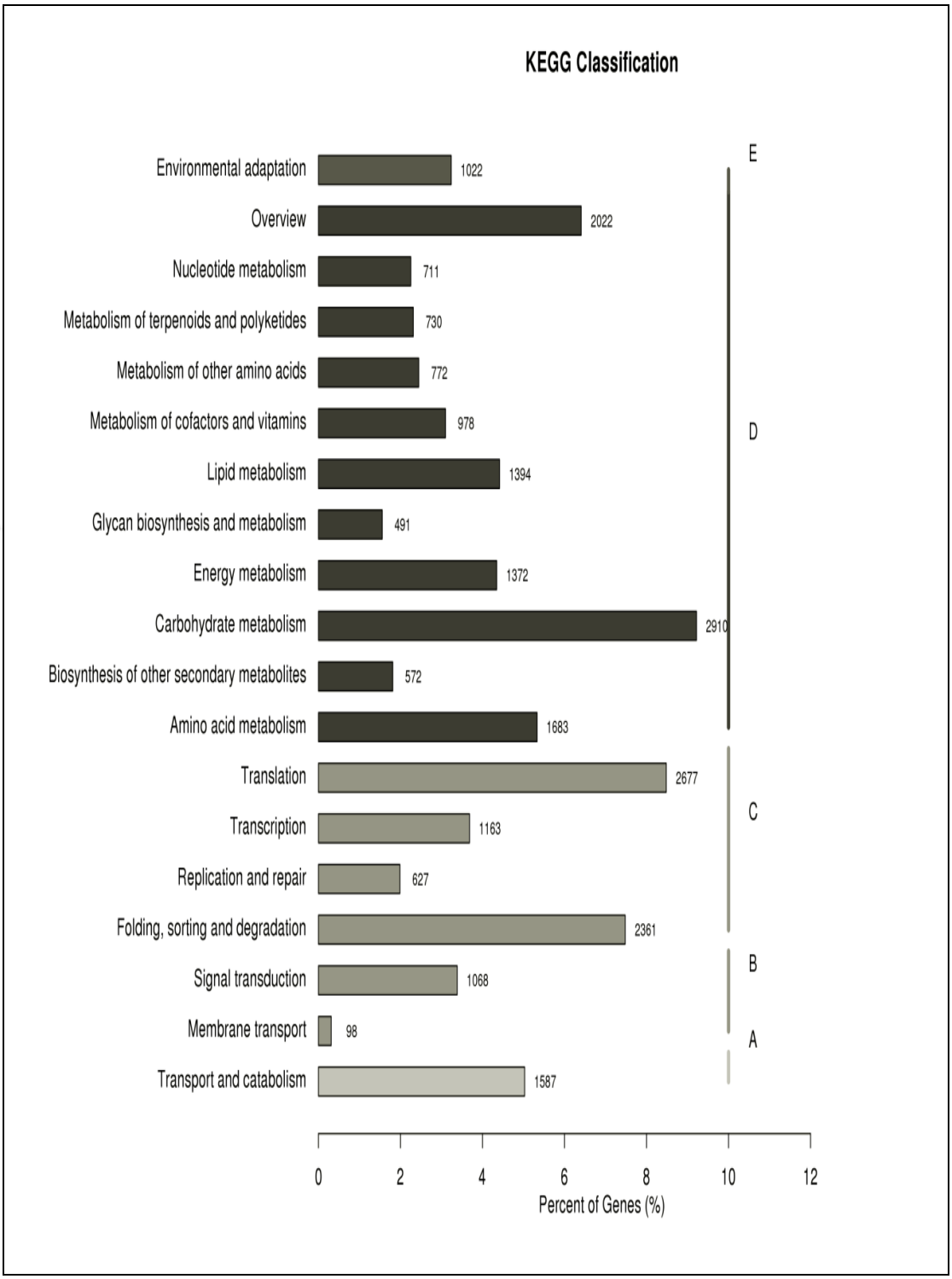


Figure 4.23. Functional classification of obtained transcripts according to KEGG database

FPKM (Fragments per Kilobase Million) is used as important criteria to evaluate next generation results. In Figure 4.24. the values in figure indicating how much of library's transcriptomes are incorporated in all sequence readings at a given depth. Accordingly, it's possible to say that stress treatment caused a change in the expression level of many transcripts.

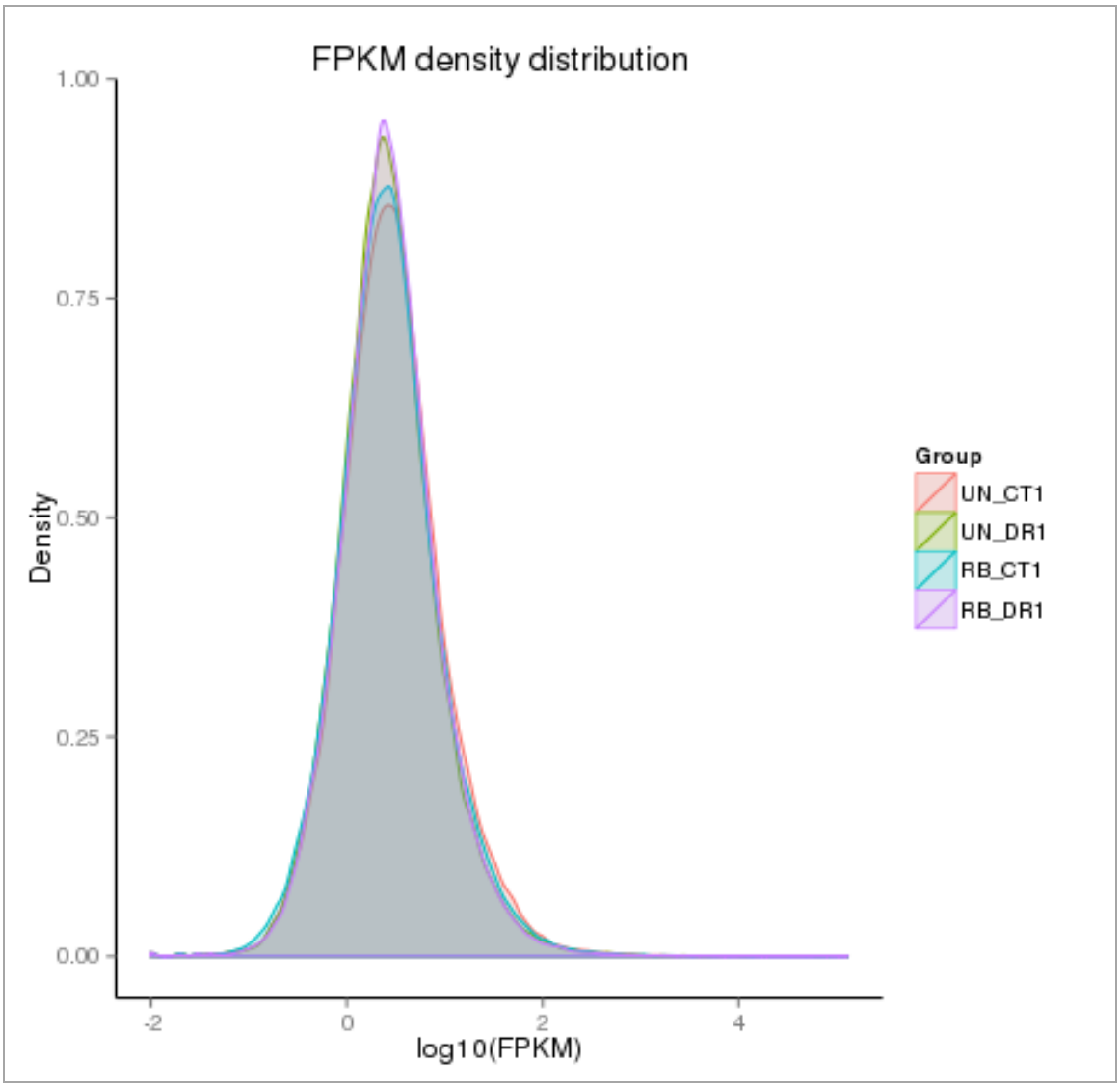


Figure 4.24. FPKM (Fragments per Kilobase Million) graphic of Unica control (UN_CT1), Unica drought (UN_DR1), Russet Burbank control (RB_CT1) and Russet Burbank drought (RB_DR1) libraries

NGS results similarity ratio of leaf libraries of 23 days drought-treated potato varieties (Unica as high tolerance and Russet Burbank as sensitive to drought stress) NGS results similarity ratio was investigated by Pearson correlation method Figure 4.25. The results demonstrate that the gene expression levels between Unica and Russet Burbank potato varieties after 23 days of drought application and also under control conditions are quite unique and the similarity ratio is low. Comparison of Pearson similarity of leaf transcriptomes obtained by NGS in drought and control conditions confirmed few similarity ratios Figure 4.25.

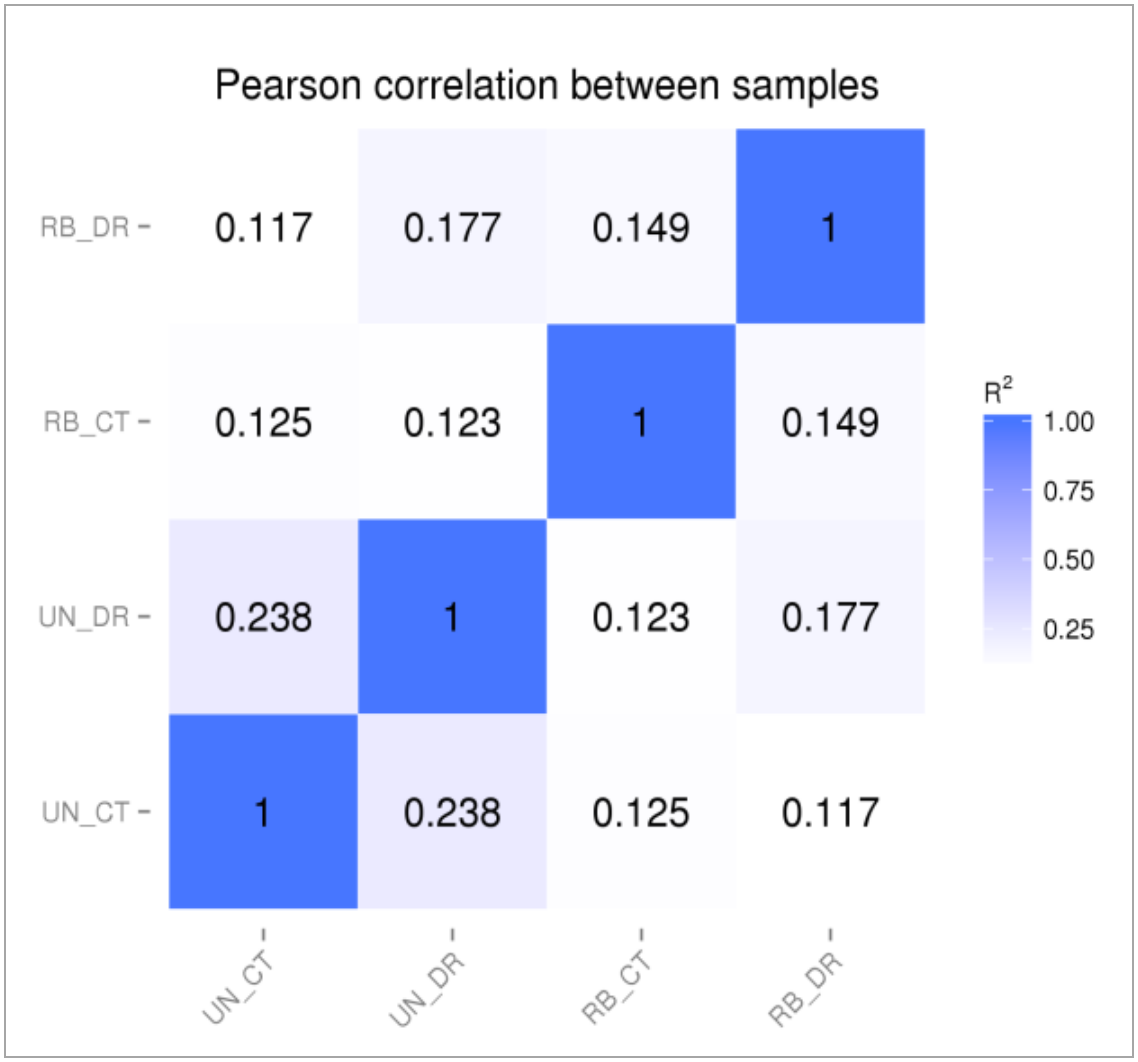


Figure 4.25. Similarity ratio of NGS results of Unica and Russet Burbank potato varieties leaf libraries investigated by Pearson correlation method

Figure 4.26. and 4.27. show Volcano plots indicating the gene expression changes in drought and control transcriptomics of Unica and Russet Burbank varieties. The graphics also display how much the transcriptomics changes matches between control

and drought of each variety and also between two varieties. In summary, expression of 1574 transcripts were either increased or decreased in Russet Burbank variety, whereas only 545 genes showed differential expression in Unica variety after drought treatment. The differences in gene expressions between two potato varieties indicate that Unica potato variety is more tolerant to drought stress.

