TRANSCRIPTOME ANALYSIS OF COMMON BEAN UNDER SALT STRESS AND FUNCTIONAL STUDY OF SALT RESPONSIVE GENES

by

Mahmut Can Hız

B.S., Molecular Biology and Genetics, Boğaziçi University, 2007

Submitted to the Institute for Graduate Studies in
Science and Engineering in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

Graduate Program in Molecular Biology and Genetics
Boğaziçi University
2015

To my great family and dearest wife…

ACKNOWLEDGEMENTS

First of all, I would like to express my gratitude to my thesis supervisor Prof. Müge Türet for her guidance, supervision and evaluations throughout my studies and for her endless efforts and contributions to the beginning of my academic life. She also deserves special thanks for her patience to an obstinate graduate student.

I would like to thank Prof. Kuyaş Buğra not just for her time and efforts to as a member of my thesis progress committee but for her valuable evaluations to all my applications and submissions as well as for her endless support and guidance to overcome the obstacles I faced. I would like to thank my thesis committee members Prof. Esra Battaloğlu, Prof. Hikmet Budak and Assist. Prof. İbrahim Yaman for their support, criticisms and valuable time.

I would also like to present my gratitude to Assoc. Prof Stefan Fuss and other faculty members for creating an opportunity for us to exchange knowledge and ideas with other researchers in our fields by making MGB-BRIDGE possible. I would also thank Prof. Yıldız Daşgan from Çukurova University, Adana and Dr. Stefanio Grillo from CNR-IGV, Italy for their hospitability and supervision during my stays in their labs.

I would like to thank my dearest colleagues Harun Niron and İsmail Tuğberk Kaya and all former and present members of PLANT, CMT and RETINA groups also for their endless support and friendship inside and outside of the lab. Aslı Uğurlu, Burçak Özeş and Neslihan Zohrap deserves special thanks for tolerating my big brother attitudes to them. I would also thank to other members of MBG from the head to the technicians and janitors.

The last but not least, I have special thanks to my great family and my dearest wife for their supports and encouragements throughout my life.

This thesis was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK 110T922) and Bogazici University Research Funds (BAP 6674).

ABSTRACT

TRANSCRIPTOME ANALYSIS OF COMMON BEAN UNDER SALT STRESS AND FUNCTIONAL STUDY OF SALT RESPONSIVE GENES

Salinity is a major abiotic stress that impairs physiology and productivity of economically important crops such as common bean. It is an important grain legume and provides 30% of the protein diet in developing countries. Several common bean genotypes have been phenotypically classified according to its diverse responses to salinity. In this study, a comprehensive high-throughput transcriptome analysis in root and leaf tissues of common bean genotypes with contrasting salt tolerance was performed under stress in hydroponic conditions. Prior to transcriptome analysis, the stress onset time was determined as the end of 3rd day of 125mM NaCl application for tolerant Ispir and susceptible TR43477 varieties following several physiological measurements. Transcriptome analysis have generated 255 million high quality reads which were assembled into 73762 all-unigenes with a mean length of 930 bp. Among the all-unigenes, 75% (55433) were assigned with Nr annotations. A total of 12001 differentially expressed genes (DEGs) were identified. Validation of the RNA-seq quantifications (RPKM values) were performed by qRT-PCR analysis. Within the selected candidate gene pool with contrasting expression levels between the two genotypes and two tissues, five genes (*Unc2*, *Unc4*, *TT12*, *CPRD2* and *β-Glu*) were cloned and transformed to Arabidopsis Col-0 ecotype to generate homozygous overexpression transgenic lines. Functional studies on these lines by both physiological assays and their impact on the expression of salt responsive genes as well as the transcription factors (TFs) regulating them, suggested a highly significant regulatory role for *TT12* and *CPRD2* genes in improvement of salt tolerance. Major contributions of these genes were implied for better management of osmotic and ionic imbalance most probably by imposing synthesis, accumulation and sequestration of osmolytes and reinforcement of the cell wall structure while minimizing damage through ROS scavenging. To do so, regulation by interacting ABA -independent and ABA-dependent expression of *DREB2A*, *MYB2* TFs and downstream *P5CS1* and *RD29B* genes were also implicated.

ÖZET

TUZ STRESİ ALTINDA TAZE FASÜLYE BİTKİSİNİN TRANSKRİPTOM ANALİZİ VE TUZA CEVAP VEREN GENLERİN İŞLEVSEL ANALİZLERİ

Tuzluluk, taze fasülye gibi ekonomik önemi olan bitkilerin fizyolojilerine ve verimliliklerine zarar veren başlıca abiyotik stres faktörlerinden birisidir. Taze fasülye, gelişmekte olan ülkelerin protein ihtiyacının %30'unu karşılayan önemli bir baklagil türüdür. Birçok taze fasülye genotipi, tuzluluğa verdikleri cevaba göre fenotipik olarak sınıflandırılmıştır. Tuza dayanıklılık açısından zıt özellikler taşıyan iki taze fasülye çeşidinin su kültürü ortamında ve tuz stresi altında, yaprak ve kök dokularından kapsamlı yüksek-çıktılı transkriptom analizi yapılmıştır. Transkriptom analizinden önce fizyolojik ölçümler yapılarak dayanıklı Ispir ve duyarlı TR43477 genotiplerinin strese giriş zamanı 125 mM tuz uygulamasının 3. gününden sonrası olarak belirlenmiştir. Transkriptom analizi sonucunda elde edilen 255 milyon yüksek kalite okuma, ortalama uzunlukları 930 baz çifti uzunluğunda 73762 transkriptte birleştirilmiştir. Bu transkriptlerin %75'i (55433) Nr protein veribankasındaki proteinler ile eşleşmiştir. Toplamda, anlatımı değişen 12001 gen tespit edilmiştir. Transkriptomdan elde edilen gen anlatım düzeyleri, qRT-PZR analizi ile doğrulanmıştır. Farklı doku ve genotiplerdeki anlatım seviyelerine bakılarak seçilen fasülye aday genlerinden 5'i (*Unc2, Unc4, TT12, CPRD2* ve *β-Glu*) klonlanabilmiş ve Arabidopsis Col-0 bitkisine aktarılarak homozigot transgenik hatlar elde edilmiştir. Bu hatlarda yapılan fizyolojik ölçümlere ve tuz stresinde anlatımı artan genlerin ve bu genleri regüle eden transkripsiyon faktörlerinin anlatımlarındaki etkilerine bakılarak yapılan işlevsel çalışmalar *TT12* ve *CPRD2* genlerinin tuza dayanıklılıkta düzenleyici roller olabileceğini ortaya koymuştur. Bu genlerin başlıca katkılarının, hücre duvarının güçlendirilmesi, oksidan zararlarının minimize edilmesi, ozmolitlerin sentezi, biriktirilmesi ve depolanması sayesinde ozmotik ve iyonik dengenin sağlanması olduğu düşünülmektedir. Bulgularımız, *DREB2A* ve *MYB2* transkripsiyon faktörlerinin ve regüle ettikleri *P5CS1* ve *RD29B* genlerinin ABA-bağımlı ve ABA-bağımsız anlatımlarınının rollerine de işaret etmektedir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

bp	Base Pairs
Ca^{+2}	Calcium ion
Cl^{-}	Chloride Ion
d	Day
g	Gravity
gr	Gram
hr	Hour
K^{+}	Potassium ion
m	Meter
mg	Milli gram
min	Minute
ml	Milli Liter
mM	Milli Molar
Na^{+}	Sodium ion
nM	Nano Molar
nt	Nucleotide
rpm	Revolutions per Minute
s	Second
µg	Micro Gram
µM	Micro Molar
µmol	Micro Mol
°C	Centigrade Degree

LIST OF ACRONYMS /ABBREVIATIONS

ABA	Abscisic Acid
Act	Actin
AP /ERF	APETALA2/Ethylene Responsive Factor
BLAST	Basic Local Alignment Seach Tool
BNS	Basal Nutrient Solution
bZIP	Basic Leucine Zipper Domain
$Ca(NO_3)_2$	Calcium Nitrate
$CaCl_2$	Calcium Chloride
CaMV	Cauliflower mosaic virus
cDNA	Complementary DNA
COG	Clusters of Orthologous Groups
Col	Colony
Col-0	Colombia Type 0
CPRD2	Cow Pea Response to Drought 2
$CuSO_4$	Cupper Sulphate
DEG	Differentially Expresse d Gene
dH2O	Distilled Water
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribo Nucleic Acid
dNTP	Deoxynucleotide tri phosphate
DREB	Dehydration responsive element binding
DW	Dry Weight
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic acid

FAD	Flavin Adenine Dinucleotide
FDR	False Discovery Rate
FW	Fresh Weight
Fwd / F	Forward
GM	Germination Medium
GO	Gene Ontology
H_3BO_3	Boric Acid
HCl	Hydrochloric acid
IDE	Insulin Degrading Enzyme
ILDW	Ispir Leaf Downregulated
ILUP	Ispir Leaf Upregulated
IRDW	Ispir Root Downregulated
IRUP	Ispir Root Upregulated
KCl	Potassium Chloride
KEGG	Kyoto Encyclopedia of Genes and Genomes
KH_2PO_4	Monopotassium Phosphate
KNO_3	Potassium Nitrate
L	Line
LB	Luria-Bertani
LC	Ispir Leaf Control
LEA	Late Embryogenesis Abundant
LS	Ispir Leaf Salt
$MgSO_4$	Magnesium Sulphate
MII	Membrane Injury Index
$MnCl_2$	Manganese(II) chloride
MS	Murashige and Skoog

MYB	Myeloblastosis
Na_2MoO_3	Sodium Molybdate
NAC	NAM ATAC CUC
NaCl	Sodium Chloride
NaFe(III)EDTA	Sodium iron EDTA
NH_4NO_3	Ammonium nitrate
NR	Non-redundant
NTC	No Template Control
OD	Optical Density
OE	Over expression
P5CS1 / P5CS2	Delta 1-pyrroline-5-carboxylate synthase
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcription PCR
RAB18	Responsive to ABA
RC	Ispir Root Control
RD26 / RD29B	Response to Drought
Rev / R	Reverse
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPKM	Reads Per Kilobase per Million Mapped reads
RS	Ispir Root Salt
RT	Reverse Transcription
RWC	Relative Water Content
SD	Standard Deviation
SEM	Standard Error of Mean
TAE	Tris Acetate EDTA

Taq	*Thermus aquaticus*
TBE	Tris Borate EDTA
TF	Transcription Factor
TLC	TR43477 Root Control
TLDW	TR43477 Leaf Downregulated
TLDW	TR43477 Root Downregulated
TLS	TR43477 Root Salt
TLUP	TR43477 Leaf Upregulated
TRC	TR43477 Root Control
TRS	TR43477 Root Salt
TRUP	TR43477 Root Upregulated
TT12	Transparent Testa 12
TW	Turfor Weight
Unc	Uncharacterized
YEP	Yeast Extract Phosphate
ZnSO4	Zinc Sulphate
β-Glu	Endo-1,3-beta-glucanase

1. INTRODUCTION

1.1. Salinity as an Abiotic Stress Factor

Plants as sessile organisms continuously battle against the adverse effects of environmental stress factors which can be biotic (bacterial, viral, fungal or herbivore attacks) or abiotic (drought, salinity, cold, heat and heavy metals etc.) in nature [1, 2]. Plants may exposed simultaneously or one at a time to these factors and face cellular and organismal dysfunction and instability. World population is increasing at an alarming rate but the agricultural production is not following similar pace [3]. It is predicted that the overall food production needs to be increased by 60% - 110% [4, 5] to feed 9.5 billion people in 2050 (faostat.fao.org). Biotic and abiotic factors are the major constrains of agricultural improvement to meet food demands of increasing population [3].