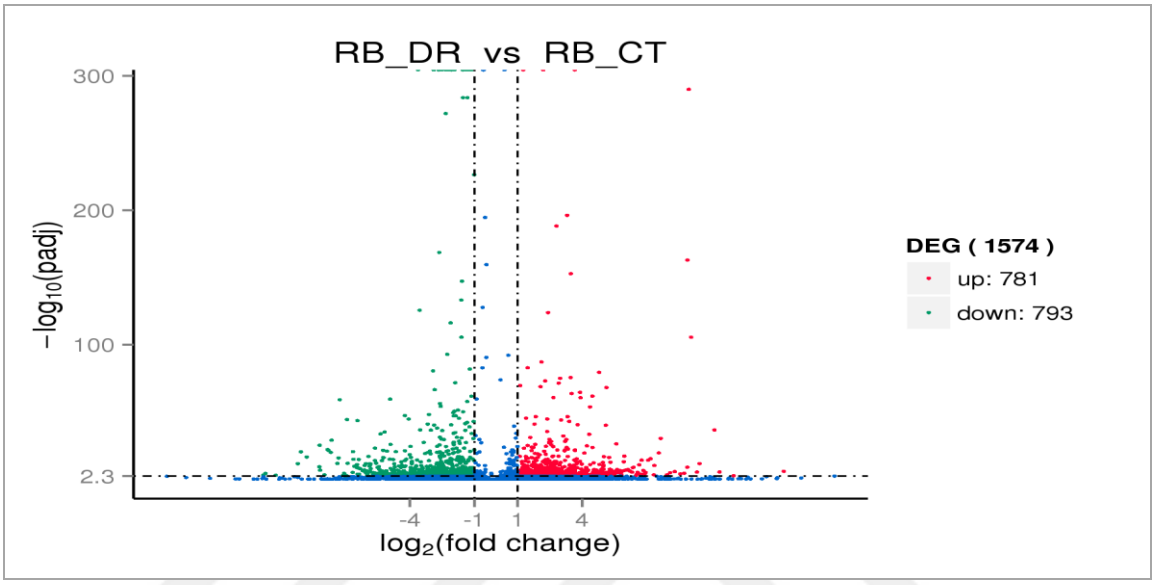


Figure 4.26. Comparison of gene expression changes in leaf transcriptomes of Russet Burbank Drought and Russet Burbank Control

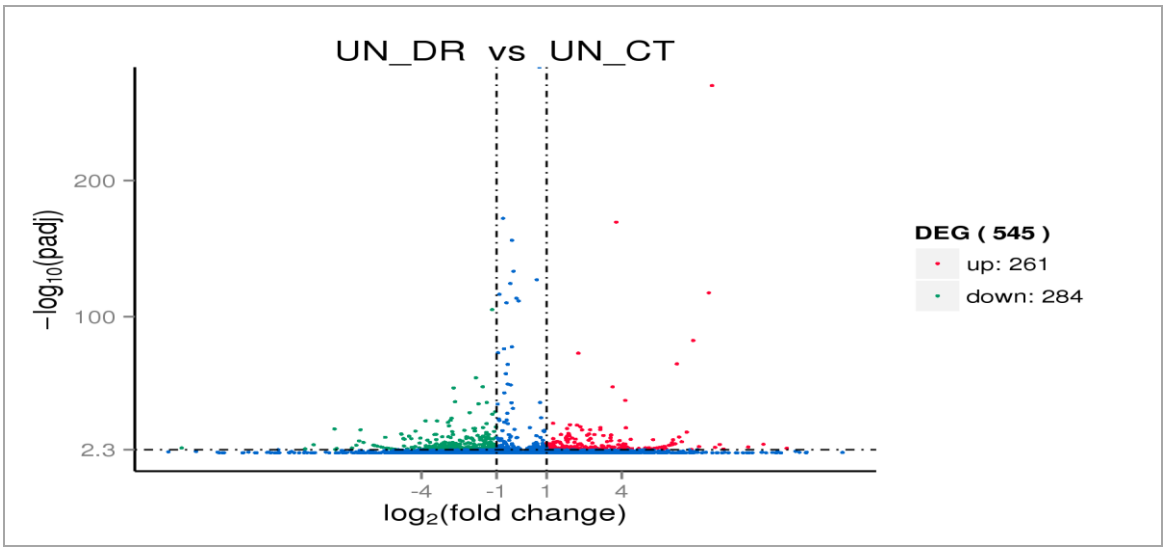


Figure 4.27. Comparison of gene expression changes in leaf transcriptomes of Unica Drought and Unica Control

The comparison of leaf transcriptomes of these two contrasting varieties under control conditions pointed out 886 genes with high levels of expression in Russet Burbank, and 650 genes in Unica (Figure 4.28.).

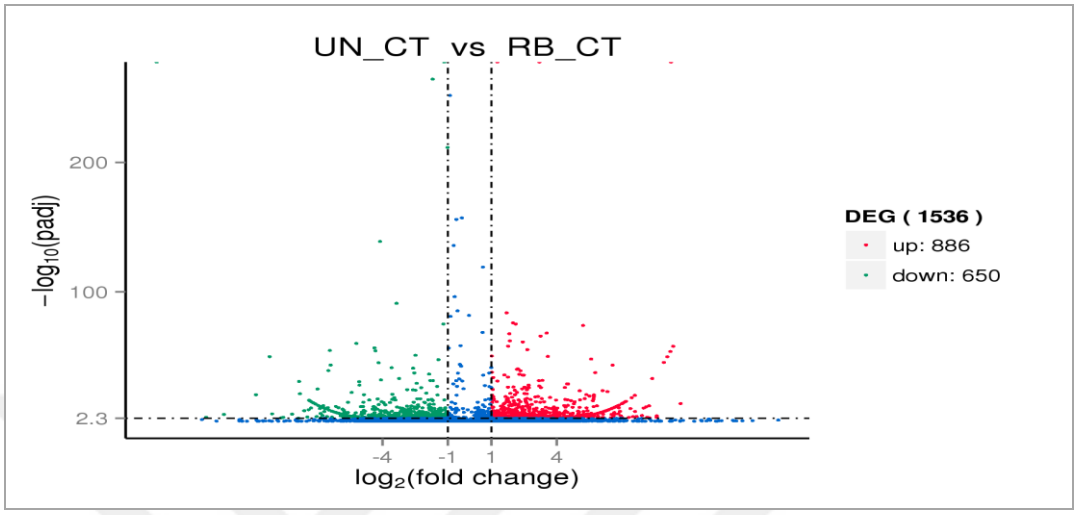


Figure 4.28. Comparison of leaf transcriptomics changes in gene expression in UN_CT and RB_CT varieties

Comparison of Unica and Russet Burbank transcripts after drought stress indicated contrasting expression levels in 952 genes (553 induced only in Unica, whereas 419 only in Russet Burbank).

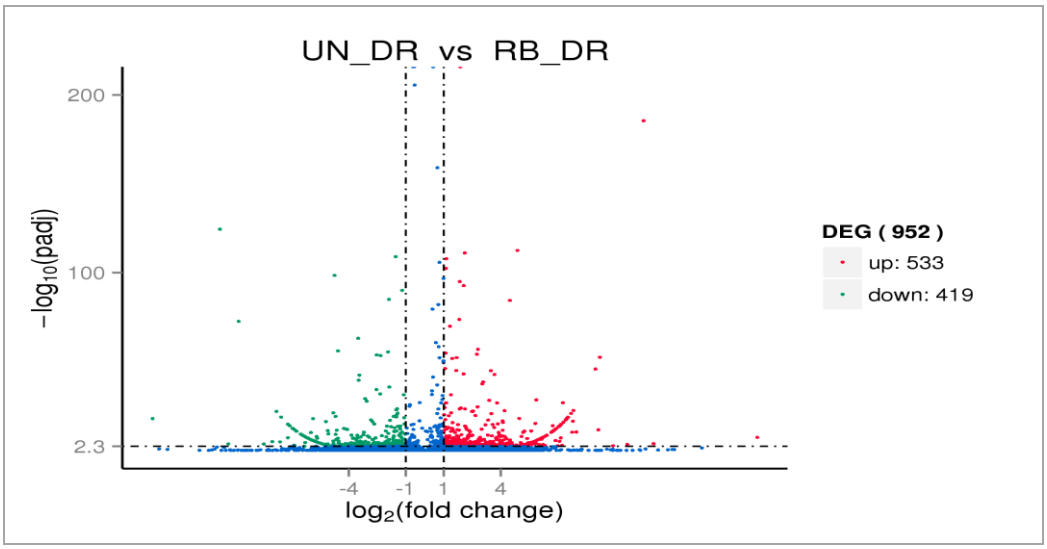


Figure 4.29. Comparison of leaf transcriptomics changes in gene expression in UN_DR and RB_DR varieties

The Venn diagrams present the number of genes that are uniquely expressed differentially within each group, with the overlapping regions showing the number of genes that are expressed in two or more groups. The sum of the numbers in each circle is the total number of genes expressed within a group, and the overlap represents the genes expressed in common between groups.

Venn diagram (Figure 4.30.) shows the number of transcripts decreased in Unica and Russet Burbank potato varieties in control and drought stress conditions. As shown, there was very little similarity in the responses of Unica and Russet Burbank. Out of 1,633 transcripts that decreased during drought stress, in total 284 genes down-regulated between Unica drought and Unica control, in total 419 genes down-regulated in Unica drought and Russet Burbank drought, in total 650 genes down-regulated in Unica control and Russet Burbank control, in total 793 genes down-regulated in Russet Burbank drought and control. An overlap of only two genes was found; the overlapped transcripts were predicted as *S.tuberosum* L. titin-like and *S.tuberosum* L. plasma membrane ATPase 4-like genes.

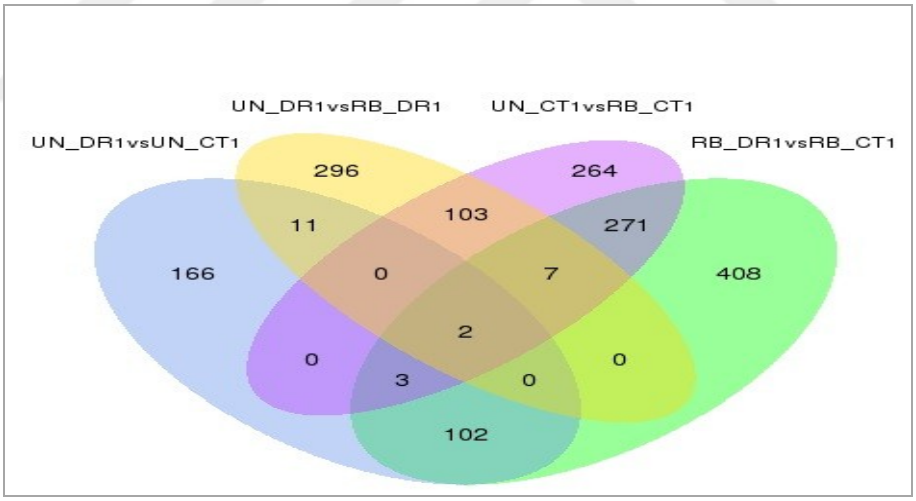


Figure 4.30. Venn diagram of control and drought stress down-regulated genes in Unica and Russet Burbank potato varieties identified on the basis of DEGs Analysis

Venn diagram Figure 4.31. shows the increased transcripts in Unica and Russet Burbank potato varieties in control and drought stress conditions. Out of 2,461 up-regulated transcripts, in total 261 genes were up-regulated between Unica drought and Unica control, 533 genes were up-regulated between Unica drought and Russet Burbank drought, 886 genes up-regulated between Unica control and Russet Burbank control, 781 genes up-regulated between Russet Burbank drought and Russet Burbank control.

There was only similarity in one gene (*S.tuberosum* L. scarecrow-like protein) between the responses of Unica and Russet Burbank.

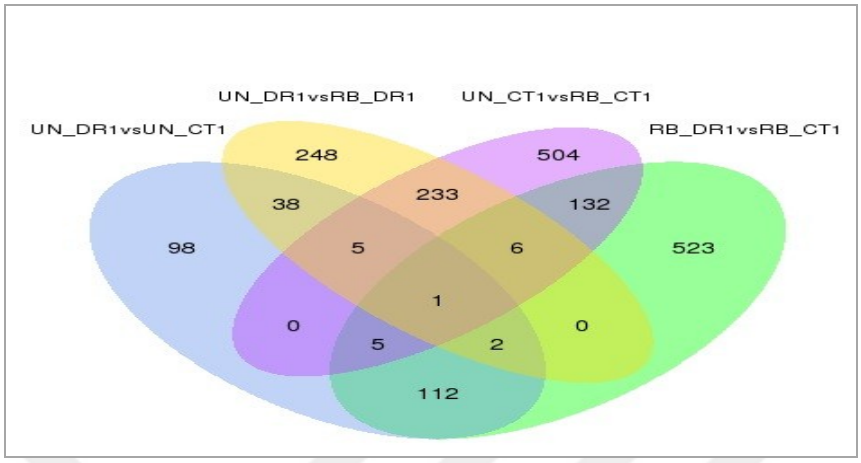


Figure 4.31. Venn diagram of control and drought stress up-regulated genes in Unica and Russet Burbank potato varieties identified on the basis of DEGs Analysis

Venn diagram in Figure 4.32. shows the total down or up-regulated genes. Out of 4,094 genes, only a little similarity was found between Unica potato variety and Russet Burbank potato variety.