Salinization is the accumulation of water-soluble ions in the soil solum (the upper part of a soil profile) to a level that effects on agricultural production, environmental health, and economic welfare. Soil is considered saline if the electrical conductivity (EC) of its saturation extract is above 4 dS/m (deciSiemens per meter) which is equivalent to approximately 40 mM NaCl [6]. Earth soil salinization is primarily due to proximity to sea, heavy rainfall and rock weathering. When such climatic and landscape features are combined with the secondary salinization created by wrong irrigation practices, bad drainage, or poor water quality, they create major deleterious effects on plant productivity [3, 7, 8]. Today 33% of the irrigated lands and 20% of the cultivated lands suffer from salinity [9]. In year 2050, salinity of arable lands is expected to reach up to 50% [10]. It is estimated that 1,5 million hectares of Turkey’s arable lands which make up 40% of the irrigated lands have production limitations due to salinity [11].

1.2. Effects of Salinity on Plants

Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, changes in plant metabolic processes and an imbalance in ion homeostasis [12]. Plants growing under saline conditions are stressed basically by two reasons. First, reduced water potential in the root zone cause water deficit which is referred as osmotic effects. Second, elevated Na^+ ions generate toxicity for cells which is also called salt specific or ion-excess effect [13-15]. The end result is tissue damage and cause reduction in germination, photosynthesis and growth rates in plants.

Increased levels of salinity leads reduction in shoot and root growth within hours of exposure [16] due to decrease in turgor pressure below the necessary threshold level [17] for cellular expansion [18]. Elevated levels of positively charged sodium (Na^+) and negatively charged chloride ($Cl^−$) ions interfere with various cellular functions by competing with other nutrients such as K^+, Ca^{+2} , and NO^{-3} creating ionic toxicity and injury in the tissues [19, 20]. Salinity also effects germination, which is a vital phase in the growth cycle, by decreasing the imbibition level due to high osmotic potential of growth environment as well as causing alterations in enzyme activities [21], protein metabolism [22] and hormonal balance [23].

1.2.1. Role of ABA and Transcription Factors in Salinity Tolerance

Abscisic acid (ABA) is a hormone that stands out as a major player in plant development as well as shaping responses to abiotic stresses including salinity. Information on physiological effects of ABA, biosynthesis/degradation pathways and to a lesser extent transcription factors activated by ABA signaling and ABA target genes have accumulated in literature over the years.

Accumulation of ABA (abscisic acid) not only increases its own biosynthesis by positive feedback loops but also upregulates the expression of cyclin-dependent kinase inhibitors to arrest cell cycle and stimulate stress regulatory proteins and osmoprotectants through ABA-induced transcription factors (TFs). Some TFs are orchestrated by ABA-dependent signaling pathways whereas others play role in salt tolerance in an ABA-independent manner [24] (Figure 1.1). Although it has been suggested that there is distinct regulation at transcription factor level based on ABA, analysis of downstream molecular mechanisms demonstrated that these two pathways are working in parallel with a crosstalk to control gene expression under abiotic stress conditions [25, 26]. ABA-Responsive Element (ABRE) motif is found in the promotor region of ABA-inducible genes [27]. ABRE binding proteins, *AREB1* and *AREB2*, belong to bZIP TF family, induced by stress conditions and play role in transcriptional activation of several proteins including LEA family protein *RD29B* [28]. *Glycine max* bZIP TF family proteins, *GmbZIP44*, *GmbZIP62* and *GmbZIP78* were reported to play role in salt and cold stress and cause upregulation of several functional genes including *P5CS1* [29] and cause an elevation in proline biosynthesis. MYB and MYC TF families are also known to be involved in ABA-dependent stress response pathways. It has been reported that *AtMYB2* overexpression induced ABA responsive genes and transgenic lines had an ABA hypersensitive phenotype [30].

ABA-independent stress response is majorly regulated by Dehydration Responsive Element Binding (DREB) motif containing TFs. They belong to *AP2*/*ERF* family TFs and have two subclasses named DREB1 and DREB2, induced by cold and drought, respectively. It was shown that several stress induced genes were upregulated in *DREB2A* transgenic Arabidopsis plant [31]. Although *DREB2A* was considered to be a member of ABA independent pathway, later on identification of ABRE-like sequences in its promoter region [32] suggested that the *DREB2A* might also act synergistically with ABRE family TFs of ABA-dependent pathway, via interacting directly [33, 34].

The *NAC* TF family may regulate genes in both ABA-dependent and ABA-independent manner. A member of this family *ERD1* was reported to be upregulated upon drought and salinity but not with ABA treatment [35, 36]. Transgenic plants overexpressing another member of this family, *RD26* was reported to upregulate ABA-inducible stress genes and show an ABA-sensitive phenotype [37].

Although there is a distinction between ABA-dependent and ABA-independent pathways based on promotor analysis of downstream genes, many studies revealed that there is a crosstalk between these pathways [24, 27, 37-39]. The *RD29A* gene has both DREB and ABRE binding sites in their promoters. It can be regulated by DREB TFs within 20 min after dehydration [40, 41], cold and salt stresses, ABA accumulation after 2 hours of dehydration and salinity also induce its expression via ABRE TFs [35]. Such crosstalk was also reported with elevated expression of ABA-dependent *RAB18*, *RD29B* and ABA-independent *COR15a*, *RD29A* genes in *OsDREB1F* transgenic rice plants [42]. The convergence of two pathways were thought to be due to the requirement of cofactors for the activation of ABA–

independent *DREB2A* via phosphorylation / dephosphorylation [43] which are dependent on ABA induced proteins like *ABI1*, *ABI2* or Ca^{+2} dependent protein kinases.[44]. Specifically, members of subclass III SnRK2 (SNF-1 protein kinase 2) kinases were reported to act as a crossing point for ABA-dependent and ABA-independent pathways [45].

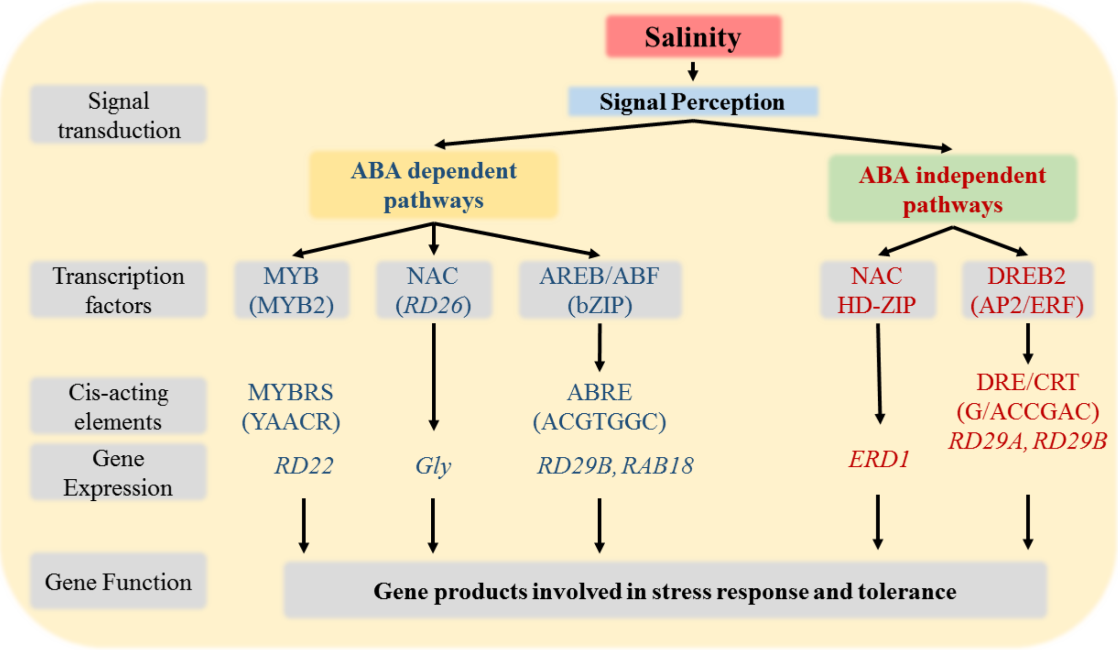


Figure 1.1. Gene networks involved in salinity stress response. Adapted from Shinozaki and Yamaguchi-Shinozaki, 2007.

1.3. Mechanisms of Salinity Tolerance in Plants

Susceptibility or tolerance responses to salt stress like any other abiotic stresses are not linear pathways, but are complex circuits involving multiple gene products, specific cellular compartments, and the interaction of additional cofactors and/or signaling molecules [46]. Plants employ various physiological and biochemical mechanisms to survive in saline environment. Maintenance of ion homeostasis via compartmentalization of ions, biosynthesis of osmoprotectants, antioxidants and polyamines, generation of nitric oxide and modulation signaling pathways are the principle mechanisms underlying salinity tolerance [47].

Ion homeostasis is crucial for all living organisms. To keep the concentration of toxic ions low while accumulating essential ions, cells need a tight control mechanism of ion flux [48]. Fundamentally, plants cope with ion toxicity by either avoiding or tolerating the influx of toxic ions [49]. Although halophytes essentially employ avoidance mechanisms, glycophytes try to tolerate salinity by minimizing osmotic stress or ion disequilibrium and alleviating the consequent secondary effects. In most crop plants (glycophytes), ionic imbalance is the primary cause of ion toxicity due to replacement of K^+ by Na^+ ions. Since normally K^+ acts as a cofactor for several enzymes and required for protein synthesis, K^+ should not be substituted by Na^+ therefore, management of cellular Na^+ concentration is critical for salt tolerance [50]. This occurs by four major mechanisms [51]; i) prevention of entry of excess sodium ions to the root cortex cells via Na^+/K^+ co-transporters such as high-affinity K^+ transporter (HKT1) by the casparian strip in the endodermis ii) excretion of Na^+ from root cells by plasma membrane Na^+/H^+ antiporters , which is regulated by salt overly sensitive (SOS) signaling and elevated Ca^{+2} levels, iii) retrieving Na^+ from the transpirational xylem stream to recirculate back into the roots by SOS1 and HKT1 and iv) sequestering of Na^+ into the vacuoles by vacuolar Na^+/H^+ antiporters (NHXs) as an cost effective strategy for osmotic adjustment.

The most important defense mechanism of plants to cope with the osmotic imbalance is synthesis of osmolytes which are polar, uncharged, soluble and non-toxic molecules even at high concentrations [47]. Proline [52], sugars [53], polyols [54] glycine betaine [55] and flavonoids are the main cellular osmolytes. Although synthesis of some of these compounds occurs in a few species, proline accumulation is a common defense to osmotic stress in most of the plant species [54]. When the proline accumulation increases upon salt stress, the concentration of amino acids such as cysteine, arginine and methionine decreases [56]. Additionally proline functions as nitrogen source at restricted conditions in nutrient uptake as well as oxygen quencher in the cytoplasm as an important anti-oxidant [57-60]. Also the intermediate molecules during proline synthesis were reported to induce deyhdrin (LEA family proteins) synthesis [61]. Under stress conditions, proline is synthesized mostly from glutamate precursor by pyrroline carboxylate synthase (P5CS) enzymes [62] in an ABA-dependent manner [63]. Thus, an elevation in the expression level of these genes was used as molecular maker under osmotic and salinity stress conditions in several reports [64, 65].

Accumulation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical (OH) are another events. It occurs under several stress conditions which is mostly caused by disruption in electron transport chain (ETC) in chloroplasts and mitochondria. Although a certain level of ROS is required for signaling and cell metabolism, their accumulation is harmful for cellular integrity and photosynthetic machinery [66]. Detoxification of ROS is mainly dependent on antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione and ascorbate peroxidases (GPX, APX) and gluthatione reductase (GR).

Generally salt signal transduction mechanism involves perception of the stress signal (ionic/osmotic imbalance) by cytoplasmic, nuclear or membrane bound receptors (G-protein coupled-receptors, ion channels, histidine kinases etc.), generation of secondary molecules (Ca^{+2}, inositol triposphate, ROS etc.), activation of signaling proteins (Ca^{+2} sensors, kinases/phosphatases, ion pumps), expression of transcription factors and major stress response genes [16].