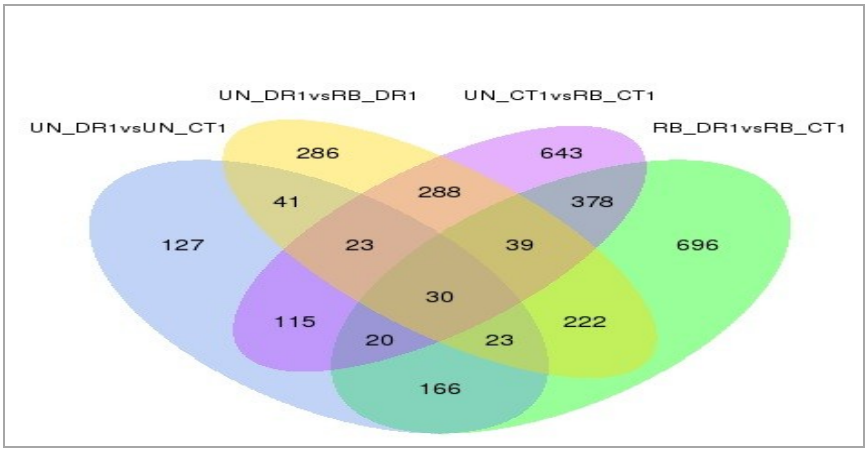


Figure 4.32. Venn diagram of control and drought stress-regulated genes (up-down) in Unica and Russet Burbank potato varieties identified on the basis of DEG analysis

The result of the cluster analysis regarding the change in expression ratios after 23 days of drought treatment of the transcripts obtained from the NGS results is given in Figure 4.33. According to this, the transcriptomics of the Unica variety, which is more tolerant to drought, have demonstrated more similarity in gene expression levels within

themselves in both control and drought conditions. However, Russet Burbank potato variety genes expression levels in control condition is more similar to Unica potato variety. The drought stress response of the Russet Burbank potato cultivar and the drought response of the Unica cultivar are quite different from each other.

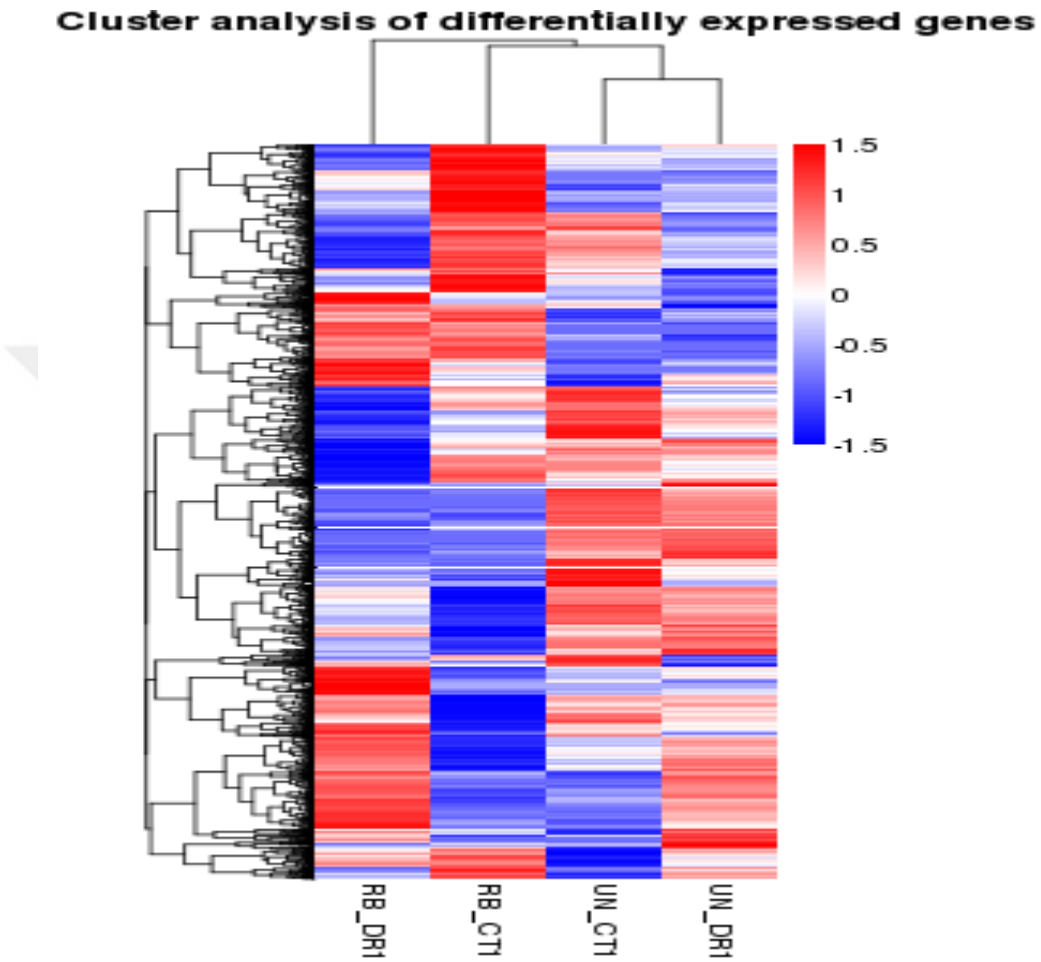


Figure 4.33. Results of cluster analysis of differentially expressed genes

Comparison of leaf transcriptomes of 23 days drought-treated Unica (high tolerant to drought) and Russet Burbank (sensitive to drought) potato varieties are given in Table 4.6. and Table 4.7.

Table 4.6. Genes with the most increased in gene expression, unchanged gene expression, or decreased in gene expression in Unica potato variety after 23 days drought treatment

Sequence code	FC log	Fold change	P value	Gene function
Increased in gene expression				
DMG400029153	10.76	1729.92	1.47E-193	Amino acid transporter
DMG400011073	10.63	1587.16	2.41E-177	Avr9/Cf-9 rapidly elicited protein 137
DMG400010207	10.39	1341.31	9.99E-150	Senescence-specific cysteine protease
DMG400005401	10.30	1262.00	1.00E-140	Sigma factor binding protein 1
DMG400004029	10.11	1103.38	4.83E-125	Anthranilate N-benzoyltransferase protein
DMG400014322	9.48	714.77	4.26E-81	CEN 1
DMG400043507	9.48	714.77	4.26E-81	Cation-transporting atpase plant
DMG400026998	9.26	611.67	1.17E-69	IS10 transposase
DMG400010258	9.03	524.43	1.00E-59	F-box family protein
DMG400018766	8.72	421.33	5.49E-48	Seed maturation protein PM41
Unchanged in gene expression				
DMG400000017	0	1	1	Beta-hydroxyisobutyryl-CoA hydrolase 1
DMG400000022	0	1	1	(+)-neomenthol dehydrogenase
DMG400000059	0	1	1	UDP-glucosyltransferase
DMG400000103	0	1	1	UDP-glucose:glucosyltransferase
DMG400000147	0	1	1	Cytochrome P450 monooxygenase
DMG400000150	0	1	1	Gene of unknown function
DMG400000154	0	1	1	NAC transcription factor
DMG400000158	0	1	1	Actin-depolymerizing factor 12
DMG400000193	0	1	1	1-aminocyclopropane-1-carboxylate synthase 2
DMG400000252	0	1	1	KDEL motif-containing protein 1
Decreased in gene expression				
DMG400026077	-9.47	-711.19	1.70E-80	Calcium-dependent protein kinase
DMG400020156	-9.52	-735.41	3.33E-83	Pectase lyase
DMG400005695	-9.55	-751.55	5.20E-85	Boron transporter
DMG400046813	-9.57	-759.62	6.50E-86	Cytochrome P450
DMG400025246	-9.60	-775.76	1.02E-87	Histone H3.2
DMG400024755	-9.63	-791.90	1.59E-89	Xyloglucan endotransglucosylase/hydrolase 1
DMG400002722	-9.83	-912.95	4.53E-103	Cellulose synthase-like A1
DMG400015005	-9.99	-1017.87	8.28E-115	Heavy metal-associated domain containing protein
DMG400020084	-10.54	-1485.95	3.53E-167	Zinc finger protein
DMG400020481	-11.05	-2115.44	3.33E-238	14 kDa proline-rich protein DC2.15

Table 4.7. Genes with the most increased in gene expression, unchanged gene expression, or decreased in gene expression in Russet Burbank potato variety after 23 days drought treatment

Sequence code	FC log	Fold change	P value	Gene function
Increased in gene expression				
DMG400026221	9.16	572.17	5.44E-61	Major pollen allergen Ory s 1
DMG400001621	9.09	543.61	9.57E-58	Phospholipase C
DMG400031042	8.23	300.86	8.32E-32	Glutamate decarboxylase isoform1
DMG402002771	8.16	286.58	4.80E-30	Anaerobic basic leucine zipper protein
DMG400011977	8.09	272.31	1.40E-28	Small heat shock protein
DMG400027611	8.05	265.17	1.06E-27	Heat shock protein 70
DMG400009580	7.75	215.19	1.74E-23	Ca2+ antiporter/cation exchanger
DMG400031523	7.54	186.63	3.09E-20	Glycine-rich cell wall structural protein 1.8
DMG400016032	7.43	172.35	9.22E-19	Glutaredoxin
DMG400016701	7.43	172.35	9.22E-19	Ulp1 protease family, C-terminal catalytic domain containing protein
Unchanged in gene expression				
DMG400000011	0	1	1	Gibberellin 20-oxidase 4
DMG400000103	0	1	1	UDP-glucose:glucosyltransferase
DMG400000154	0	1	1	NAC transcription factor
DMG400000193	0	1	1	1-aminocyclopropane-1-carboxylate synthase 2
DMG400000200	0	1	1	Acyl-CoA thioesterase family protein
DMG400000271	0	1	1	Monooxygenase
DMG400000284	0	1	1	1-aminocyclopropane-1-carboxylate synthase
DMG400000360	0	1	1	Malic enzyme
DMG400000439	0	1	1	Actin-11
DMG400000466	0	1	1	Phosphatidylcholine-sterol acyltransferase
Decreased in gene expression				
DMG400014104	-7.23	-149.87	9.24E-131	Patatin-2-Kuras 4
DMG400020017	-7.36	-164.73	6.94E-18	Lichenase
DMG400023634	-7.36	-164.73	6.94E-18	Ring finger protein
DMG400015350	-7.52	-182.92	1.08E-19	Monooxygenase
DMG400013198	-7.65	-201.12	8.48E-22	Systemin receptor SR160
DMG400039214	-7.65	-201.12	8.48E-22	Arachidonic acid-induced DEA1
DMG400028244	-7.72	-210.21	1.06E-22	Short chain alcohol dehydrogenase
DMG400019119	-7.78	-219.31	1.32E-23	Oxidoreductase
DMG400035689	-8.55	-373.94	3.68E-40	Flavonoid glucoyltransferase UGT73N1
DMG400018989	-8.80	-446.71	5.49E-48	Cyclin-dependent kinase inhibitor 1

The comparison shows that between two potato varieties used in the treatment, there are increasing or decreasing in gene expression levels, as well as the expression levels of some genes show a reverse change after 23 days of drought treatment. Examples of transcripts that the gene expression levels among varieties have changed similarly (increased in 10,734 genes or decreased in 7,057 genes) and an expression level has changed reversely after drought stress is summarized in Table 4.8.

Table 4.8. Examples of transcripts that the gene expression levels were changed similarly (increased or decreased) and the gene expression levels were changed reversely (increased in one variety while decreased in the other) among varieties

Sequence code	Gene function	Unica fold change	Russet Burbank fold change
Similarly increased in gene expression levels			
DMG400033042	Abc transporter	50.98	72.38
DMG400025797	Wound-responsive AP2 like factor 1	43.84	32.72
DMG400034273	Phosphatidylinositol n-acetylglucosaminyltransferase subunit p	36.70	32.72
DMG400005062	Peroxidase	29.56	32.72
DMG400007771	Protein transport Sec1a	29.56	32.72
DMG400014592	Glutamine synthetase	29.56	32.72
DMG400028277	Glutamate-ammonia ligase	29.56	24.79
DMG401001731	Ascorbate peroxidase	23.44	30.60
DMG400019580	Homeobox-leucine zipper protein ATHB-40	22.42	32.72
DMG400025837	Nucleobase ascorbate transporter	22.42	24.79
DMG400016742	Protein phosphatase 2C AHG3 homolog	20.12	28.11
DMG400011102	Transcription factor HBP-1b	15.28	24.79
DMG400011250	MYB transcription factor	15.28	24.79
DMG402023061	HVA22 c	15.28	24.79
DMG400011649	Monovalent cation:proton antiporter	15.28	16.86
DMG400033077	ABC transporter A family member 2	15.28	16.86
DMG400031137	Ripening regulated protein DDTFR8	13.54	15.85
DMG401028907	Heat shock protein 83	13.40	9.82
DMG400024644	101 kDa heat shock protein	11.33	19.31
DMG400045602	Pentatricopeptide repeat-containing protein	9.96	8.85
DMG401013246	Ubiquitin-protein ligase	5.18	3.60
DMG400023801	Armadillo repeat-containing protein	4.66	3.41
DMG400001708	RING-H2 finger protein ATL1Q	4.61	3.17
DMG400017240	Desacetoxyvindoline 4-hydroxylase	4.34	5.40

Table 4.8. (continue) Examples of transcripts that the gene expression levels were changed similarly (increased or decreased) and the gene expression levels were changed reversely (increased in one variety while decreased in the other) among varieties

DMG400016590	Stress enhanced protein 2	2.05	3.71
Similarly decreased in gene expression levels			
DMG400014304	Peptidoglycan-binding LysM domain-containing protein	-192.02	-130.13
DMG400023522	Histone H4	-137.44	-412.59
DMG400014442	Flavonoid 3-hydroxylase	-73.77	-49.42
DMG400022517	Extensin Dif10	-37.38	-25.21
DMG401018646	O-methyltransferase	-37.38	-25.21
DMG400031025	Microtubule-associated protein	-19.19	-57.49
DMG400016123	Anthocyanidine rhamnosyl-transferase	-19.19	-41.35
DMG400002552	Delta TIP	-18.67	-28.21
DMG400014598	AP2 domain-containing transcription factor	-10.10	-33.28
DMG400013024	Indole-3-acetic acid amido synthetase	-10.10	-17.14
DMG400017203	Primary amine oxidase	-9.51	-17.28
DMG400004842	Beta-galactosidase	-6.43	-12.13
DMG400046343	3-hydroxy-3-methylglutaryl-coenzyme A reductase 1	-5.42	-3.73
DMG400000757	Salicylic acid-binding protein 2	-5.32	-7.34
DMG401010822	MYC1	-5.27	-4.35
DMG400030415	Expansin18	-5.16	-7.50
DMG400005750	PIN1-like auxin transport protein	-4.87	-3.41
DMG400008801	Eukaryotic translation initiation factor 2 gamma subunit	-4.65	-2.93
DMG402023368	Amine oxidase	-4.51	-4.39
DMG400000503	IS10 transposase	-4.49	-4.27
DMG400019000	Photoreceptor-interacting protein	-2.74	-1.94
DMG400023391	Ferredoxin-3, chloroplast	-2.43	-2.27
DMG400002682	Phytosulfokine peptide	-2.39	-2.27
DMG400026409	Ribulose bisphosphate carboxylase small chain 2B, chloroplastic	-1.76	-3.19
DMG400023973	Aminoadipate-semialdehyde dehydrogenase	-1.38	-1.31
Reversely changed in gene expression levels			
DMG400016032	Glutaredoxin	172.35	-3.00
DMG400032278	TdcA1-ORF2 protein	100.95	-1.07
DMG400019017	BHLH transcriptional regulator	65.26	-49.42
DMG400024476	Heat-shock protein	58.12	-105.92
DMG400028733	Serine/threonine-protein kinase cdk9	58.12	-41.35
DMG400005388	Basic helix-loop-helix protein BHLH5	58.12	-1.02
DMG401003552	NAC domain protein	50.98	-41.35
DMG400011722	Universal stress protein 2	50.98	-3.73
DMG400000064	WRKY transcription factor 23	50.98	-1.39
DMG400019408	WRKY transcription factor	43.84	-33.28

Table 4.8. (continue) Examples of transcripts that the gene expression levels were changed similarly (increased or decreased) and the gene expression levels were changed reversely (increased in one variety while decreased in the other) among varieties

DMG400004953	Homeotic protein knotted-1	43.84	-17.14
DMG400006844	Subtilase	43.84	-11.86
DMG400004926	Early-responsive to dehydration protein	43.84	-1.26
DMG400029741	NAC domain-containing protein	43.84	-1.02
DMG400009255	Small heat-shock protein homolog protein	36.70	-7.24
DMG400006433	Myb family transcription factor	29.56	-17.14
DMG400011776	1-aminocyclopropane-1-carboxylic acid oxidase	29.56	-17.14
DMG400022208	Endo-1,4-beta-glucanase	15.28	-105.92
DMG400021290	Heat shock protein DnaJ	15.28	-1.26
DMG400003377	Retrotransposon gag protein	8.14	-5.53
DMG400027857	DNA-3-methyladenine glycosylase	3.57	-162.41
DMG400034476	WRKY33	1.00	-25.21
DMG400042864	WD-repeat protein	1.00	-25.21
DMG400022151	Chaperone protein DNAj	-7.71	6.65
DMG402023899	Early-responsive to dehydration 7	-10.10	3.61
DMG400021849	Retrotransposon	-10.10	8.93
DMG400018212	Germin E protein	-19.19	16.86
DMG400033618	Heat shock protein 40	-28.29	16.86
DMG400028700	Dehydration-responsive element-binding protein 2G	-28.29	24.79
DMG400030387	Protein phosphatase 2C 51	-28.29	24.79
DMG400024661	Brassinosteroid LRR receptor kinase	-46.48	16.86
DMG400000505	Alpha-DOX1	-46.48	24.79
DMG402020076	Desiccation-associated protein	-46.48	32.72
DMG400015235	Ubiquitin-protein ligase	-55.58	16.86
DMG400047336	ACRE 132	-73.77	40.65

As it can be seen in Table 4.8. the great majority of transcriptomes that their gene expressions were changed reversely among varieties after 23 days of drought treatment are related to stress response, suggesting that varieties react at different levels to drought stress. For example, “heat-shock protein”, “universal stress protein 2”, “WRKY transcription factor 2”, “early-responsive to dehydration protein” and “heat-shock protein DnaJ” genes expression levels were increased in Unica as a drought tolerant variety after 23 days of drought treatment, whereas in Russet Burbank (sensitive variety to drought), genes expression levels were either diminished at different levels or stayed unchanged. In contrary, the expression level of “heat-shock protein 40”, “dehydration-responsive element binding protein 26”, “desiccation-associated protein” and “ubiquitin-protein ligase” were increased at different levels in Russet Burbank,

nevertheless the expression levels of these genes have either decreased or never changed in the Unica variety.

Table 4.9. Examples of unchanged gene expression transcriptomes in potato varieties after 23 days of drought treatment

Sequence code	Gene function	Unica	Russet Burbank
DMG400032200	1,3-beta-glucan synthase	1.29	1.61
DMG400004274	Acetyl-CoA synthetase	-1.30	1.06
DMG400020878	Actin 2	-1.88	1.96
DMG400018565	Alcohol dehydrogenase	-1.68	-1.02
DMG400004962	Alpha-tubulin	-1.60	-1.71
DMG400009938	Beta-tubulin	-1.75	-1.81
DMG400022387	DNA mismatch repair protein muts2	1.31	-1.30
DMG400020772	Elongation factor 1-alpha	-1.02	-1.51
DMG400008885	Fatty acyl-CoA reductase 2	1.56	-1.97
DMG400002716	Gamma-tubulin complex component	1.25	-1.16
DMG400026784	Inorganic pyrophosphatase	-1.30	-1.77
DMG400026456	Malic enzyme	-1.05	-1.39
DMG400012857	Microtubule-associated protein MAP65-1b	-1.17	-1.34
DMG400007872	NBS-LRR protein	1.22	1.01
DMG400008591	O-methyltransferase	-1.43	-1.55
DMG400004547	Proteinase inhibitor type-2 P303.51	1.85	-1.55
DMG400004052	Serine/threonine protein phosphatase 2a regulatory subunit A	1.42	1.91
DMG400026491	Sigma factor	1.26	1.05
DMG400029926	Tubulin beta chain	-1.01	-1.91
DMG400017725	Vacuolar H+-pyrophosphatase	-1.27	-1.12

In Table 4.9. examples of the level of gene expression considered unchanged (-2<fold change>2) that were selected from 20,200 genes was given. It is important to note that “alpha-tubulin”, “beta-tubulin”, and “elongation factor 1-alpha” gene expression levels were unchanged in both potato varieties upon stress treatment. The “elongation factor 1-alpha” among the unchanged gene was used as reference gene for qRT-PCR studies. Some genes expressed only in one of the potato variety, are listed in Table 4.10.

Table 4.10. Examples of genes that were expressed only in Unica or only in Russet Burbank

Sequence code	Gene function	Unica	Russet Burbank
DMG400004029	Anthranilate N-benzoyltransferase protein		1103.38
DMG400034095	Dehydrin DH2a		278.58
DMG400041272	Phosphate transporter PHO1 homolog 10		159.62
DMG400004618	Invertase inhibitor		119.96
DMG405025785	Dynamin		72.38
DMG400009710	Na(+)/H(+) antiporter		56.52
DMG400025672	HECT; Ubiquitin		32.72
DMG400003706	Dehydration-responsive element-binding protein 2G		24.79
DMG400029236	Metalloendoproteinase 1		16.86
DMG400040222	Dehydration-responsive element-binding protein 1B		8.93
DMG400010135	Ethylene-responsive element-binding family protein	93.81	
DMG400005375	Oxidoreductase	72.40	
DMG400000252	KDEL motif-containing protein 1	50.98	
DMG400020795	Cationic peroxidase 1	36.70	
DMG400010770	Glutaredoxin	15.28	
DMG400037204	WD-40 repeat family protein	15.28	
DMG400046464	MAPKKK19	15.28	
DMG400022482	Heat shock protein DnaJ	8.14	
DMG400043954	'chromo' domain containing protein	8.14	
DMG400045376	WRKY transcription factor	8.14	

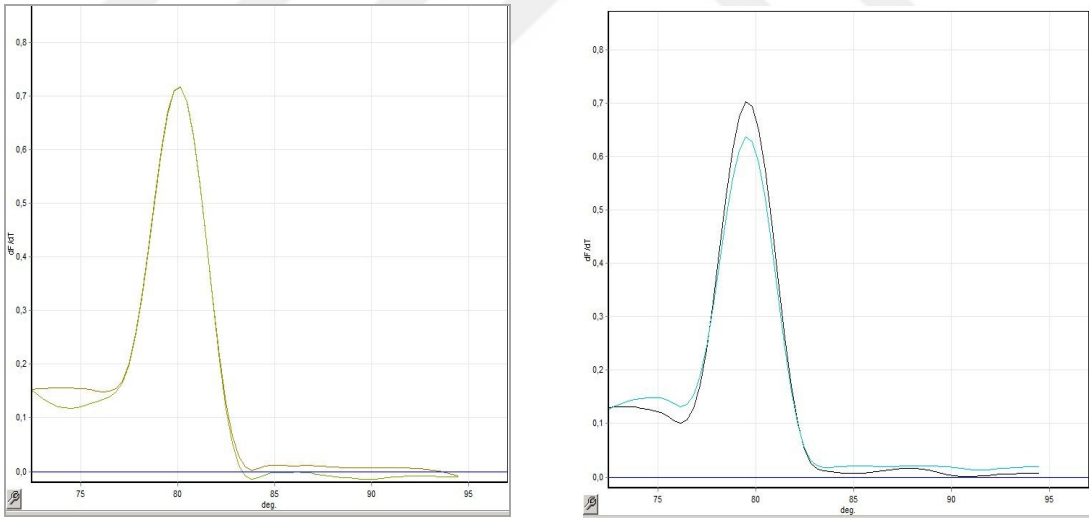
4.5 Verification of gene expression by real-time PCR (qRT-PCR)

Thirteen transcripts differing in gene expression levels (increasing or decreasing) were selected from leaf tissues among varieties of Unica as tolerant to drought and Russet Burbank as less tolerant to drought stress and the change in gene expression levels obtained from NGS results were verified by qRT-PCR method. For this purpose selected genes and gene expression levels were given in Table 4.11. In this context, attention was paid to the selection of the transcripts whose gene expression increased similarly, decreased similarly, or changed in reverse (increased in Unica, decreased in Russet Burbank or increased in Russet Burbank, decreased in Unica). Because in the NGS results, expression level of the “elongation factor EF-1 alpha” (EF) did not change, it was used as a reference gene.

Table 4.11. Transcripts and gene expression levels used for qRT-PCR validation of NGS results

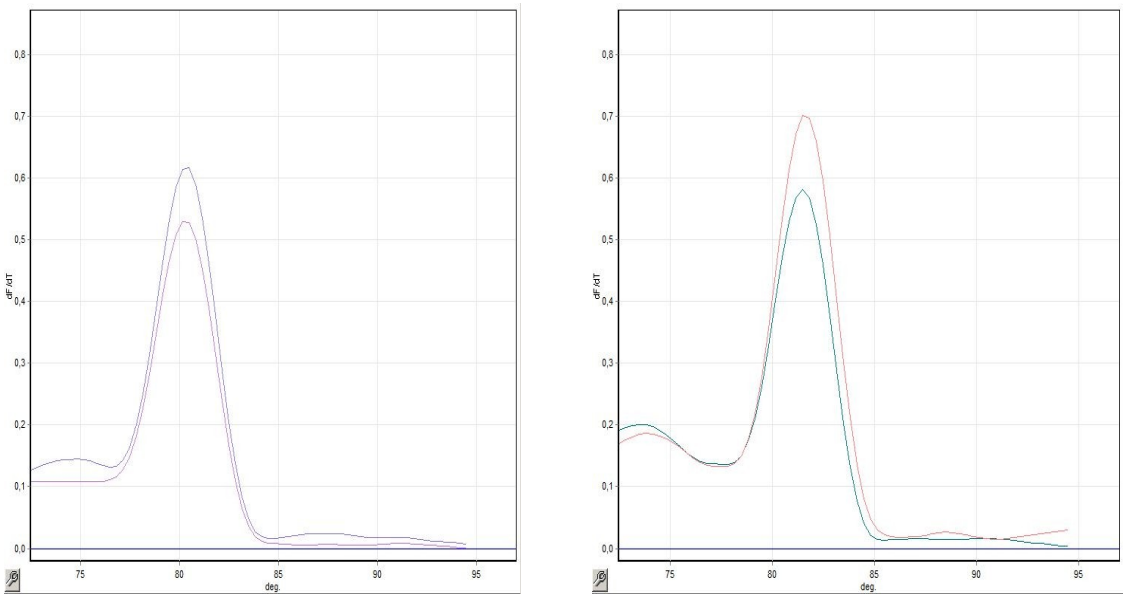
Transcripts	(UN_DR1 vs UN_CT1)	(RB_DR1 vs RB_CT1)
Superoxide dismutase [Cu-Zn], chloroplastic-like isoform X2 **(SOD)**	-19.63	-28.61
Plastidial pyruvate kinase 4, chloroplastic-like **(Plast)**	-33.42	65.73
Tyrosine/DOPA decarboxylase 1-like **(DOPA)**	-29.39	-47.45
Transcription factor bHLH68-like isoform X1 **(bHLH)**	-32.83	-15.07
Transcription factor MYB1R1-like **(MYB)**	-31.63	-34.94
L-ascorbate peroxidase 1, cytosolic-like **(Ascorb)**	-0.05	11.95
Heat shock cognate 70 kDa protein 1-like **(HSP)**	1.12	14.05
Protein EARLY RESPONSIVE TO DEHYDRATION 15-like **(Early)**	11.69	31.24
Protein GAST1-like **(GAST)**	-34.41	-52.92
Auxin-responsive protein IAA17-like **(IAA)**	19.35	33.97
Ethylene-responsive transcription factor ERF106-like **(ERF)**	46.78	16.51
Cryptochrome-2-like **(Crypto)**	36.36	0.83
AP2/ERF and B3 domain-containing transcription repressor TEM1-like **(AP2)**	-1.20	-24.54