1.4. *Phaseolus vulgaris* as a Salt Sensitive Crop

As a member of grain legumes, common bean (*Phaseolus vulgaris* L.) is a major source of human dietary proteins, minerals, vitamins, and represents nearly half of the consumed grain legumes worldwide [67]. Common bean is also vital in agriculture as it forms root nodules via symbiotic associations with nitrogen fixing bacteria [67]. Although there exist an extensive genetic diversity for salt tolerance in plant taxa, most crops are salt sensitive or hypersensitive plants (glycophytes). Among those, *Phaseolus vulgaris* L. (common bean) is known to be the most susceptible species to salinity. Even at 1 dS/m saline soil which considered as slightly saline, reduction in common bean productivity can reach up to 20% [68].

In the first decade of 21st century, more than 17 million metric tons of common bean was produced annually in the world. China leads common bean production in the world and Turkey is the third biggest producer (http://faostat3.fao.org). Production in Turkey is highly dominated by middle Blacksea region Production in Turkey is highly dominated by middle

Blacksea region and can reach up to 2-4 dS/m during irrigated seasons due to high salinity level of Kızılırmak river as the major water resource [69, 70].

The common bean variety ‘‘Ispir’’ used in this study is known to be a very old local genotype named after Ispir county of Erzurum province where it has been produced more than 150 years. The variety has been registered in 2008 and patented as ‘‘Geographic Trademark’’ by Turkish patent office (www.tpe.gov.tr). The ability of the variety to tolerate high salt concentrations without compromising germination time is well documented [71]. Another variety used in this study was TR43477 which was reported to be sensitive to salt stress [72]. In this study, a comprehensive transcriptome analysis under salinity conditions was performed to reveal the differences in response to salt stress in these two varieties of common bean one with its known salt tolerance and the other with its sensitivity.

1.5. Transcriptome Analysis

As the environmental stress factors change, plants develop many strategies to adapt and survive. Several omics approaches such as genomics, transcriptomics, proteomics, and metabolomics have been employed to analyze the changes in plants at the molecular level (reviewed in [73]). Among them genomics creates a basis for transcriptomics and proteomics studies by generating gene annotations. Reference genome of several legumes have been published in the last decade including *Glycine max* (soybean) [74], *Lotus japonicus* (Lotus) [75], *Medicago truncatula* (barrel medic) [76], Cajanuc cajan (pigeon pea) [77], *Cicer arietinum* (chickpea) [78] and finally *Phaseolus vulgaris* (common bean) [79]. However, for the genome data to be meaningful, understanding the plant response mechanisms against environmental stressors, the expression level of transcripts and proteins should be investigated [80]. Transcriptome studies are the first step to understand these mechanisms and widely employed for decades by using expressed sequence tags (ESTs), suppression subtractive hybridization (SSH) and microarray approaches [81]. Although these approaches generate valuable information about expression profile of plants, they did not allow for to compare and detect all the genes in the plant genome [74, 82, 83]. More recently next generation sequencing technologies such as RNA-seq enables high-throughput analysis of whole transcriptome and quantifies the gene expression levels with low cost even without the need of prior sequence information. The accuracy of results in detection of gene expression levels has proven to be higher than previous approaches when results from proteomic studies were compared [84].

1.5.1. Transcriptome Studies on Leguminous Plants

Reports on RNA- sequencing data and expression profiling on both model plants and agriculturally important crops to identify genes involved in stress responses were increased dramatically in recent years [85-93]. Transcriptome studies on legumes was also increased recently. The collection of ESTs generated by several other methods from different organs of legumes related to environmental stress factors such as phosphorus starvation, rust disease resistance, and drought stress [94, 95] can not be underestimated. However, there are still few studies on transcriptome analysis under abiotic stress conditions in these species.

There are only four transcriptomic studies in the literature on common bean performed by high-throughput RNA-sequencing approach. These can be listed as general transcriptome assembly [96], data-mining of host resistance gene like sequences within the transcriptome [97], analysis of sulfur metabolism in developing seeds [98] and analysis of drought responsive genes via *de novo* assembly of short reads [99]. Although, there are several reports on the physiological responses to salt stress in common bean [7, 71], transcriptome profiling in common bean covering whole genome analysis has not been reported, yet. Thus, we used the Illumina high-throughput RNA- sequencing platform for comparative *de novo* transcriptome analysis of leaf and root tissues of salt tolerant (Ispir) and salt susceptible (TR43477) common bean varieties. DEGs and related pathways were determined by comprehensive analysis of RNA-seq data from both tissues of both genotypes grown under saline and control hydroponic conditions. Based on sequencing data, functional annotations of unigenes, and their characterization with gene ontology and metabolic pathway analysis, a potential pool of candidate genes was generated for functional characterization.

1.6. Functional Characterization by Biotechnological Approaches

The information filtered out from the omics studies revealed that there are dozens of potential candidate genes which may have role in response to adverse effects of stress in plants [91, 100-102]. Generation of transgenic and mutant lines, regulation of gene expression and genetic alterations are extensively used approaches to study the impacts of genes under a particular biotic and abiotic stress condition [103-106]. Overexpression of genes in model organisms is one of the majorly employed technique to perform functional studies. For example, the effect of wheat *NF-YA10* gene under salt stress in Arabidopsis [107], tomoato *WRKY1* gene against pathogen attack in tobacco [108], rice calmodulin-like gene *OsMSR2* under salt and drought stress in Arabidopsis [109] and sheep grass transcription factor *LcMYB1* under salt stress in Arabidopsis [104] were successfully demonstrated.

In this study, the salt responsive candidate genes were selected from the upregulated transcripts of the *P. vulgaris*. Among the 13 candidate genes, and five of them were transferred to Arabidopsis plants under the control of 35S cauliflower mosaic virus (CMV) promoter to generate homozygous overexpression lines. The two of these were novel genes (*Unc2* and *Unc4*) without a significant similarity to the proteins in public databases (Nr database, accessed in August, 2014). The other three were shown significant similarity to transparent testa 12 (*TT12*) in *Medicago truncatula*, cowpea clones responsive to dehydration 2 (*CPRD2*) genes in cowpea and endo-1,3-beta-glucosidase (*β-Glu*) of *Glycine max.*

It was reported that *TT12* acts as a vacuolar flavonoid/H^+-antiporter active in proanthocyanidin (PA)-accumulating cells of the seed coat [110]. It is a MATE transporter protein in tonoplastic membrane and mostly associated with seed color formation [111] as well as transport of monomeric glycosylated flavanols as PA precursors [111]. However its role under abiotic stress conditions has not been evaluated, yet.

CPRD2 was identified in cowpea as a dehydration responsive protein [112] of berberine-bridge (BBE) flavanoprotein subgroup containing an FAD binding domain and has a putative role in isoquinoline alkaloid biosynthesis (a secondary metabolite) [113]. There is a great diversity in the number of BBE-like proteins among species. For example, while *Physcomitrella patens* has only one BBE-like enzyme, this number reaches to 28 in Arabidopsis and 64 in *Populus trichocarpa* genome [114]. It was reported that BBE-like enzymes were expressed in root elongation, maturation, and proliferation as well as embryonal development. Up to 400 fold increase in their expression level was detected under osmotic stress and pathogen attack in Arabidopsis [115]. This finding was also supported by other studies in different species [116, 117]. Recently, it was demonstrated that these proteins play role in oxidation of mono lignols which indicates that they may also have a role in cell wall metabolism in response to abiotic stresses [118].

Glycosyl hydrolase superfamily protein *β-Glu* catalyzes the hydrolysis of 1,3-β-D-beta- glucosidic linkages in 1,3-β-D-glucans. Glucans are the most abundant molecule in fungal cell wall and marine macro-algae [119]. In plants *β-glu* also play role in cell wall biogenesis [120] and observed to be upregulated during salinity [121-123]. Additionally, degradative activity for fungal cell walls during pathogen attacks was suggested [124, 125]. Functional identification of these candidates in our study highlighted their role in response to salt stress.

2. PURPOSE

Salt stress tolerance is a complex trait and involves activities of vast array of genes. Understanding the underlying mechanism of tolerance requires cumulative knowledge at the molecular level. For this purpose we have performed,

(i) to determine differentially expressed salt response genes using comprehensive comparative transcriptome analysis of two common bean genotypes with contrasting salt stress response using two different tissue types
(ii) to characterize and annotate these genes and identify the metabolic pathways in which they are involved
(iii) to characterize certain salt responsive candidate genes through generation of overexpression transgenic lines in Arabidopsis plant using physiological parameters and quantitative expression analysis of salt stress associated regulatory genes to uncover their role in tolerance mechanism.

3. MATERIALS

3.1. General Enzymes, Kits and Reagents

Enzymes, kits and reagents in Table 3.1 were purchased from Thermo Fisher Scientific, (Wilmington, DE) unless stated otherwise.

Table 3.1. List of enzymes, kits and reagents.

Name	**Brand**
Total RNA Extraction Kit	74104, RNeasy Plant Mini Kit, Qiagen, Hilden, Germany
Gel Extraction Kit	K0691, GeneJET Gel Extraction Kit
Plasmid Isolation Kit	740615, Nucleospin Plasmid Isolation Kit, Macherey-Nagel, Germany
First Strand cDNA Synthesis Kit	K1672, Maxima First Strand cDNA Synthesis Kit for RT-PCR with Dnase
High-Fidelity DNA polymerase	F-530S, Phusion, Thermo Fisher Scientific, Wilmington, DE
qPCR Master Mix	K0222, Maxima SYBR Green/ROX qPCR Master Mix, K-6252 AccuPower 2X Greenstar Master Mix, Bioneer, Daejon, Korea
DNA ladder	SM0311, GeneRuler 1 kb DNA Ladder SM0321, GeneRuler 100 bp Plus DNA Ladder SM0241, GeneRuler 100 bp DNA Ladder
DNA loading Dye	R0611, DNA Gel Loading Dye (6X)
RNA Ladder	SM1821, RiboRuler High Range RNA Ladder
RNA Loading Dye	R0641, RNA Gel Loading Dye (2X)
BP Clonase	11789-020, Gateway BP Clonase II Enzyme mix
LR Clonase	11791-100, Gateway LR Clonase II Enzyme mix
DNA Polymerase	18038042, *Taq* DNA Polymerase
Magnesium Chloride	R0971, MgCl2 (magnesium chloride) (25 mM)
dNTPs	R0191, dNTP Mix (10 mM each)

3.2. Chemicals, Plastics and Glassware

All chemicals were purchased from *Sigma-Aldrich* (USA), *Merck* (Germany), *Applichem* (Germany) or *Duchefa* (Netherlands) unless stated otherwise. Tips and tubes, sterile plates and falcon tubes were ordered from *Axygen* (USA), *Interlab, and BD Biosciences* (USA), respectively. All glassware, tips and tubes were sterilized by autoclaving at 121°C for 20 minutes.

3.3. Buffers and Solutions

Table 3.2. List of common buffers and solutions.

Solution Name	**Concentration**
10X TBE buffer	1M Tris base, 1M Boric Acid, 20mM EDTA
50X TAE buffer	2M Tris base, 5.71% (v/v) Glacial acetic acid, 50 mM EDTA, pH:8.0
1X TE buffer	10mM Tris HCl, 1mM EDTA, pH:8
LB Medium	1% (w/v) Tryptone, 0.5% (w/v) NaCl, 0.5% (w/v) Yeast extract, pH: 7.5
LB Agar Medium	1% (w/v) Tryptone, 0.5% (w/v) NaCl, 0.5% (w/v) Yeast extract, 1,5% (w/v) Bacteriological Agar, pH:7.5
YEP Medium	1% (w/v) Peptone, 0.5% (w/v) NaCl, 1% (w/v) Yeast extract, pH: 7.5
YEP Agar Medium	1% (w/v) Peptone, 0.5% (w/v) NaCl, 1% (w/v) Yeast extract, 1% (w/v) Bacteriological Agar, pH:7.5
MS Agar Medium	0.44% (w/v) Murashige Skoog Basal Medium, 1% (w/v) Agar Type A
Kanamycin	50 mg/ml in sterile distilled water
Rifampicin	50 mg/ml in sterile distilled water
Spectinomycin	50 mg/ml in sterile distilled water
Gentamicin	30 mg/ml in sterile distilled water
Proline Reaction Mixture	0.6% (w/v) Sulfosalicyclic acid, 40% (v/v) Glacial acetic acid, 40% (v/v) Acidic ninhydrin (2.5% (w/v) Ninhydrin (1,2,3-indantrione monohydrate), 60% Glacial acetic acid, 2.4 M orthophosphoric acid)
Proline Stock Solution	5 mg/mL Proline in distilled water

Table 3.3. Modified Hoagland solution for hydroponic growth of common bean [72].

Macronutrients	**Concentration (mM)**
$Ca(NO_3)_2$	3
$MgSO_4$	1
K_2SO_4	0.9
KH_2PO_4	0.2
Micronutrients	**Concentration (nM)**
Fe-EDDHA	0.1
H_3BO_3	10
$MnSO_4$	1
$ZnSO_4$	1
$CuSO_4$	0.1
$(NH)_6Mo_7O_{24}$	0.01

Table 3.4. Hydroponics growth and germination medium for *A.thaliana* [126].

Macronutrients	**Germination Medium (GM) Final conc (mM)**	**Basal Nutrient Solution (BNS) Final conc (mM)**
NH_4NO_3	-	2
KNO_3	-	3
$CaCl_2$	0.75	0.1
KCl	1	2
$Ca(NO_3)_2$	0.25	2
$MgSO_4$	1	2
KH_2PO_4	0.2	0.6
NaCl	-	1.5
Micronutrients	**Germination Medium (GM) Final conc (µM)**	**Basal Nutrient Solution (BNS) Final conc (µM)**
NaFe(III)EDTA	50	50
H_3BO_3	50	50
$MnCl_2$	5	5
$ZnSO_4$	10	10
$CuSO_4$	0.5	0.5
Na_2MoO_3	0.1	0.1

3.4. Equipments

Table 3.5. List of equipments.

Name	**Model & Brand**
Thermal Cycler	C1000 Thermal Cycler, Bio Rad, USA Runik Thermal Cycler, Sacem Life Technologies, Turkey
Agarose Gel Electrophoresis	Minicell Primo EC320, Thermo Fisher Scientific, USA
Power Supply	164-5050 PowerPac Basic, Bio Rad, USA EC250-90, Thermo Fisher Scientific, USA
Gel Documentation System	Gel Doc XR Bio Rad, USA
Real-Time PCR System	PikoReal96, Thermo Fisher Scientific, USA
Spectrophotometer	NanoDrop 1000, Thermo Fisher Scientific, USA
Micro-centrifuge	miniSpin Plus, Eppendorf, Germany Centrifuge 5414R, Eppendorf, Germany
Ultra-centrifuge	Allgra X-22, Beckman, USA J2-MC Centrifuge, Beckman, USA J2-21 Centrifuge, Beckman, USA
Rotors	JS-7.5 Beckman,USA JA-14 Beckman, USA
pH Meter	HI 83141, Hanna, USA
Pipettes	Pipetman Classic, Gilson, USA
Multi-Channel Pipettes	Transferpette, TreffLab, Switzerland
Vortex	NM110, Nuve, Turkey
Magnetic Stirrer	RCT basic, IKA, Germany
Balances	AY123, Sartorius, Germany
Plant Growth Chamber	JSPC-960, JSR, Korea
Incubator	EN500, Nuve, Turkey

Table 3.5. List of equipments (cont.).

Name	**Model & Brand**
Microwave Oven	MD551, Arcelik, Turkey
Autoclaves	Mac-601, Eyela, Japan ASB260T, Astell, UK
Deep Freezer (-20°C)	A2021-D, Arcelik, Turkey
Deep Freezer (-80°C)	Forma 860-ULT, Thermo Fisher Scientific, USA
Ice machine	AF20, Scotsman, Italy
Scanner	GT15000, Epson, Japan
Portable Area Meter	LI-3000A, Licor, USA
Chlorophyl Meter	SPAD-502, Minolta, Japan
Pressure Chamber	Water Status Console 3000, Soilmoisture, USA
Porometer	AP4, Delta-T Devices, UK
EC Meter	OrionStar A122, Thermo, USA
Atomic Absorption Spectrometer	SpectrAA 220-FS, Varian, USA

3.5. Biological Material

3.5.1. Plant Material

Seeds of Ispir (salt-tolerant) and TR43477 (salt-susceptible) common bean varieties were kindly provided by Prof. Yıldız Daşgan from Çukurova University Department of Horticulture, Adana, Turkey. Wild type *Arabidopsis thaliana* ecotype Col-0 seeds were kindly provided by Dr. Giorgia Batelli from Institute of Plant Genetics (IGV), National Research Council (CNR), Portici, Italy.

3.5.2. Bacterial Strains

Escherichia coli, DH5α strain was used for bacterial cloning. *Agrobacterium tumafaciens*, GV3101 strain which is resistant to 100µg/mL rifampicin and 30µg/mL gentamicin was used for *in planta* transformation.

3.5.3. Plasmids

pDONR 207 and pGWB 412 [127] vectors were kindly provided by Dr. Giorgia Batelli from Institute of Plant Genetics (IGV), National Research Council (CNR), Portici, Italy and used as donor and destination vectors, respectively, in Gateway cloning system detailed in Section 4.4.

3.5.4. Primers

The list of all primers used in this study has been presented in Appendix A.

4. METHODS

4.1. Determination of Salt Stress Onset Time in Common Bean

4.1.1. Plant Growth Conditions and Salt treatments

The seeds of Ispir and TR43477 common bean (*Phaseolus vulgaris* L.) varieties were surface sterilized in 5% hypochlorite solution for 5 min. and rinsed three times with distilled water. The seeds were transferred to the vermiculite containing plug trays and germinated at 24/20°C cycle with a 16-hrs light/8-hrs dark photoperiod under 300 μmol m^{-2} s^{-1} light intensity and 50-60% relative humidity. The trays were watered daily with the 1X modified Hoaglands solution (Table 3.3). Five seedlings at the trifoliolate stage were transferred (Figure 4.1a) to three liter pods by wrapping the hypocotyl of the seedlings with sponge (Figure 4.1b, c) for support.

Twenty-four pods were prepared for each variety for the hydroponic growth and salt treatments (Figure 4.1d). Plants were kept under the same conditions as in the germination periods. Five days after the transfer, half of the pods from each variety were left as control and the rest were exposed to gradually increasing NaCl concentrations starting from 50 mM at the first day, increased to 100 mM at the second day and reached to 125 mM at the third day gradually for salt acclimatization to prevent plasmolysis [128]. Plants were exposed to 125 mM NaCl for five more days in hydroponics solution. Leaf and root samples were collected from each group at the first, third and the fifth day after salt acclimatization to measure physiological and morphological changes.

Five gr of each tissue sample was flash frozen in liquid nitrogen and stored at -80°C for RNA isolations. Seven different parameters of physiological analysis were performed to four individual plants of each group at the first, third and fifth day of 125 mM NaCl application as described in following sections.

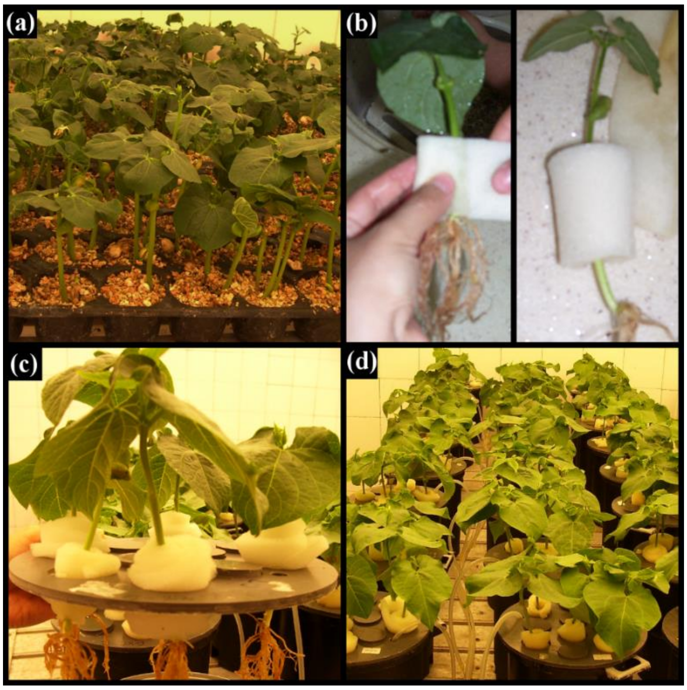


Figure 4.1. Hydroponic growth of common bean. (a) Germination in vermiculite. (b) Wrapping hypocotyls with sponge. (c) Transfer to hydroponics culture and (d) application of salt in hydroponics culture.

4.1.2. Visual Symptoms Analysis

One-to-five visual symptom scale was used to evaluate the canopy injury level [72]. Three independent observers evaluated the plants according to the following ranking scale, 1- no injury or negligible, 2- slightly wilted or yellowed, 3- wilted or yellowed moderately, 4- wilted or dried mostly (50-80%) and 5- wilted or dried severely or totally. (Figure 4.2)

Figure 4.2. One-to-five visual symptom scale.

4.1.3. Canopy and Root Fresh and Dry Weight

The canopy and root fresh weights were measured separately and they were incubated at 65°C aerated oven for 48 hours for dry weight measurements.

4.1.4. Leaf Area (cm^2/plant)

All leaves of a plant were used to measure leaf area by Licor (Nebreska, USA) LI-3000A portable area meter according to the manufacturer's instructions.

4.1.5. Relative Water Content (RWC)

Fourth leaves from the top were used to calculate RWC according to the formula; RWC(%)=(FW-DW)/(TW-DW)X100 where FW indicates fresh weight, DW indicates dry weight and TW indicates turgid weight [129]. Turgid weight were determined after floating the sample on distilled water for 5h at room temperature. Dry weights were measured after the samples were oven dried for 48 hrs at 65°C.

4.1.6. Stomatal Conductance (µmol H2O m^{-2} s^{-1})

Water vaporization rates from stoma (stomatal conductance) were measured by Porometer AP4 (Delta-T Devices, Cambridge, UK) from the third leaf from the top of each plants and the decrease of vaporization rates were the reflection of stomatal closure due to salt stress.

4.1.7. Membrane Injury Index (%)

Membrane injury index (MII) was calculated by measuring the amount of electrolyte leakage in leaf samples. Five circular leaf discs of same diameter (1cm diameter) were cut out of the second leaf from the top in each plant and were immersed in 10 ml of deionized water for five hours at room temperature. The electrical conductivity (EC) of the released electrolytes from the leaf discs were measured by portable EC meter (Thermo Fisher Scientific, Wilmington, DE) before and after they were autoclaved for 10 min to release total electrolytes to water. Membrane injuries were calculated as the percentage injury index following the formula. Membrane-Injury-Index=[1(1-T1/T2)/(1-C1/C2)]x100 where C1 and C2 represent electrical conductivity of control group before and after autoclaving, respectively; T1 and T2 represent electrical conductivity of treated group before and after autoclaving, respectively.[130, 131].

4.1.8. Determination of K^+, Ca^{+2}, Na^+ and Cl^- Ion Ratios

To determine, the changes in K^+, Ca^{+2}, Na^+ and Cl^- ion concentrations, dry canopies and roots of the plants were burned to ash at 550°C for 5 hrs and the ashes were dissolved in 3,3% (v/v) HCl. The K^+, Ca^{+2} and Na^+ concentration were measured by the Varian 220 FS atomic absorption spectrometer in the emission mode. The Cl^- concentration was measured according to the Mohr method which is a colorimetric detection method by titration of chloride ions in the presence of with potassium chromate and silver chromate [132].

4.1.9. Statistical Analysis of Physiological Parameters

Stress onset time of both varieties to salt stress were determined by analyzing mean values of the above parameters for 1st, 3rd and 5th days of 125mM salt application by student’s *t*-test.

4.2. Transcriptome Analysis of Common Bean

4.2.1. Sample Collection and Pooling

Root samples from two control (RC1, RC2) and two salt treated (RS1, RS2) plants of Ispir variety were pooled as two biological replicates as schematized in Figure 4.3c to analyze the reproducibility of the transcriptome analysis. The Pearson correlation coefficient between RPKM values of these replicates were significantly high (Figure 4.3a, b). Thus, the leaf and the root samples from four control and treated plants of TR43477 variety (TLC, TLS and TRC, TRS) and the leaf samples of Ispir (LC, LS) variety were pooled separately as described in Figure 4.3d and sequenced without replicates. In total, 10 samples were subjected to transcriptome analysis.