As shown in Figure 4.34 and 4.35, the examples of melting curves indicates specific expression for all primers confirmed by qRT-PCR.



a b

Figure 4.34. Melting curve of Auxin (a) Cryptochrome (b)



a b

Figure 4.35. Melting curve of EF primer (a) and Heat-shock protein (HSP) (b)

Figure 4.36 shows the melting curve in overall showing the amplification of different genes of a qRT-PCR assay performed on a 96-well plate system. Each peak represents the melting curve of an amplicon of a different gene and confirms the authenticity of performed the qRT-PCR study

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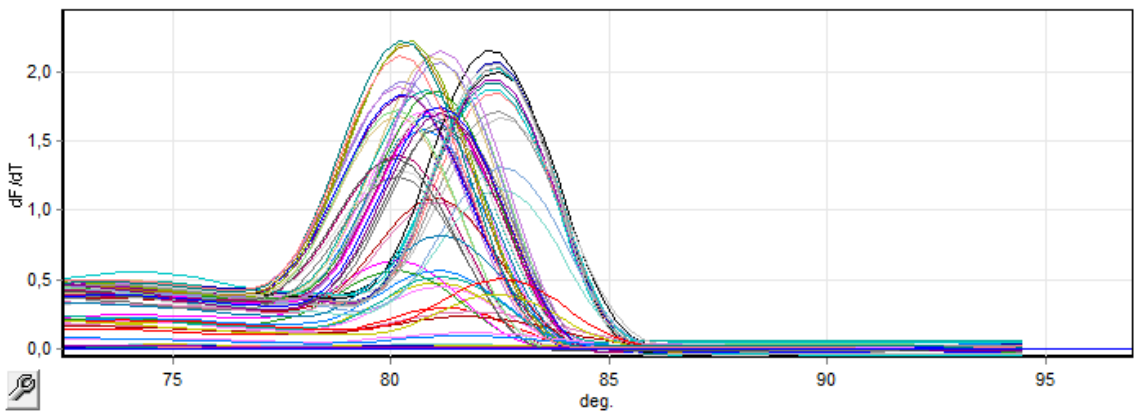
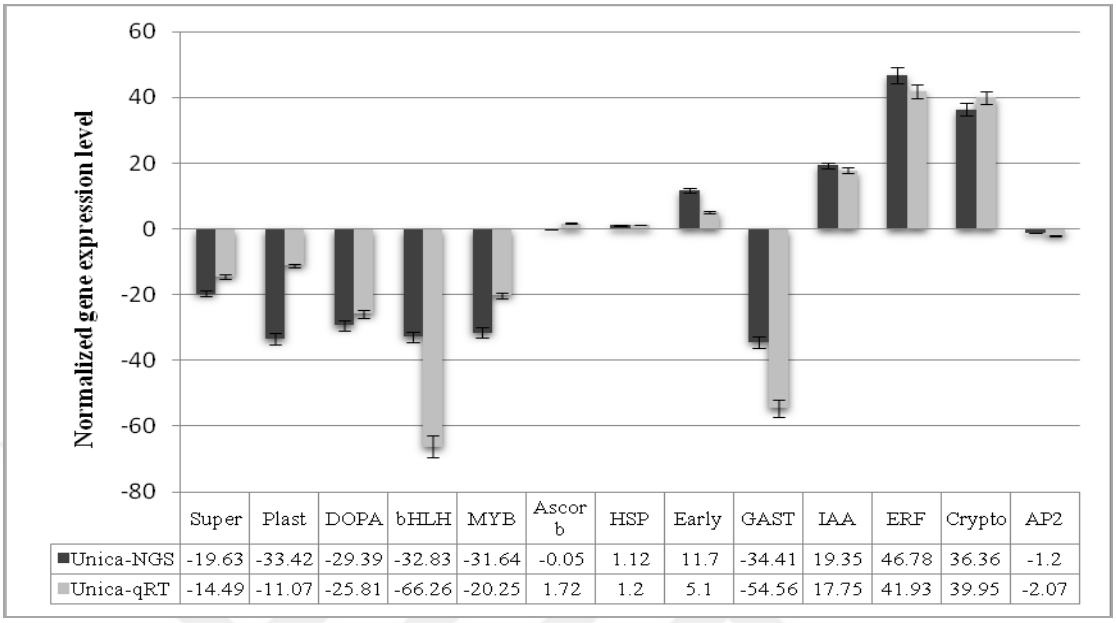


Figure 4.36. An example of the melting curves where qRT-PCR results were evaluated

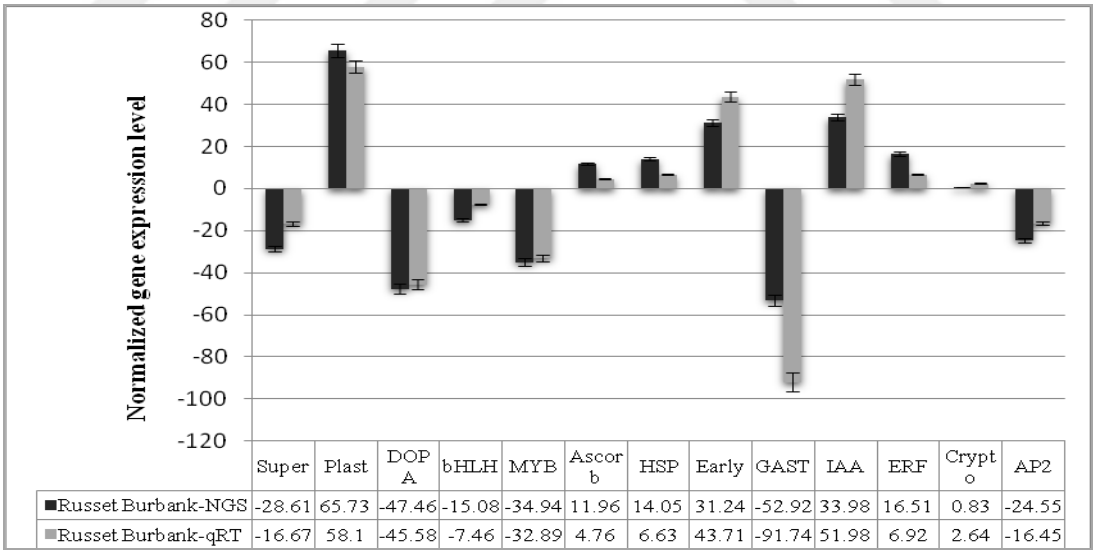
The graphs comparing gene expression differences detected in the NGS and obtained by real-time PCR (qRT-PCR) are given in Figure 4.37. (Unica) and Figure 4.38. (Russet Burbank). Differences in gene expression were calculated as folds increased or decreased compared to the control leaf samples after 23 days drought treatment. The

results indicate nice overlapping of NGS and qRT-PCR and confirming the reliability of gene expression changes



	Super	Plast	DOPA	bHLH	MYB	Ascorb	HSP	Early	GAST	IAA	ERF	Crypto	AP2
Unica-NGS	-19.63	-33.42	-29.39	-32.83	-31.64	-0.05	1.12	11.7	-34.41	19.35	46.78	36.36	-1.2
Unica-qRT	-14.49	-11.07	-25.81	-66.26	-20.25	1.72	1.2	5.1	-54.56	17.75	41.93	39.95	-2.07

Figure 4.37. A comparative graph of the results of NGS and real-time PCR (qRT-PCR) related to Unica potato variety



	Super	Plast	DOPA	bHLH	MYB	Ascorb	HSP	Early	GAST	IAA	ERF	Crypto	AP2
Russet Burbank-NGS	-28.61	65.73	-47.46	-15.08	-34.94	11.96	14.05	31.24	-52.92	33.98	16.51	0.83	-24.55
Russet Burbank-qRT	-16.67	58.1	-45.58	-7.46	-32.89	4.76	6.63	43.71	-91.74	51.98	6.92	2.64	-16.45

Figure 4.38. A comparative graph of the results of NGS and qRT-PCR related to Russet Burbank potato variety

CHAPTER V

DISCUSSION AND CONCLUSION

This thesis was aimed to compare the transcriptomic results of two potato varieties, one with high tolerance to drought and the other sensitive to drought stress, before and after drought treatment by NGS.

For this purpose, first physiological effects of drought stress on two potato varieties were evaluated, and then genes with increased or decreased expression in leaf tissue after drought treatment were identified by NGS approach. Increased and decreased expression in genes considered to be related to drought stress response confirmed by qRT-PCR approach.

Because the potato is a plant with high water use efficiency and tuber formation, especially the water stress directly affects the yield. In the literature, the drought tolerance of the potato is described as genotypes that are less restrictive than the others in terms of yield or irrigation in dry conditions. In arid conditions, a plant looks physiologically and morphologically very abnormal in the above-ground part of the soil may undergo less loss when assessed for yield. According to the information obtained from literatures Unica was selected as drought tolerant and Russet Burbank as drought sensitive (Cabello et al., 2012, 2013; Schafleitner et al., 2007b; Stark et al., 2013).

5.1 Comparison of transcriptomes by NGS approach

Graphs showing classification of all transcriptomes obtained in the NGS results in terms of metabolic pathways in different data banks after annotation are given in Figure 4.21. (GO data bank), Figure 4.22. (KOG database) and Figure 4.23. (KEGG database). According to the GO database, it seems that most of the genes are involved in cellular functions and metabolic activities, but a significant portion of transcripts are located in the metabolic pathways of response to stimulus and signaling as expected after drought. According to KOG classification, an important part of all transcripts also play a role in signal transduction mechanisms and defense mechanism. Finally, the KEGG database results show the high presence of transcripts, especially those that play a role in environmental adaptation. These results demonstrate that drought treatment changes the

metabolic pathways involved in signal transduction and stress response at the transcriptomic level, as expected.

Cluster analysis results Figure 4.33. revealed that the responses of Unica and Russet Burbank potato varieties under drought stress are quite different from each other and the Volcano graphics support the results similarly in Figures 4.26. and 4.29. it has been shown that the differences in gene expression of Unica variety are similar within themselves after control and drought treatment. It means that Unica variety as a tolerant plant to drought did not respond too much to stress conditions in gene expression level as expected, while the level of gene expression in Russet Burbank as a sensitive variety only under control conditions were showed similarity with Unica (Figure 4.33.). Cluster analysis results of Russet Burbank as a sensitive variety show that post-stress transcript has minimal similarities with the other three clusters.

Figure 5.1. and 5.2. show the metabolic pathways of Unica and Russet Burbank varieties according to GO database with a quantitative increase in transcript after drought treatment. Accordingly, drought treatment in Russet Burbank potato variety led to the activation of metabolic pathways (“response to water” and “response to stress”) as it was expected in drought stress conditions Figure 5.1. In the Unica variety, with a higher tolerance to drought, there has been a little quantitative increase in gene expression of “response to water” metabolic pathways, and the other metabolic pathways remained active.

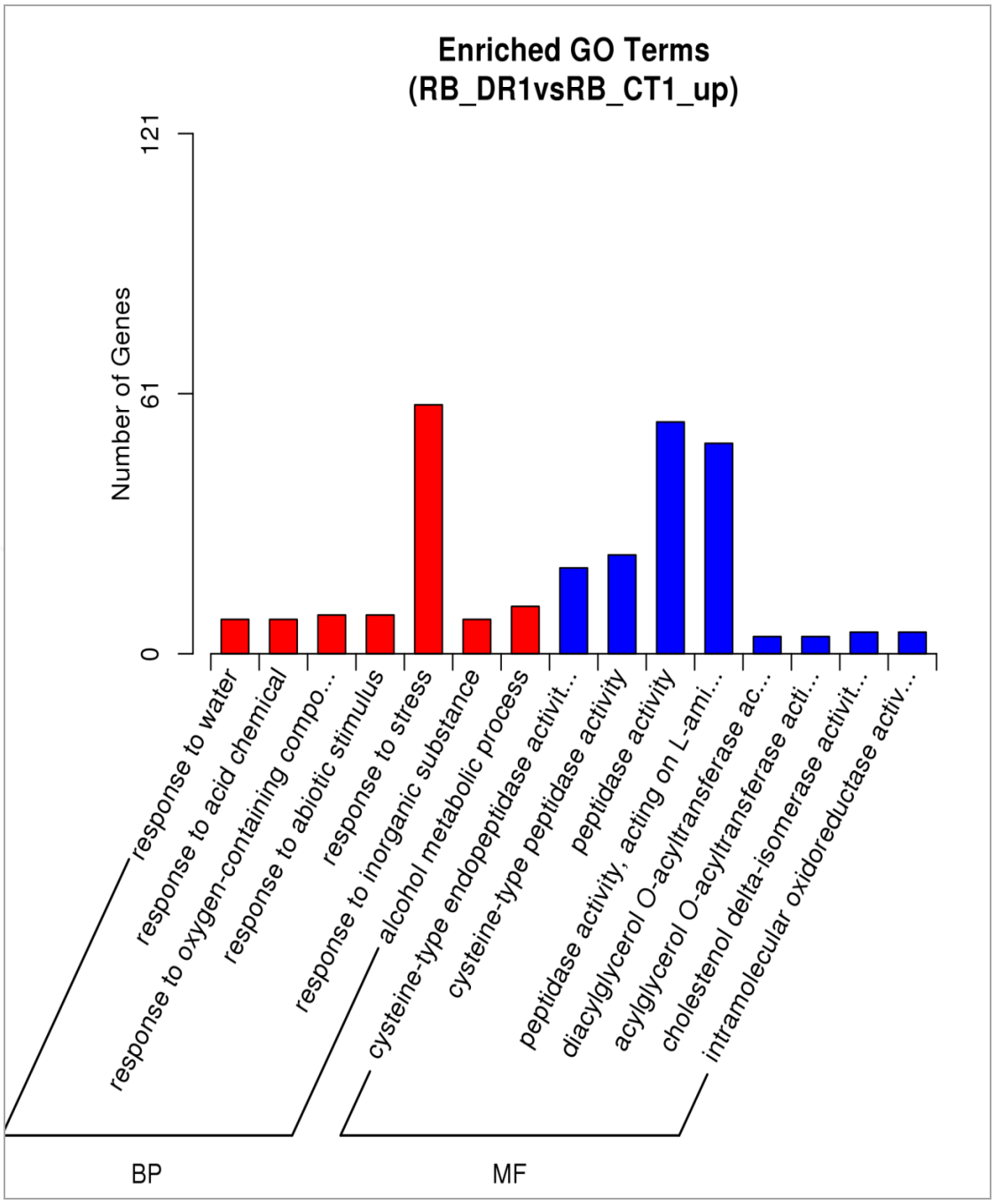


Figure 5.1. Metabolic pathways that their transcripts increased quantitatively in Russet Burbank variety classified by GO database after drought treatment biological pathway (BP), molecular function (MF)