4.2.2. Total RNA Extraction

Hundred mg of leaf or root tissue of each common bean variety from both control and salt treated groups were homogenized with mortar and pestle in liquid nitrogen to extract total RNA by RNeasy Plant kit (Qiagen, Hilden, Germany) according to the instructions manual. Total RNA samples were quantified using Nanodrop 1000 (Thermo Fisher Scientific, Wilmington, DE) spectrophotometer and RNA integrities were assessed by running 500 ng RNA samples of each tissue in 1% denaturing agarose gel electrophoresis.

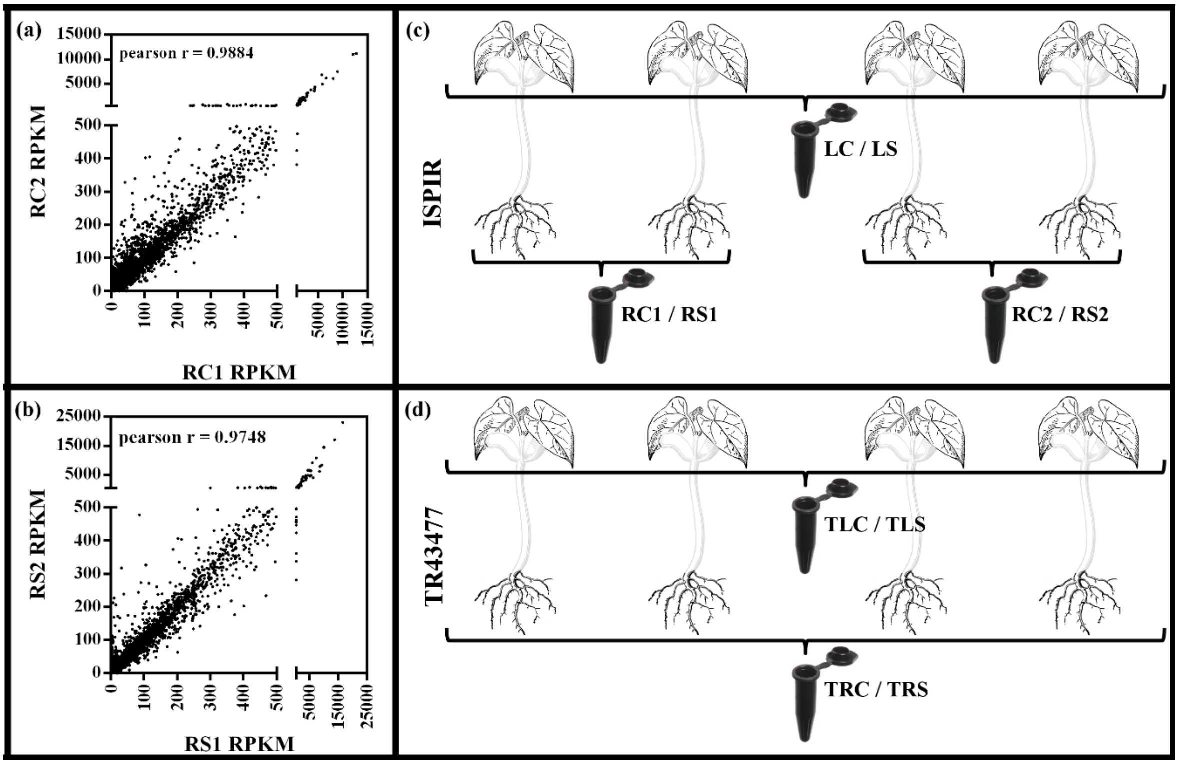


Figure 4.3. Scheme of sample pooling strategy and correlation analysis of root samples RPKM values. Correlation analyses within RC (a) and RS (b) samples. Black lines indicate the pooling of leaf and root samples from Ispir (c) and TR43477 (d) varieties.

4.2.3. cDNA Library Construction and Sequencing

Fourty µg of total RNA from each sample was used to generate cDNA library and sequenced by BGI (Shenzen, China) using Illumina HiSeq2000 platform. RNA integrity was analyzed by 2100 Bioanalyzer (Agilent, California, USA) before cDNA library construction at BGI. Total RNA was treated with DNase I and poly(A)+ RNA was enriched by oligo dT magnetic beads. The poly(A)+ RNA were digested to 200-700 nt long fragments and first strand cDNA was synthesized with random hexamers. After the RNase H treatment second strand cDNA was synthesized by DNA polymerase I. The double stranded cDNAs were purified with QiaQuick PCR purification kit (Qiagen, Hilden, Germany). The sequencing adaptors were ligated to double stranded DNA after end repair by single adenine nucleotide addition. The quality of the cDNA libraries were analyzed by agarose gel electrophoresis and adaptor ligated cDNAs were enriched with PCR amplification. The insert size and the concentration were analyzed with Bioanalyzer 2100 (Agilent, California, USA) and qPCR before sequencing on a flow cell using Illumina HiSeq2000 sequencing instrument.

4.2.4. *De novo* Assembly and Data Analyses

The raw sequence data were filtered by trimming adapters and removing reads with unknown bases more than 5% and/or reads which contain more than 20% bases with Phred quality score less than 10. The duplicated reads were also filtered. Using the Trinity software [133] the clean reads from each sample were assembled *de novo* by setting the k-mer length of the software to 25 to generate subtranscriptomes. The so called “all-unigene” sequences which were no longer be extended on either end were generated by pooling the contigs from each subtranscriptome and assembling them with Trinity software. Sequence orientations and protein coding regions were predicted by BLASTx analyses of the all-unigene sequences against the following databases; NCBI non-redundant (Nr) protein, Swiss-Prot, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Cluster of Orthologous Groups (COG). Protein coding regions were predicted using the most similar hit in BLASTx alignment results. The ESTscan software [134] was used to predict the sequence orientation and protein coding regions of the all-unigenes that cannot be annotated in any of the databases. All-unigenes were annotated with the most similar sequence by BLASTx with an *E*-value cut-off of 10^{-5} against the Nr database. The BLASTx results were classified by the

WEGO [135] software to understand the gene function distributions of common bean species at macro level using Gene Ontology (GO) annotations [136]. The BLASTx analysis against the KEGG pathway database was also performed to assign putative metabolic pathways to all-unigenes.

Relative expression values of all-unigenes were estimated using the RPKM (reads per kilobase per million) values which were calculated according to the following formula; RPKM = (10^9 x C)/(N x L) where C stands for the number of reads mapped to a gene; N stands for total mapped reads in the experiment; and L stands for gene length in base-pairs for a gene. The clean reads mapped to more than one unigene were not counted to calculate RPKM. Eight RPKM (four from root libraries two from leaf libraries) values were calculated for every all-unigene, by mapping eight transcriptomes using the SOAP2 [137] software with a maximum of three mismatches. Genes with a false discovery rate (FDR) [138] less than 10^{-3} and the absolute ratio of $\log^{-2}$ (RPKM-tr / RPKM-cont) ≥ 1 were defined as a differentially expressed (DEGs). The GO terms and the KEGG pathways that were enriched within the DEGs were identified by publicly available agriGO [139] and FatiGO [140] softwares by setting the FDR and the adjusted *P*-values lower than 0.05 for the agriGO and the FatiGO softwares respectively functional significance of the enriched terms were calculated by hypergeometric test using the common bean transcriptome as background.

4.2.5. qRT-PCR Analyses

The relative expression levels estimated by transcriptome analysis were validated by quantitative Reverse Transcription PCR (qRT-PCR) analyses of 43 all-unigenes (Table A.4) using the *insulin degrading enzym*e (*IDE*, Phvul.001G133200.1) [GenBank: FE702602.1] and the *actin-11* (*Act-11*, Phvul.008G011000.1) [GenBank: CV529679.1] genes of common bean as stably expressed internal references under salt stress [141].

Four individual plants from control and salt treated groups were used as biological replicates and three technical replicates for each biological replicate were used in qRT-PCR analyses. One μg of total RNA from each sample was used as template for first strand cDNA synthesis following the instructions of Maxima First Strand cDNA Synthesis Kit for RT-PCR with DNase (Thermo Fisher Scientific, Wilmington, DE). For the selected 43 genes, specific primers (Table A.4) were designed using the Primer Design module of CLC Main Workbench (version 6.0) software keeping the product length between 100-246 bp and annealing temperatures between 50-60°C. qRT-PCR analyses were performed by PikoReal 96 Real-time PCR system (Thermo Fisher Scientific, Wilmington, DE) using 10 ng first strand cDNAs as template and AccuPower 2X Greenstar master mix (Bioneer, Daejon, Korea) with 0.25 pmol forward and reverse primers in a 10 μl solution. Following the initial denaturation at 95°C for five min., 40 cycles of denaturation at 95°C for 10 s and 60 s of merged annealing-extension steps were performed at optimized temperatures (Table A.4) for each primer pairs. For each gene, relative expression levels were normalized to the geometric average of Ct (threshold cycle) values of *Act-11* and *IDE* and calculated with the $2^{-\Delta\Delta Ct}$ method. The Student's *t*-test was applied to determine the level of significance between relative expressions of the control and the treated samples.

4.3. Selection of Genes for Functional Characterization Under Salt Stress

The transcriptome analysis revealed the expression of thousands of genes under salt stress. For functional analysis the main focus was on the upregulated transcripts and the pathways which were significantly enriched in GO and KEGG analysis. The candidate genes were selected among those transcripts, which were upregulated in both Ispir and TR43477 varieties and the ones, which were upregulated in Ispir but not changed or downregulated in TR43477 varieties upon salt treatment (Table 4.1) as well as the ones which were classified under the significantly enriched GO and KEGG pathways (Table 4.2).

The coding sequences of the selected genes were determined by the BLASTx analysis against the Nr and common bean transcriptome database (phytozome.org) (Table 4.1). The transcripts which lack predicted coding sequences were eliminated from the list. Gene specific primers were designed for each selected gene by the Primer design module of CLC Main Workbench (version 6.0) (Table A.1).

Table 4.1. The list of selected candidates for cloning and functional characterization.

Gene ID (Transcriptome)	**Annotation**	**geneID (Phytozome.org)**	**LS vs. LC**	**RS vs. RS**	**TR-LS vs. TR-LC**	**TR-RS vs. TR-RC**
CL7815.Contig2_All	FAD-binding Berberine family protein (**CPRD2**)	Phvul.002G200600.1	Up	Up	Up	Down
Unigene24388_All	Uncharacterized protein (**Unc2**)	Phvul.002G211900.1	--	Up	--	Up
Unigene14206_All	Endo-1,3-beta-glucanase (**β-Glu**)	Phvul.011G077900.1	Up	Up	--	--
Unigene27640_All	Uncharacterized protein (**Unc4**)	Phvul.003G209000.1	Up	Up	Up	Up
Unigene4657_All	PREDICTED: protein TRANSPARENT TESTA 12-like (**TT12**)	Phvul.011G104200.1	Up	--	Up	--
Unigene8197_All	Vigna angularis mRNA for galactinol raffinose 6-galactosyltransferase	Phvul.001G214300.1	--	Up	--	--
CL6934.Contig2_All	Uncharacterized protein	Phvul.008G047500.1	Up	Up	--	--
CL6394.Contig2_All	PREDICTED: anthoc yanin 5-aromatic acyltransferase-like	Phvul.008G032400.1	Up	Up	Up	Up
Unigene38692_All	PREDICTED: 2-aminoethanethiol dioxygenase	Phvul.001G114800.1	--	Up	--	--
Unigene27315_All	PREDICTED: G-type lectin S-receptor-like serine/threonine-protein kinase At5g24080-like	Phvul.007G077300.1	Up	Up	Up	Up
Unigene28084_All	PREDICTED: E3 ubiquitin-protein ligase RNF181-like	Phvul.001G255200.1	Up	Up	Up	Up
Unigene17632_All	PREDICTED: universal stress protein A-like protein-like	Phvul.001G225300.1	Up	Up	Up	--
Unigene1626_All	PREDICTED: uncharacterized protein LOC100778355	Phvul.011G110800.1	Up	Up	Up	Down

Table 4.2. The GO and The KEGG classifications of the selected genes.

geneID (Phytozome.org)	**Annotation**	**GO-ID**	**Gene Ontology Functional Class**
Phvul.002G200600.1	FAD-binding Berberine family protein **(CPRD2**)	GO:0055114	oxidation-reduction process
Phvul.002G211900.1	Uncharacterized protein (**Unc2**)	unknown	unknown
Phvul.011G077900.1	Glycine max endo-1,3-beta-glucanase (**β-Glu**)	GO:0004553	hydrolase activity
Phvul.003G209000.1	Uncharacterized protein (**Unc4**)	unknown	unknown
Phvul.011G104200.1	PREDICTED: protein TRANSPARENT TESTA 12-like (**TT12**)	GO:0005215	transporter activity
Phvul.001G214300.1	Vigna angularis mRNA for galactinol raffinose 6-galactosyltransferase	GO:0009312	oligosaccharide biosynthetic process
Phvul.008G047500.1	Uncharacterized protein	unknown	unknown
Phvul.008G032400.1	PREDICTED: anthocyanin 5-aromatic acyltransferase-like	GO:0016740	transferase activity
Phvul.001G114800.1	PREDICTED: 2-aminoethanethiol dioxygenase	GO:0010382	cellular cell wall macromolecule metabolic process
Phvul.007G077300.1	PREDICTED: G-type lectin S-receptor-like serine/threonine-protein kinase At5g24080-like	GO:0004674	protein serine/threonine kinase activity
Phvul.001G255200.1	PREDICTED: E3 ubiquitin-protein ligase RNF181-like	GO:0008270	zinc ion binding
Phvul.001G225300.1	Universal stress protein A-like protein	GO:0006950	response to stress
Phvul.011G110800.1	Putative wound-induced protein	GO:0009611	response to wounding

4.4. Cloning and transformation of selected genes to *Arabidopsis thaliana*

Transgenic *A. thaliana* plants were generated using the Gateway® cloning technology (Thermo Fisher Scientific, Wilmington, DE). This system requires a two-step cloning reaction. Firstly, the gene of interest was cloned to a donor vector with a site-specific recombination reaction facilitated by BP clonase® enzyme. Secondly the insert was sub-cloned to an expression vector with LR clonase® enzyme with another site-specific recombination reaction.

4.4.1. Vectors

The donor vector used in this study was pDONR 207 which is a 5585 bp long plasmid with gentamicin resistance gene for bacterial selection and the *att*P1 and *att*P2 sites for site-specific recombination of insert. The expression vector was pGWB412 [127] which is a 11711 bp long N-terminal FLAG-tagged vector with two copies of 35S cauliflower mosaic virus (CaMV) constitutive promoters, a spectinomycin resistance gene for bacterial selection, a kanamycin resistance gene for plant selection and the *att*R1 and *att*R2 sites for site-specific recombinational entry of insert.

4.4.2. Preparation of Inserts

The RNA extraction and cDNA synthesis were performed as indicated in Section 4.2.2 and 4.2.5 . The cloning system requires 30 bp long *att*B adaptor ligation to both ends of the gene of interest for recombination reaction. Thus, the adaptors were added to the insert with two step nested PCR reaction. The first PCR reaction was performed with primers specific to the C and N terminus of the open reading frame of the gene of interest (Table A.1) with an overhang of first 15 bases of the *att*B1 and *att*B2 adaptors. The reaction mix was contained 0.5µM each primers, 0.2 mM dNTP, 3% DMSO and one unit Phusion High-Fidelity DNA Polymerase in 1X HF buffer (Thermo Fisher Scientific, Wilmington, DE). The PCR product was run on a 1% agarose gel and the expected bands were excised and purified by GeneJet^{TM} Gel Extraction kit (Thermo Fisher Scientific, Wilmington, DE) according to the instructions manual. The second PCR reaction was performed using the gel purified product of the previous reaction as template. The second step PCR mix included 0.5µM from each universal *att*B primers (Table A.1), 0.2 mM dNTP, 3% DMSO and one unit Phusion High-Fidelity DNA Polymerase in 1X HF buffer (Thermo Fisher Scientific, Wilmington, DE). The product of the second PCR reaction also run on 1% agarose gel and the adaptor ligated final product was extracted for subsequent cloning reactions. The cycles and annealing temperatures were stated in Table 4.3.

Table 4.3. Nested PCR conditions for preparation of insert.

Primary PCR			
Temperature(°C)	**Step**	**Time**	**Cycle**
98	Initial Denaturation	3'	1x
98	Denaturation	20"	11x
52	Annealing	30"	
72	Extension	75"	
98	Denaturation	20"	20x
72	Annealing/Extension	80"	
72	Final Extension	5'	1x
Secondary PCR			
Temperature(°C)	**Step**	**Time**	**Cycle**
98	Initial Denaturation	3'	1x
98	Denaturation	20"	13x
45	Annealing	30"	
72	Extension	75"	
98	Denaturation	20"	20
72	Annealing/Extension	80"	
72	Final Extension	7'	1x

4.4.3. Site-specific recombination of Inserts

The insert with the flanking *att*B1 and *att*B2 sites were cloned into pDONR 207 vector with a recombination reaction (Figure 4.4a). The reaction volume was 10 µl and carried out in TE buffer (pH:8) (Table 3.2) in the presence of 0.3 µg vector, 0,5 µg insert and 2µl BP clonase enzyme. Although not stated in the instructions manual of the Gateway system, it was realized that the BP reaction works poorly if the concentration of gel purified insert is less than 100 ng/ul. This reaction created an entry clone with the insert flanked by the *att*L1 and *att*L2 sites in pDONR 207 backbone (Figure 4.4a). Entry clone was transformed to E.coli DH5α and the insert was sequenced for verification with vector and gene specific primers (Table A.2). The insert was sub-cloned from the entry clone to pGWB412 destination vector under the control of double CaMV 35S constitutive promoter by LR recombination reaction (Figure 4.4b). Total of 10 µl reaction in TE buffer (pH:8) contained 0.3 µg entry and expression vector and 2µl of LR clonase enzyme. Both the BP and the LR reactions were incubated at 25°C for 1h and terminated with incubation at 37°C for 10 min following addition of 2µg of Proteinase-K.

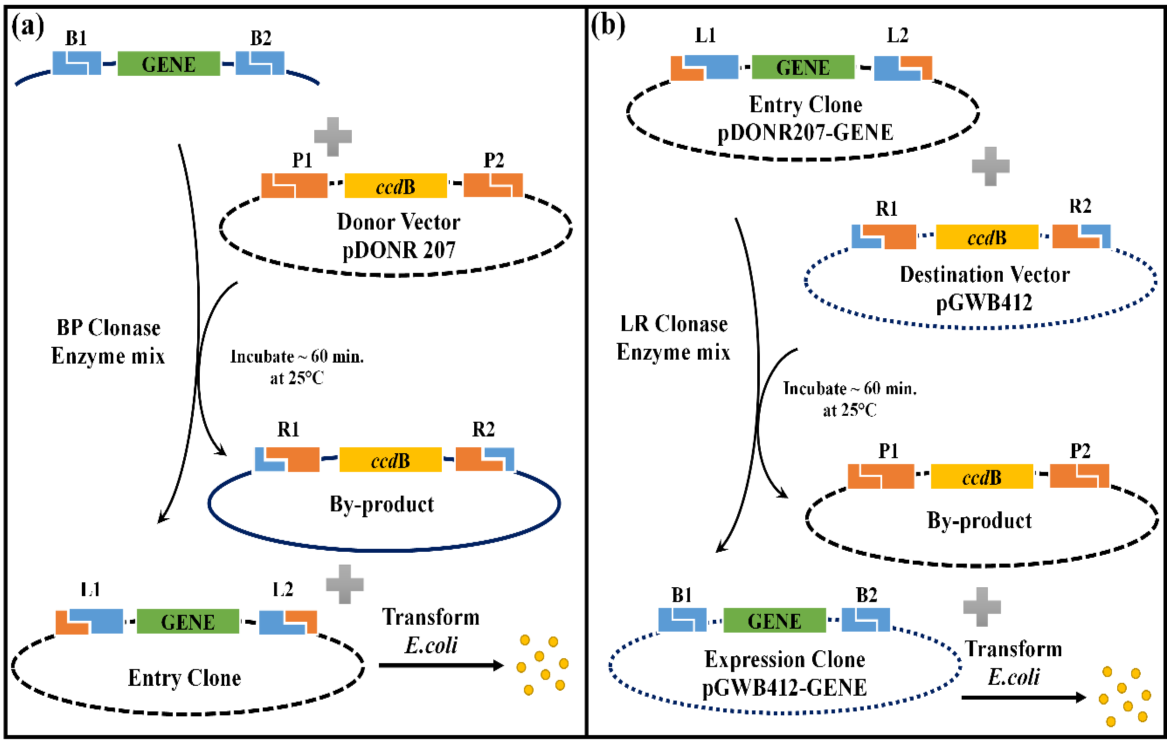


Figure 4.4. Two-step Gateway cloning of gene of interest. (a) Construction of Entry clone by BP reaction. (b) Construction of expression clone by LR reaction. *ccd*B is lethal gene for E.coli DH5α (Regenerated based on scheme in bioinforx.com).

4.4.4. Bacterial Transformation of the Entry the and Epression vectors.

Competent *E. coli* DH5α [142] and *Agrobacterium tumefaciens* GV3101 [143] strains were prepared as described in van Die *et al*., 1983 and Holters *et al*., 1978, respectively and stored at -80°C prior to transformation. Two µl of the BP or LR clonase reaction mix or 0.1 µg purified plasmid was added into pre-thawed 100 µL aliquot of competent *E. coli* suspension and incubated on ice for 30 min. The suspension was heat shocked at 42°C for 90 s and transferred to ice for 5 min. Following incubation on ice 0.9 ml of LB medium was added to the suspension and the cells were grown 1hr at 37°C on a shaker at 200 rpm. The cells were spread on selective LB agar medium (Table 4.4) and incubated overnight at 37°C. A hundred µl aliquot of competent *A.tumafaciens* cells were thawed on ice for 5 min and the suspension was submerged into liquid nitrogen for 5 min after the addition of 0.1 µg expression vector. The cells were incubated at 37°C before the addition of 0.9 ml YEP medium. The bacterial culture was grown for 4 hrs at 28°C on 200 rpm rotational shakering and spread on selective (Table 4.4) YEP medium to grow at 28°C for 2 days.

Table 4.4. Antibiotics used to prepare selective mediums after transformation.

	Medium	**pDONR 207**	**pGWB 412**
E. coli	LB	Kanamycin (50 µg/ml)	Spectinomycin (50 µg/ml)
A. tumafaciens	YEP	-	Spectinomycin (50 µg/ml) Rifampicin (100 µg/ml) Gentamicin (30 µg/ml
A. thaliana	½ Strength MS	-	Kanamycin (75 µg/ml)

4.4.5. Verification of Transformation with Colony PCR

Five colonies were picked by sterile micropipette tips for the verification of transformation using colony PCR. The tips were submerged in 10 µl sterile distilled water and 0.1 ml of proper medium separately (LB or YEP). The medium was transferred to shaker for incubation at proper temperature (28 or 37 °C) and 2 µl of the solution was used as template for PCR reaction. The PCR reaction was performed with 0.5 units *Taq* DNA polymerase (Thermo Fisher Scientific, Wilmington, DE), 0.2 µM gene specific primers (Table A.3), 0.4 mM dNTPs, 2mM $MgCl_2$ and 1X *Taq* buffer (Thermo Fisher Scientific, Wilmington, DE). The PCR products were run on 1% agarose gel electrophoresis and visualized under UV light. The positive clones were propagated in 5 ml medium (LB or YEP) and plasmids were isolated using the Nucleospin Plasmid Isolation Kit (Macherey-Nagel, Germany). All the selected clones were sequenced (Macrogen, Korea) and the results were BLASTx analyzed against *P.vulgaris* reference genome (Phytozome 10.3v) and our *de novo* assembled transcriptome sequences for verification.