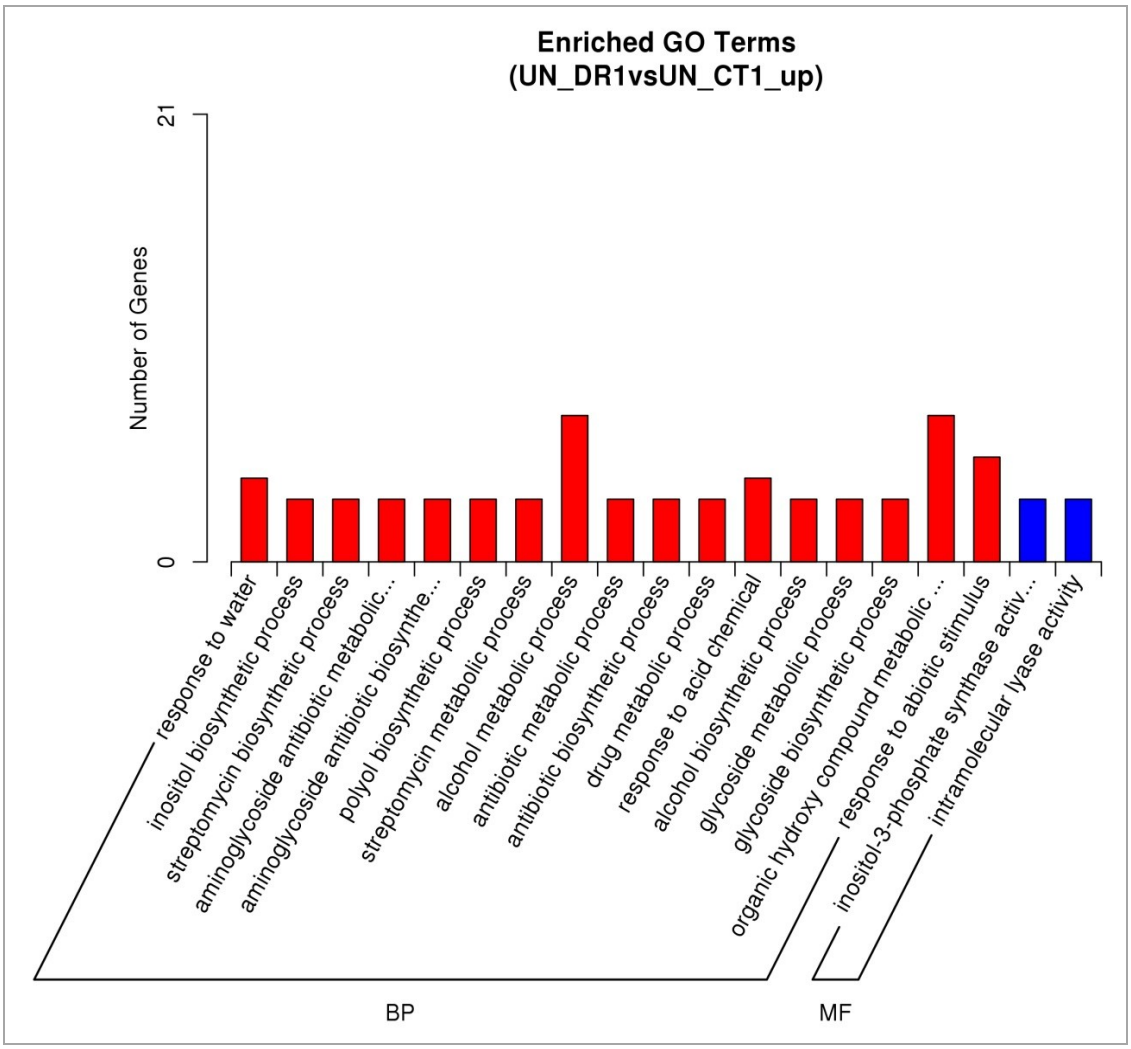


Figure 5.2. Metabolic pathways that their transcripts increased quantitatively in Unica variety classified by GO database after drought treatment biological pathway (BP), molecular function (MF)

Figure 5.3. and 5.4. show change in gene expressions which altered their metabolic pathways after 23 days drought treatment in Unica (high drought tolerance) and Russet Burbank (sensitive to drought). Accordingly, while drought treatment causes increase in genes involved in redox ("oxidation-reduction") reactions in Russet Burbank variety Figure 5.3. This increase in Unica variety was mainly observed in the genes responsible from carbon metabolism Figure 5.4.

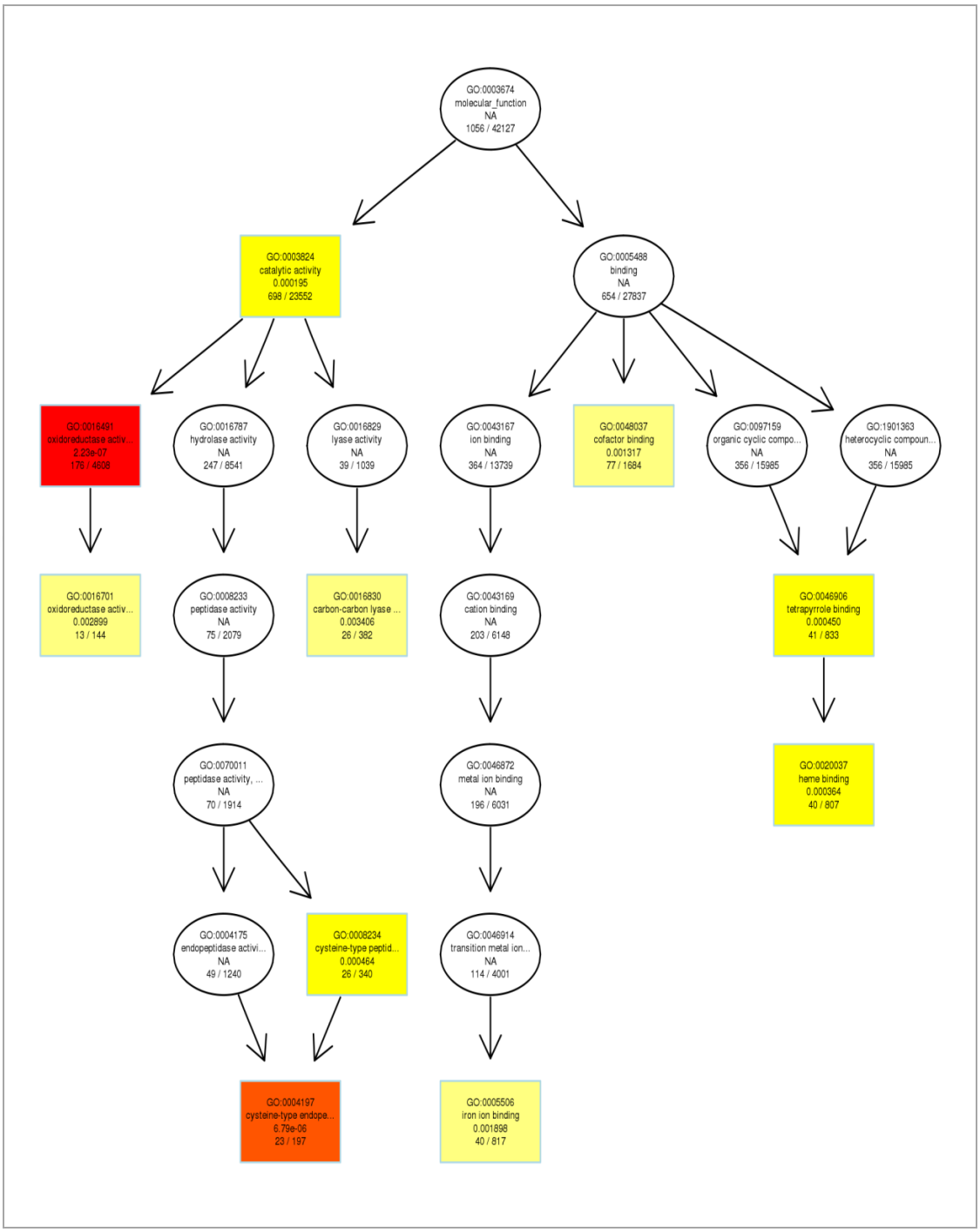


Figure 5.3. Metabolic pathways activated in Russet Burbank after 23 days of drought treatment

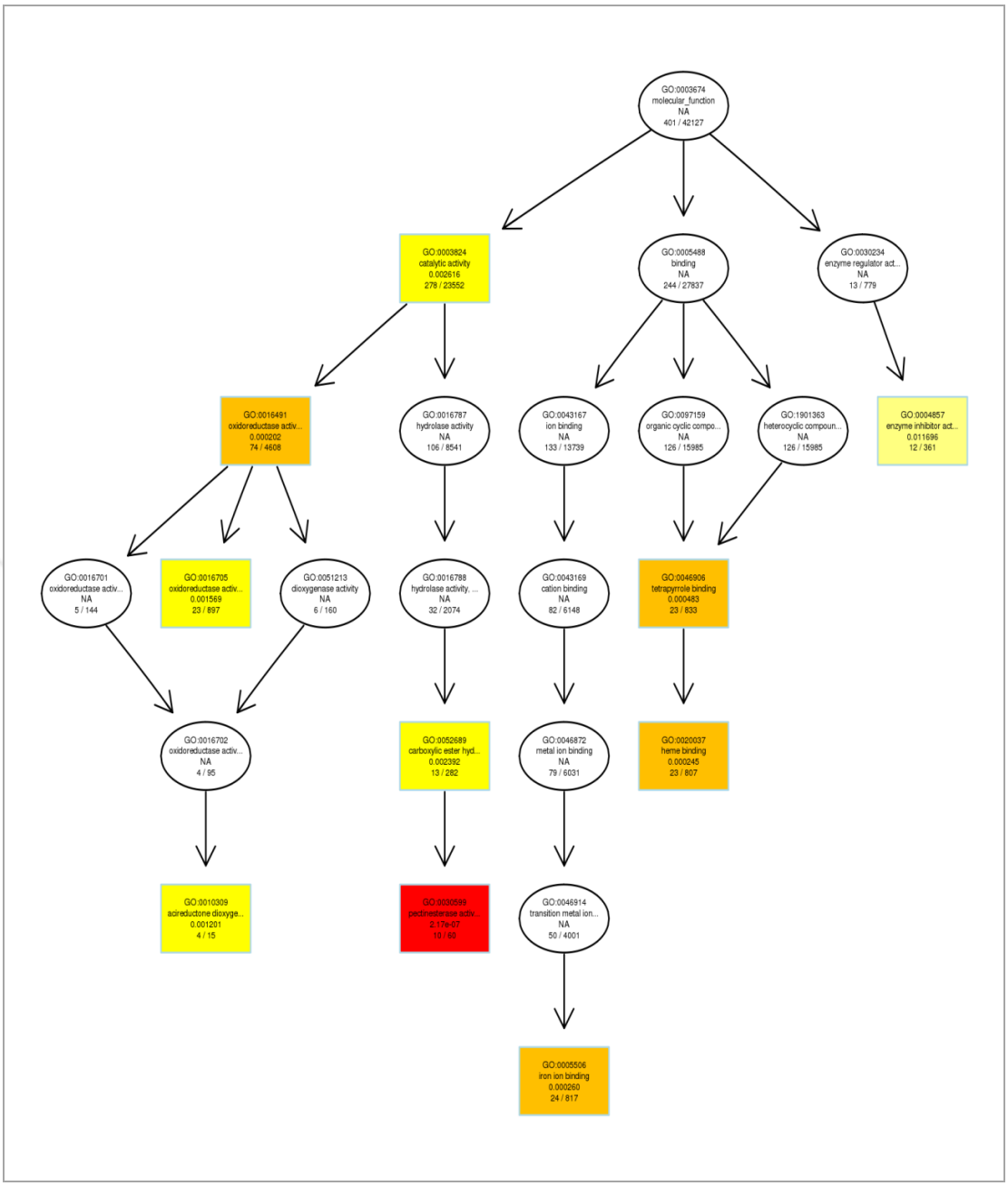


Figure 5.4. Metabolic pathways activated in Unica after 23 days drought treatment

Similar results can be seen after drought treatment when comparing the metabolic pathways in which the quantity of transcripts increases among varieties Figure 5.5. Basically the greatest difference between two varieties was seen as genes involved in increasing redox ("oxidation-reduction") reaction in Russet Burbank as sensitive variety.

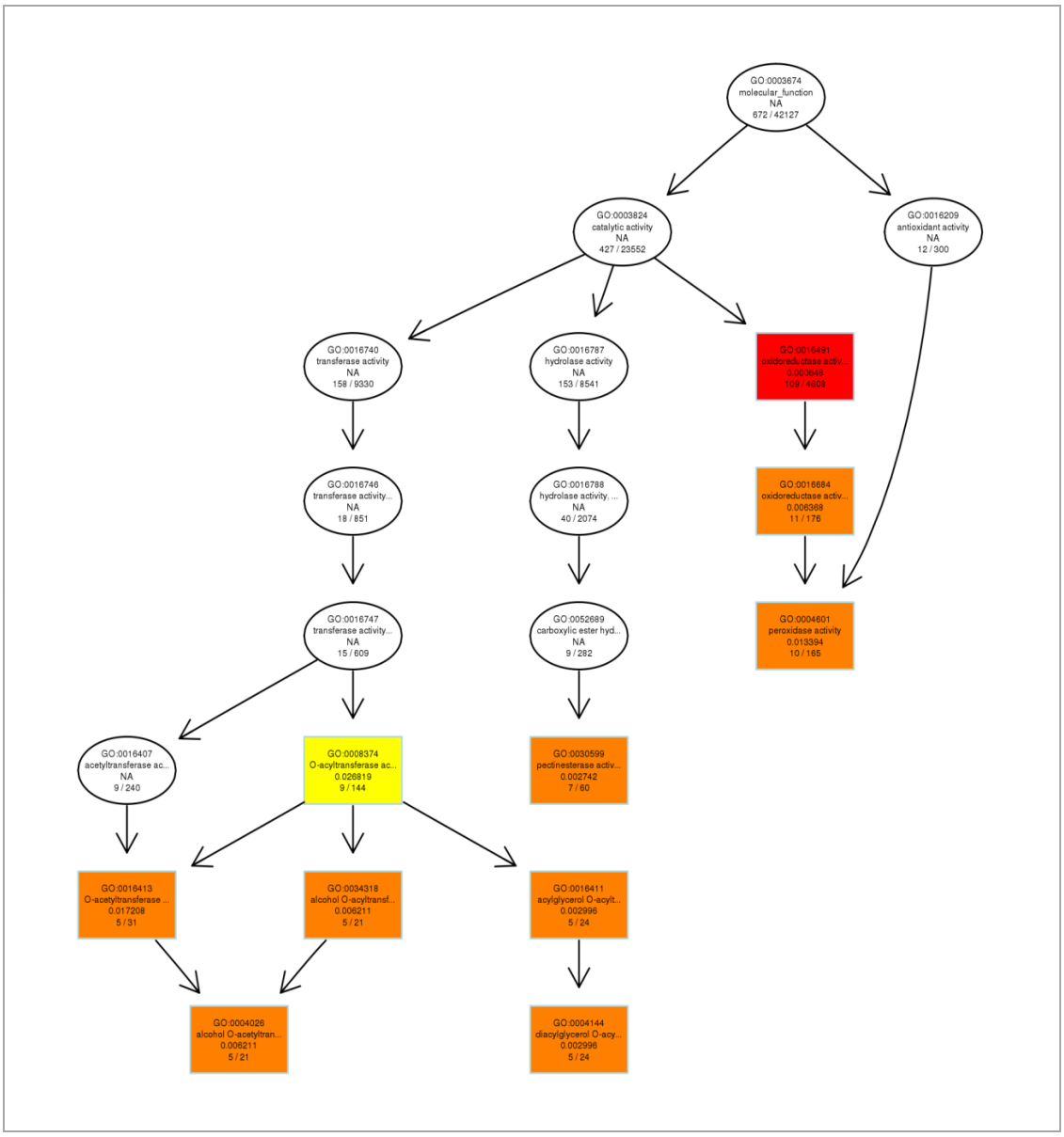


Figure 5.5. Comparison of the metabolic pathways in which quantity of transcripts in Unica and Russet Burbank varieties increased after drought treatment

Figure 5.6. shows the differences between two potato varieties when performed under the same comparative control conditions, it reveals that again the main difference is redox potential.

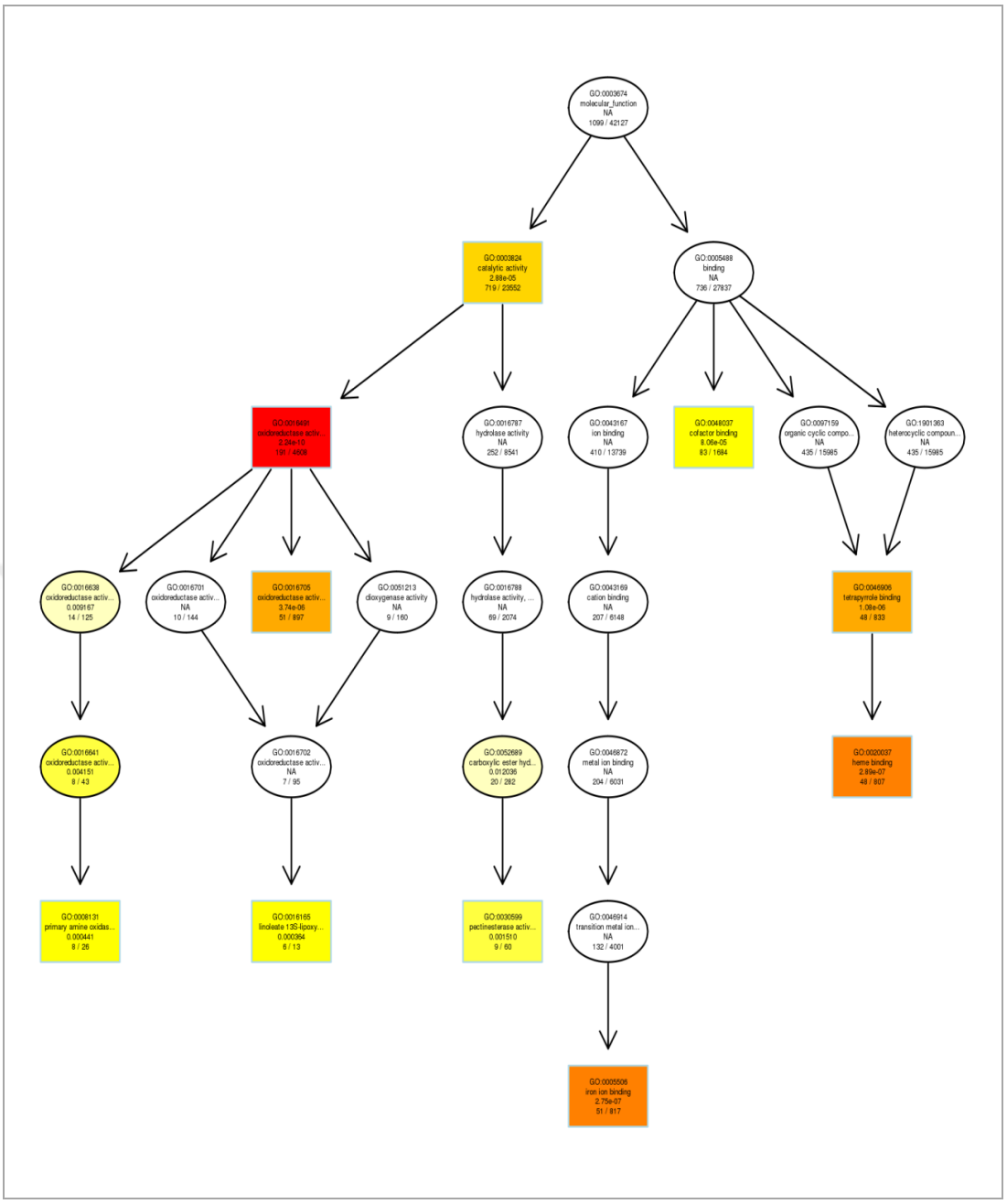


Figure 5.6. Comparison of the metabolic pathways in which the quantity of transcripts in Unica and Russet Burbank varieties increased before the treatment of drought (control condition)

CHAPTER VI

CONCLUSION

The objective of this thesis was to compare the leaf transcriptome of two potato varieties (higher tolerance and sensitive) that differ in terms of drought tolerance. In this respect, significant differences between two varieties were revealed. Four libraries were sequenced through Illumina Hiseq 2500 system, and a total 340,573,814 unique reads achieved, which almost 94 % of reads had ≥ Q30. In total 71.7 % of reads were belong to *S.tuberosum* L. Information obtained from KEGG database, showed that 1022 transcripts belong to environmental adaptation. The Venn diagram indicated that a little similarity between Unica and Russet Burbank, in down-regulated expression only 2 genes were in common (*S.tuberosum* L. titin-like and *S.tuberosum* L. plasma membrane ATPase 4-like), whereas in up-regulated expression only one gene was in common (*S.tuberosum* L. scarecrow-like protein). Out of 4,094 genes, only 30 genes were in common. Cluster analysis presented the different behavior of Russet Burbank compare to Unica potato variety under drought stress. Drought treatment in Russet Burbank led to increase in genes responsible for redox ("oxidation-reduction"), whereas; in Unica the increase was mainly in genes responsible for carbon metabolism. The most important obstruction was the fact the potato is a heterozygous tetraploid plant, and as a result, the gene expression level change highly depends on the number of gene copies. The main target of transcriptomic comparisons of plants investigated up to now were to determine how gene expressions or metabolic activities of plants changes under stress conditions.

REFERENCES

Anonymous., "The European Cultivated Potato Database", https://www.europotato.org/display_description.php?variety_name=Russet%20Burbank , 15 June 2016.

Ashburner, M. Ball, C.A. Blake, J.A. Botstein, D. Butler, H. Cherry, J.M. Davis, A.P. Dolinski, K. Dwight, S.S. Eppig, J.T. and Harris, M.A., "Gene Ontology: tool for the unification of biology", ***Nature Genetics*** *25*(1), 25-29, 2000.

Barnabás, B. Jäger, K. and Fehér, A., "The effect of drought and heat stress on reproductive processes in cereals", *Plant, Cell and Environment*, *31*(1), 11-38, 2008.

Bartels, D. and Souer, E., "Molecular responses of higher plants to dehydration", ***Plant Responses to Abiotic Stress*** 9-38, 2004.

Bartels, D. and Sunkar, R., "Drought and salt tolerance in plants", ***Critical Reviews in Plant Sciences*** *24*(1), 23-58, 2005.

Bates, L.S., Waldren, R.P. and Teare, I.D., "Rapid determination of free proline for water-stress studies", ***Plant and Soil*** *39*(1), 205-207, 1973.

Battaglia, M., Solórzano, R.M., Hernández, M., Cuéllar-Ortiz, S., García-Gómez, B., Márquez, J. and Covarrubias, A.A., "Proline-rich cell wall proteins accumulate in growing regions and phloem tissue in response to water deficit in common bean seedlings", ***Planta*** *225*(5), 1121-1133, 2007.

Blum, A., Plant breeding for stress environments, ***CRC Press***, Florida, 1988.

Cabello, R., De Mendiburu, F., Bonierbale, M., Monneveux, P., Roca, W. and Chujoy, E., "Large-scale evaluation of potato improved varieties, genetic stocks and landraces for drought tolerance", ***American Journal of Potato Research*** *89*(5), 400-410. 2012.

Cabello, R., Monneveux, P., De Mendiburu, F. and Bonierbale, M., "Comparison of yield based drought tolerance indices in improved varieties, genetic stocks and landraces of potato (*S.tuberosum L.* L.)", ***Euphytica*** *193*(2), 147-156, 2013.

Chaves, M.M., "Effects of water deficits on carbon assimilation", ***Journal of experimental Botany*** *42*(1), 1-16, 1991.

Chaves, M.M., Flexas, J. and Pinheiro, C., "Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell", ***Annals of Botany*** *103*(4), 551-560, 2009.

Chaves, M.M., Maroco, J.P. and Pereira, J.S., "Understanding plant responses to drought: from genes to the whole plant", ***Functional Plant Biology*** *30*(3), 239-264. 2003.

Cominelli, E., Conti, L., Tonelli, C. and Galbiati, M., "Challenges and perspectives to improve crop drought and salinity tolerance", ***New Biotechnology*** *30*(4), 355-361, 2013.

Deikman, J., Petracek, M. and Heard, J.E., "Drought tolerance through biotechnology: improving translation from the laboratory to farmers' fields", ***Current Opinion in Biotechnology*** *23*(2), 243-250, 2012.

Demiral, T. and Türkan, I., "Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment?", ***Journal of Plant Physiology*** *161*(10), 1089-1100, 2004.

Dunwell, J.M., "Transgenic approaches to crop improvement", ***Journal of Experimental Botany*** *51*(suppl_1), 487-496, 2000.

Farooq, M. Wahid, A. Kobayashi, N. Fujita, D. and Basra, S.M.A., "Plant drought stress: effects, mechanisms and management", ***Agronomy for Sustainable Development*** *29*(1), 185-212, 2009.

Fernandez, G.C., "Effective selection criteria for assessing plant stress tolerance", **In** ***Proceedings of the international symposium on adaptation of vegetables and other food crops in temperature and water stress*** 257-270, August,1992.

Finn, R.D. Tate, J. Mistry, J. Coggill, P.C. Sammut, S.J. Hotz, H.R. Ceric, G. Forslund, K. Eddy, S.R. Sonnhammer, E.L. and Bateman, A., "The Pfam protein families database", ***Nucleic Acids Research*** *36*(suppl_1), 281-288, 2007.

Flexas, J., Bota, J., Loreto, F., Cornic, G. and Sharkey, T.D., "Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants", ***Plant Biology*** *6*(03), 269-279, 2004.

Flexas, J., DIAZ-ESPEJO, A.N.T.O.N.I.O., GalmES, J., Kaldenhoff, R., Medrano, H. and RIBAS-CARBO, M.I.Q.U.E.L., "Rapid variations of mesophyll conductance in response to changes in CO2 concentration around leaves", ***Plant, Cell and Environment*** *30*(10), 1284-1298, 2007.

Flowers, T.J., "Improving crop salt tolerance", ***Journal of Experimental botany*** *55*(396), 307-319, 2004.