4.5. Transformation of the Selected Genes to *A.thaliana*

4.5.1. Plant Growth and Seed Collection

The wild type Col-0 *Arabidopsis thaliana* seeds were surface sterilized with 5% hypochlorite solution and rinsed three times with distilled water. Seeds were embedded in half strength Murashige and Skoog (MS) agar medium (Table 3.2) and incubated at 4°C for 48 hrs. The plates were transferred to JSPC-960 (JSR, South Korea) growth chamber under 16/8-hours light/dark photoperiod and 50-60% relative humidity at 22°C. After a week growth, the seedlings were transferred to turf and watered every second day with half strength basal nutrient solution (BNS) [126] (Table 3.4). The irrigation was stopped once 60-80% of the cliques become yellow or brown. The seeds were collected after the whole plants dried totally.

4.5.2. *In planta* Transformations by Floral Dip Method

When the Arabidopsis plants, grown in JSPC-960 chamber, formed the primary bolts (21-24 days old), they were clipped to encourage proliferation of many secondary bolts to obtain high number of inflorescences (32-35 days old) for floral dip method [144] (Figure 4.5a). For the application of this method firstly an agrobacterium primary culture was prepared by growth of the transformant agrobacterium cells in selective (Table 4.4) YEP medium overnight at 28°C with constant agitation at 200 rpm. This primary culture was diluted hundred times in 0.5 L YEP medium and grown overnight under same conditions.

The culture was centrifuged at 3000 g for 10 min and the pellet was resuspended in 200 ml of transformation buffer containing 5% sucrose, 55nM benzylaminopurine (BAP) and 0.02% Silwet L-77 or Tween-20. The inflorescences were dipped in transformation buffer (Figure 4.5b) for 5 min and the plants were covered with a plastic wrap (Figure 4.5c) to keep them humid in dark horizontally for 24 hrs. The plants were unwrapped and left to continue their growth to set seeds (Figure 4.5d). Seeds were embedded in selective (Table 4.4) half strength MS medium agar plates and exposed to light for 4-6 hours. Then transferred to dark chamber for 48 hrs and followed by growing under normal conditions (Section 4.1.1) for two more days for the selection of transgenic seedlings. The seedlings with green cotyledon [145] were selected as transgenics (Figure 4.5e) and transferred to turf and grown under normal conditions (Section 4.1.1) for seed collection.

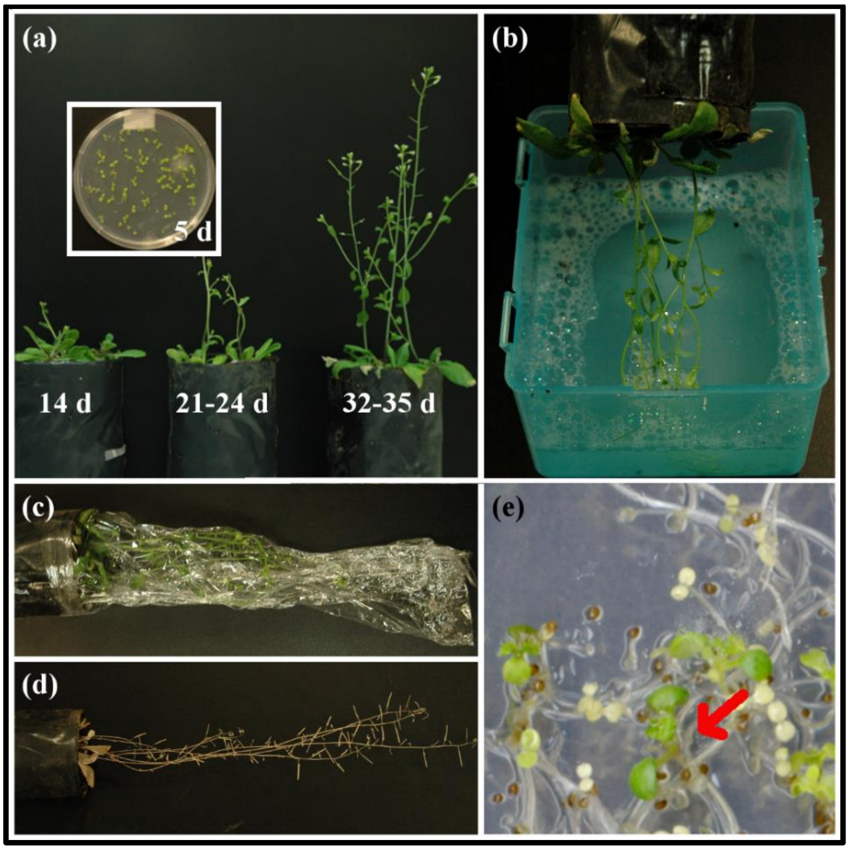


Figure 4.5. *In planta* transformation to *A.thaliana* and transformant selection. (a) Growth stages of *A.thaliana*. (b) Floral dipping in transformation buffer. (c) Wrapping the plant to keep humidity. (d) Plants before harvest. (e) Kanamycin selection seedlings.

4.5.3. Selection of Homozygous Transgenic Lines and Verification of *In planta* Transformation

Homozygous third generation transgenic Arabidopsis (T3) lines were needed to be generated to continue with functional analysis of the transgenics using physiological parameters. A hundred seeds from the transformant plants (T1) were plated on half strength MS agar medium containing 75µg/mL kanamycin. The seedlings were further grown in turf in growth chamber as indicated in Section 4.5.1. While first generation transgenic plants (T1) were expected to be heterozygous for the transgene, second generation (T2) plants were expected to show 1/3 homozygosity in a Mendelian inheritance fashion. Therefore, one hundred seeds of T2 generation were grown under same conditions as T1 and the number of transgenics and non-transgenics have been counted. When the T3 lines were grown from T2 seeds they were expected to be 100% transgenic and used in further phenotype analysis after verification of transgene expression by qRT-PCR.

The expression of the gene of interest in transgenic Arabidopsis lines was verified by RT-PCR at T1 generation. Following the RNA isolations the single stranded cDNAs were synthesized as indicated in Section 4.2.5. The PCR amplifications were performed as indicated in Section 4.4.5 using gene specific primers (Table A.3) and 20 ng cDNA as template. The products were run on 1% agarose gel and visualized under UV light to detect expected bands for the determination of transgenic lines.

4.6. Phenotype Analysis of Transgenic Overexpression Lines Under Salt Stress

4.6.1. Germination Assay

Thirty seeds from each homozygous transgenic line and wild type Arabidopsis (Col-0) were embedded on half strength MS agar control plates and 150 mM NaCl containing plates. After 48 hours of stratification at 4°C, the plates were transferred to the growth chamber and the number of seedlings only with dicotyledon leaves and primary roots (Figure 4.6) were counted every day for a duration of 7 days starting 24 hrs after transfer to growth chamber.

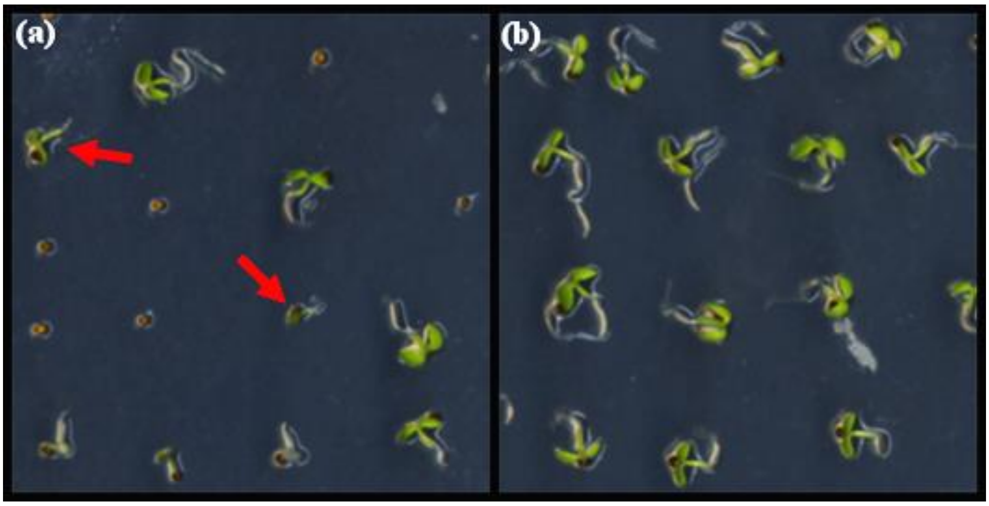


Figure 4.6. Germination assay. (a) Seedlings growing on MS medium with 150 mM NaCl starting to emerge cotyledon leaves and primary roots (indicated with red arrow). (b) Seedlings growing on MS medium with no NaCl.

4.6.2. Root Elongation Assay

Ten seeds from each homozygous transgenic line and Col-0 Arabidopsis were embedded over a straight line (Figure 4.7) on half strength MS agar control plates and 150 mM NaCl containing plates. The plates were left at 4° for 48 hours for stratification and then transferred to growth chamber in a vertical position. The plates were scanned after 14 days of incubation in growth chamber and the root lengths were measured by the Image J software (National Institute of Health, version 1.6.0) [146].

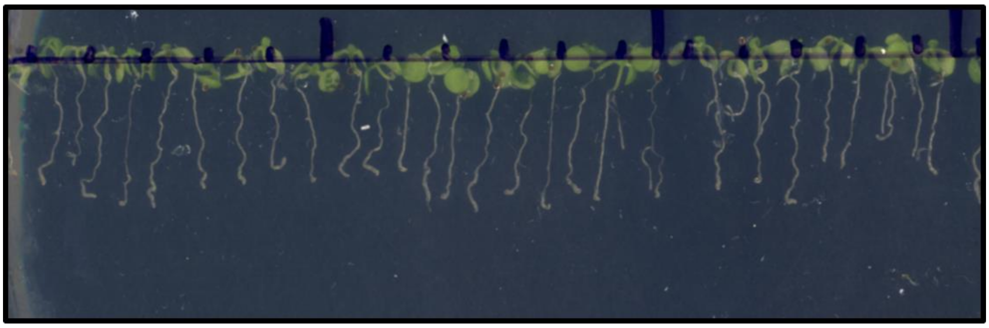


Figure 4.7. A representative image for the measurement of root elongation in transgenic Arabidopsis lines.

4.6.3. RWC and Proline Content Measurements in Hydroponics Culture

The homozygous transgenic overexpression lines were grown in hydroponics system which was designed as follows (Figure 4.8) [126]: four seeds of each transgenic line and wild type Arabidopsis (Col-0) were embedded (Figure 4.8c) in half strength MS medium (Table 3.4) poured into the drilled caps (Figure 4.8a) of 0.5 ml microcentrifuge tubes, after the caps were placed on the 24-well plates (Figure 4.8b and d). The tip boxes were filled with liquid germination (GM) medium (Table 3.4) until it reaches to the caps and the covers of the boxes were placed on them. The boxes were incubated in dark at 4°C for 48 hours. They were transferred to growth chamber (Figure 4.8e) under growth conditions described in 4.5.1. Once the roots started emerging below the caps after four days (Figure 4.8f), the GM medium was replaced with BNS (Table 3.4) medium gradually with 33% increments in three days. The level of the medium was kept under strict control and filled up daily to prevent loss of contact with the agar medium in the caps. The BNS medium was replaced every four days until salt application. The control group was continued to be grown under the same regime (Figure 4.8g). For the treatment group plants, the salt application started 21 days after transfer to the growth chamber. Salt concentration was increased gradually starting with 50 mM daily increments until it reached to 150 mM and continued for seven days at this final concentration. At the end of the treatments, both groups of plants (treated and control group) were sacrificed to continue with physiological measurements.