Gene Ontology Consortium, "Gene ontology consortium: going forward", ***Nucleic Acids Research*** *43*(D1), 1049-1056, 2015.

Gong, L. Zhang, H. Gan, X. Zhang, L. Chen, Y. Nie, F. Shi, L. Li, M. Guo, Z. Zhang, G. and Song, Y., "Transcriptome profiling of the potato (*S.tuberosum L.* L.) plant under drought stress and water-stimulus conditions", ***PLoS One*** *10*(5), p.e0128041, 2015.

Grabherr, M.G. Haas, B.J. Yassour, M. Levin, J.Z. Thompson, D.A. Amit, I. Adiconis, X. Fan, L. Raychowdhury, R. Zeng, Q. and Chen, Z., "Full-length transcriptome assembly from RNA-Seq data without a reference genome", ***Nature Biotechnology*** *29*(7), 644-652, 2011.

Haas, B.J. Papanicolaou, A. Yassour, M. Grabherr, M. Blood, P.D. Bowden, J. Couger, M.B. Eccles, D. Li, B. Lieber, M. and MacManes, M.D., "De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis", ***Nature Protocols*** *8*(8),1494-1512, 2013.

Hassanpanah, D., "Evaluation of potato cultivars for resistance against water deficit stress under in vivo conditions", ***Potato Research*** *53*(4), 383-392, 2010.

Jogaiah, S. Govind, S.R. and Tran, L.S.P., "Systems biology-based approaches toward understanding drought tolerance in food crops", ***Critical Reviews in Biotechnology*** *33*(1), 23-39, 2013.

Jun-Wei, W.A.N.G., Feng-Ping, Y.A.N.G., Xu-Qing, C.H.E.N., LIANG, R.Q., ZHANG, L.Q., Dong-Mei, G.E.N.G., ZHANG, X.D., Ya-Zhen, S.O.N.G. and ZHANG, G.S., "Induced expression of DREB transcriptional factor and study on its physiological effects of drought tolerance in transgenic wheat", ***Acta Genetica Sinica*** *33*(5),468-476, 2006.

Kanehisa, M. Araki, M. Goto, S. Hattori, M. Hirakawa, M. Itoh, M. Katayama, T. Kawashima, S. Okuda, S. Tokimatsu, T. and Yamanishi, Y., "KEGG for linking genomes to life and the environment", ***Nucleic Acids Research*** *36*(suppl_1),480-484, 2007.

Lahlou, O. and Ledent, J.F., "Root mass and depth, stolons and roots formed on stolons in four cultivars of potato under water stress", ***European Journal of Agronomy*** *22*(2), 159-173, 2005.

Lahlou, O., Ouattar, S. and Ledent, J.F., "The effect of drought and cultivar on growth parameters, yield and yield components of potato", ***Agronomie*** *23*(3), 257-268, 2003.

Lawlor, D.W. and Cornic, G., "Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants", ***Plant, Cell and Environment*** *25*(2), 275-294, 2002.

Lawlor, D.W., "Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities", ***Journal of Experimental Botany*** *64*(1), 83-108, 2012.

Lefèvre, I., Ziebel, J., Guignard, C., Hausman, J.F., Gutiérrez Rosales, R.O., Bonierbale, M., Hoffmann, L., Schafleitner, R. and Evers, D., "Drought impacts mineral contents in Andean potato cultivars", ***Journal of agronomy and crop science*** *198*(3), pp.196-206, 2012.

Levy, D., Coleman, W.K. and Veilleux, R.E., "Adaptation of potato to water shortage: irrigation management and enhancement of tolerance to drought and salinity", ***American Journal of Potato Research*** *90*(2), 186-206, 2013.

Liu, F., Jensen, C.R., Shahanzari, A., Andersen, M.N. and Jacobsen, S.E., "ABA regulated stomatal control and photosynthetic water use efficiency of potato (*S.tuberosum L.* L.) during progressive soil drying", ***Plant Science*** *168*(3), 831-836, 2005.

Ludlow, M.M. and Muchow, R.C., "A critical evaluation of traits for improving crop yields in water-limited environments", ***Advances in Agronomy*** *43*,107-153, 1990.

Martin, J.A. and Wang, Z., "Next-generation transcriptome assembly", ***Nature Reviews Genetics*** *12*(10), 671-682, 2011.

McCue, K.F. and Hanson, A.D., "Drought and salt tolerance: towards understanding and application", ***Trends in Biotechnology*** 8,358-362, 1990.

McWilliam J, The dimensions of drought, In Baker F (ed), Drought resistance in cereals, Wallingford, UK, **CAB International**, 1989.

Medrano, H., Escalona, J.M., Bota, J., Gulias, J. and Flexas, J., "Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter", ***Annals of botany*** *89*(7), 895-905, 2002.

Mir, R.R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R. and Varshney, R.K., "Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops", ***Theoretical and Applied Genetics*** *125*(4), 625-645, 2012.

Mitra, J., "Genetics and genetic improvement of drought resistance in crop plants", ***Current Science*** 758-763, 2001.

Monneveux, P., Ramírez, D.A. and Pino, M.T., "Drought tolerance in potato (S. tuberosum L.): Can we learn from drought tolerance research in cereals?", ***Plant Science*** *205*, 76-86, 2013.

Nayyar, H. and Gupta, D., "Differential sensitivity of C 3 and C 4 plants to water deficit stress: association with oxidative stress and antioxidants", ***Environmental and Experimental Botany*** *58*(1), 106-113, 2006.

Onder, S., Caliskan, M.E., Onder, D. and Caliskan, S., "Different irrigation methods and water stress effects on potato yield and yield components", ***Agricultural Water Management*** *73*(1), 73-86, 2005.

Ort, D.R., "When there is too much light", ***Plant physiology*****,** *125*(1), 29-32, 2001.

Pennisi, E., "The blue revolution, drop by drop, gene by gene", ***Science*** *320*(5873), 171-173, 2008.

Price, A.H., Cairns, J.E., Horton, P., Jones, H.G. and Griffiths, H., "Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses", ***Journal of Experimental Botany*** *53*(371), 989-1004, 2002a.

Price, A.H., Steele, K.A., Moore, B.J. and Jones, R.G.W., "Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: II. Mapping quantitative trait loci for root morphology and distribution", ***Field Crops Research*** *76*(1), 25-43, 2002b.

Ramírez, D.A., Rolando, J.L., Yactayo, W., Monneveux, P. and Quiroz, R., "Is Discrimination of 13C in Potato Leaflets and Tubers an Appropriate Trait to Describe Genotype Responses to Restrictive and Well-Watered Conditions?" ***Journal of Agronomy and Crop Science*** *201*(6), 410-418, 2015.

Rampino, P., Pataleo, S., Gerardi, C., Mita, G. and Perrotta, C., "Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes", ***Plant, Cell and Environment*** *29*(12), 2143-2152, 2006.

Rao, K.M., Raghavendra, A.S. and Reddy, K.J. eds., Physiology and molecular biology of stress tolerance in plants, ***Springer Science and Business Media*** Berlin, 2006.

Robertson, G., Schein, J., Chiu, R., Corbett, R., Field, M., Jackman, S.D., Mungall, K., Lee, S., Okada, H.M., Qian, J.Q. and Griffith, M., "De novo assembly and analysis of RNA-seq data", ***Nature Methods*** *7*(11), 909-912, 2010.

Rontein, D., Basset, G. and Hanson, A.D., "Metabolic engineering of osmoprotectant accumulation in plants". ***Metabolic Engineering*** *4*(1), 49-56. 2002.

Schafleitner, R., "Growing more potatoes with less water", ***Tropical Plant Biology*** *2*(3-4), 111-121, 2009.

Schafleitner, R., Gutierrez, R., Espino, R., Gaudin, A., Pérez, J., Martínez, M., Domínguez, A., Tincopa, L., Alvarado, C., Numberto, G. and Bonierbale, M., "Field screening for variation of drought tolerance in *S.tuberosum L.* L. by agronomical, physiological and genetic analysis", ***Potato Research*** *50*(1), 71-85, 2007a.

Schafleitner, R., Rosales, R.O.G., Gaudin, A., Aliaga, C.A.A., Martinez, G.N., Marca, L.R.T., Bolivar, L.A., Delgado, F.M., Simon, R. and Bonierbale, M., "Capturing candidate drought tolerance traits in two native Andean potato clones by transcription profiling of field grown plants under water stress", ***Plant Physiology and Biochemistry*** *45*(9), 673-690, 2007b.

Shin, D., Moon, S.J., Han, S., Kim, B.G., Park, S.R., Lee, S.K., Yoon, H.J., Lee, H.E., Kwon, H.B., Baek, D. and Yi, B.Y., "Expression of StMYB1R-1, a novel potato single MYB-like domain transcription factor, increases drought tolerance", ***Plant Physiology*** *155*(1), 421-432, 2011.

Shinozaki, K. and Yamaguchi-Shinozaki, K., "Gene networks involved in drought stress response and tolerance", ***Journal of Experimental Botany*** *58*(2), 221-227,2007.

Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M., "Regulatory network of gene expression in the drought and cold stress responses", ***Current Opinion in Plant Biology*** *6*(5), 410-417, 2003.

Siddique, M.R.B., Hamid, A. and Islam, M.S., "Drought stress effects on water relations of wheat", ***Botanical Bulletin of Academia Sinica*** *41*, 2000.

Stark, J.C., Love, S.L., King, B.A., Marshall, J.M., Bohl, W.H. and Salaiz, T., "Potato cultivar response to seasonal drought patterns", ***American journal of potato research*** *90*(3), 207-216, 2013.

Szabados, L. and Savouré, A., "Proline: a multifunctional amino acid. ***Trends in Plant Science*** *15*(2), 89-97, 2010.

Tarn, T.R., Tai, G.C.C., De Jong, H., Murphy, A.M. and Seabrook, J.E.A., "Breeding potatoes for long-day, temperate climates", ***Plant Breeding Reviews*** *9*, 217-332, 1992.

Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., Van Baren, M.J., Salzberg, S.L., Wold, B.J. and Pachter, L., "Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation", ***Nature Biotechnology*** *28*(5), 511-515, 2010.

Tuberosa, R., "Phenotyping for drought tolerance of crops in the genomics era", ***Frontiers in Physiology*** *3*, 2012.

Urano, K., Kurihara, Y., Seki, M. and Shinozaki, K., "Omics analyses of regulatory networks in plant abiotic stress responses", ***Current Opinion in Plant Biology*** *13*(2), 132-138, 2010.

Walworth, J.L. and Carling, D.E., "Tuber initiation and development in irrigated and non-irrigated potatoes", ***American Journal of Potato Research*****,** *79*(6), 387-395, 2002.
Wang, W., Vinocur, B. and Altman, A., "Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance", ***Planta*** *218*(1), 1-14, 2003.

Wang, Z., Fang, B., Chen, J., Zhang, X., Luo, Z., Huang, L., Chen, X. and Li, Y., "De novo assembly and characterization of root transcriptome using Illumina paired-end sequencing and development of cSSR markers in sweet potato (Ipomoea batatas)", ***BMC Genomics*** *11*(1),726, 2010.

Watkinson, J.I., Hendricks, L., Sioson, A.A., Vasquez-Robinet, C., Stromberg, V., Heath, L.S., Schuler, M., Bohnert, H.J., Bonierbale, M. and Grene, R., "Accessions of *S.tuberosum L.* ssp. andigena show differences in photosynthetic recovery after drought stress as reflected in gene expression profiles", ***Plant Science*** *171*(6), 745-758, 2006.

Wu, C.A., Lowry, D.B., Nutter, L.I. and Willis, J.H., "Natural variation for drought-response traits in the Mimulus guttatus species complex", ***Oecologia*** *162*(1), 23-33, 2010.

Wu, Y. and Cosgrove, D.J., "Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins", ***Journal of Experimental Botany*** *51*(350), 1543-1553, 2000.

Xu, P., Liu, Z., Fan, X., Gao, J., Zhang, X., Zhang, X. and Shen, X., "De novo transcriptome sequencing and comparative analysis of differentially expressed genes in Gossypium aridum under salt stress", ***Gene*** *525*(1), 26-34, 2013.

Young, M.D., Wakefield, M.J., Smyth, G.K. and Oshlack, A., "Gene ontology analysis for RNA-seq: accounting for selection bias", ***Genome Biology*** *11*(2), 14, 2010.

Zhu, X., Gong, H., Chen, G., Wang, S. and Zhang, C., "Different solute levels in two spring wheat cultivars induced by progressive field water stress at different developmental stages", ***Journal of Arid Environments*** *62*(1), 1-14, 2005.

APPENDIX-A List of chemicals

TRIzol Reagent	Thermo Fisher Scientific
Agarose	SIGMA
EtBr	Thermo Fisher Scientific
Gene Ruler	Thermo Fisher Scientific
Omniscript RT Kit	QIAGEN
RNase inhibitor	Thermo Fisher Scientific
DreamTaq DNA Polymerase	Thermo Fisher Scientific
dNTP mix	Thermo Fisher Scientific
Oligo dT primer	Thermo Fisher Scientific

CURRICULUME VITAE

Mohammad Hussain AZIMI was born on November 25, 1990 in Ghazni, Afghanistan. After completing his high school, he attended to YÖS Exam (University Entrance Exam for Foreigner in Republic of Turkey) in 2010. He completed his B.Sc. in Food Engineering from SAKARYA University, Turkey. He enrolled in Graduate School of Natural and Applied Sciences, Department of Agricultural Genetic Engineering at Niğde Ömer Halisdemir University, Niğde, Turkey to pursue his master education under the guidance of Dr. Zahide Neslihan ÖZTÜRK GÖKÇE. During his master thesis research he worked on comparison of drought stress response of potato varieties at the transcriptomic level. He knows English, Turkish and Persian languages.

ACHIEVMENT(s) OBTAINED DURING THESIS RESEARCH

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