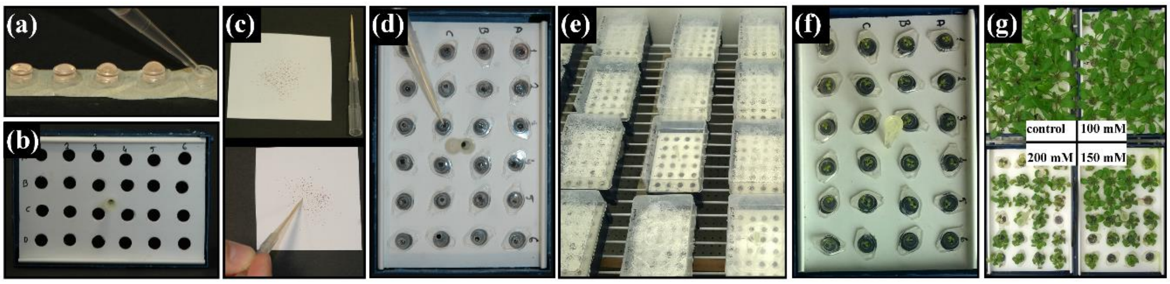


Figure 4.8. Hydroponics growth of *A. thaliana* lines. (a) ½ MS medium was poured in drilled caps. (b) The stand for the caps and storage for BNS medium. (c) Selection and (d) transferring of seeds. (e) Plants in growth chamber. (f) Seedling stage. (g) Optimization.

Following salt treatment in hydroponic culture the plants from each line were sacrificed and one rosette from each four plants of every transgenic line and control group were used to calculate RWC with formula given Section 4.1.5.

Proline concentrations were measured as indicated in Ábrahám, E., C. Hourton-Cabassa and L. Erdei 2010 from a single rosette of each four plants of transgenic line and control group. The leaves were grinded in liquid nitrogen and suspended in 5µl of 3% (w/v) sulfosalicyclic acid per 1 mg fresh weight. After centrifugation for 5 min. at room temperature at full speed in a microcentrifuge 12 µl of the supernatant was mixed with 60 µl reaction mixture (Table 3.2) and incubated at 96°C for 1hr. The chromophore was extracted with 120 µl of toluene and the absorbance was measured at 520 nm in Nanodrop 1000 (Thermo Fisher Scientific, Wilmington, DE) using toluene as reference. A standard curve with OD values of proline standard changing between 10 to 500 µg/ml has been drawn. The concentration of the samples were calculated according to the slope of the standard curve.

4.6.4. qRT-PCR Analysis of Stress-associated Genes in Transgenic Lines

Total RNA extraction and cDNA synthesis were performed as indicated in Section 4.2.1 and 4.2.5. The stress associated *DREB2A*, *MYB2* and *RD26* transcription factors and the *RAB18, RD29B, P5CS1* and *P5CS2* response genes [141] were selected to analyze with qRT-PCR. The qRT-PCR amplifications were performed with gene specific primers (Table A.5) as indicated in 4.2.5 using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Wilmington, DE).

5. RESULTS

5.1. Determination of Salt Stress Onset Time in Common Bean

The aim of the analyses performed in this section was to determine the salt stress onset time in common bean varieties to apply later for the transcriptome studies. The analyses were performed by measuring the parameters listed in Section 4.1 using four individual plants of salt treated and control group which were grown in hydroponic condition as described in Section 4.1.1. The bar graphs were generated from the mean value of each group and the error bars indicated the standard deviations (SD).

5.1.1. Visual Symptoms Scale of Common Bean under Salt Stress

Three independent observers evaluated the plants according to the visual scale described in 4.1.2. and Figure 4.2 to avoid bias in evaluation. Pictures of each control and treatment group was taken for visual examination before treatment and at the 1st, 3rd and 5th, days of salt treatments (Figure 5.1a, c). Although in Ispir leaves there was negligible wilting and no drying or damage even at the 5th day of 125 mM salt treatment (scale 1,75) (Figure 5.1c), TR43477 leaves have shown significant wilting (scale 3,5) at the 3rd day of salt treatment and at the 5th day damages on the leaf skins in the form of dry patches were observed (scale 3,75-4,25) (Figure 5.1c).

5.1.2. Analysis of Changes in Biomass

One of the most significant adverse effects of salinity is growth inhibition [12]. Changes in the canopy and root, fresh and dry weights were used to analyze the effect of salinity on biomass to assess the salt stress onset time. The results of salt treated and control group of plants (Figure 5.2 and Figure 5.3) have indicated a significant difference at the 3rd day of 125 mM salt treatment for both Ispir and TR43477 varieties.

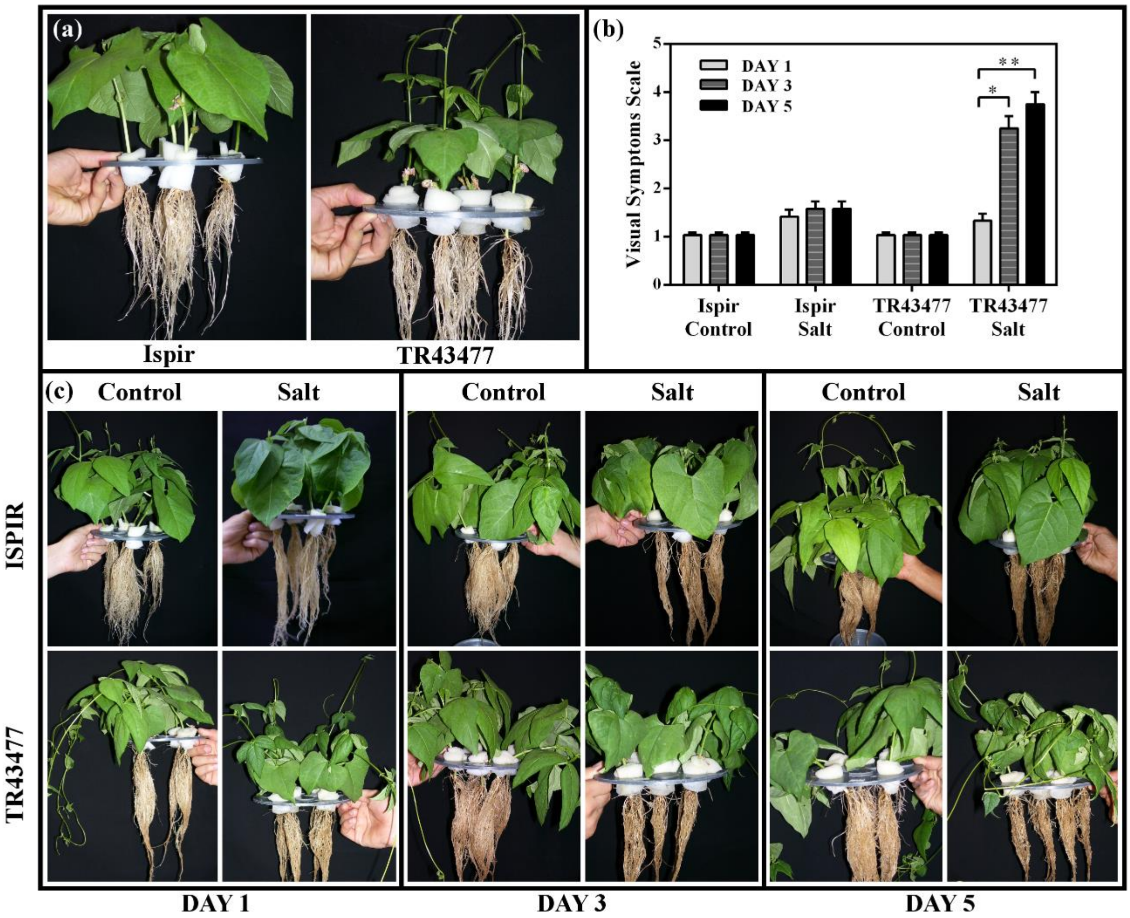


Figure 5.1. Visual symptoms scaling of plants grown in hydroponics culture. (a) Plants before salt application, (b) Bar graph of visual symptoms scale evaluations. (b) Pictures of treatment and control groups of tolerant and susceptible variety at 1st, 3rd and 5th days. Error bars indicates SD ("*": $p<0.05$, "**": $p<0.01$).

Leaf area is considered to be another indicator of biomass [17] and turgosity level of leaf tissues. As shown in Figure 5.4a and b, the difference between salt treated and control groups of Ispir was minimal even at the 3rd day of salt treatment, whereas the difference was significantly high in TR43477 due to severe wilting and reduction in growth rate (Figure 5.1c).

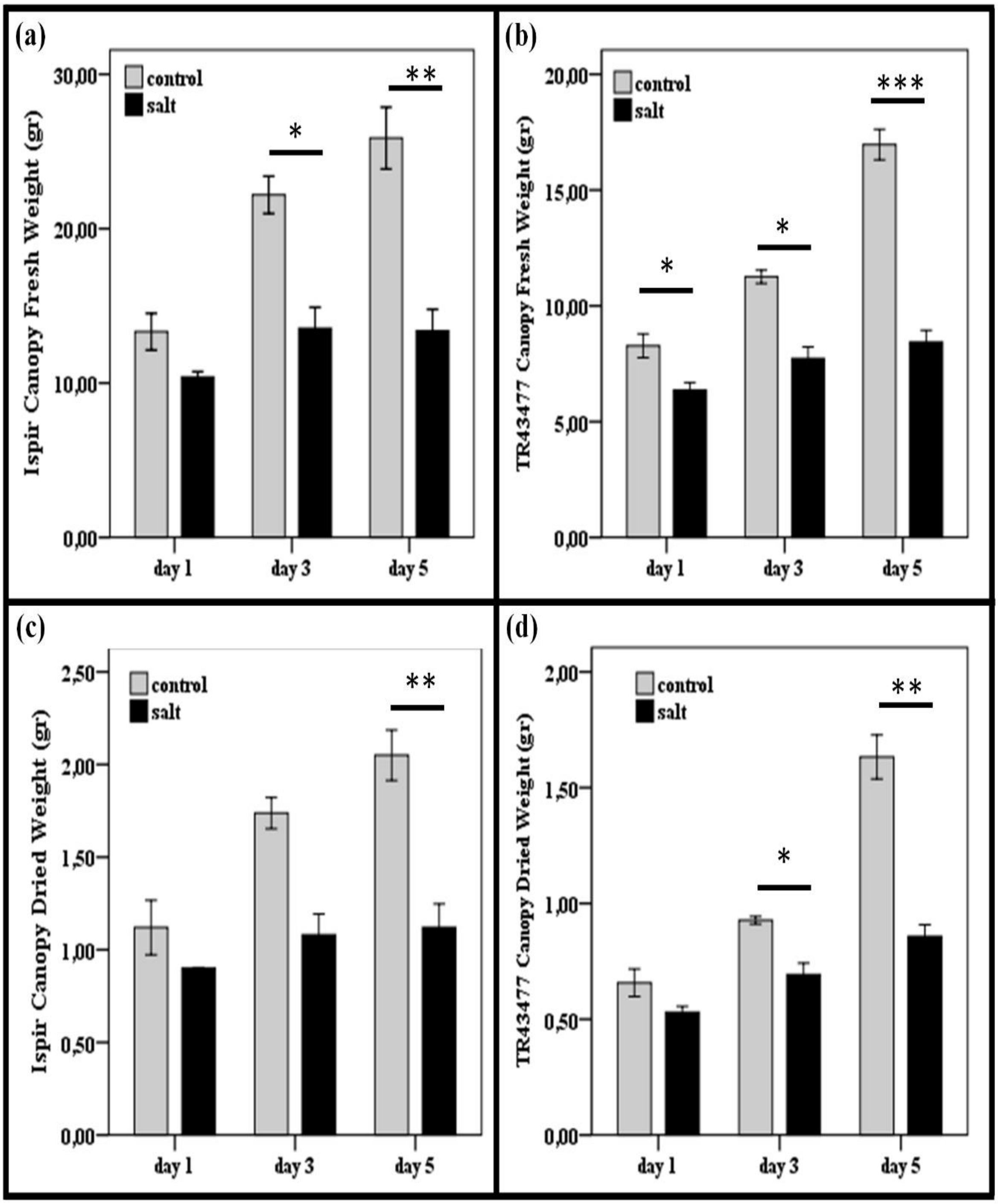


Figure 5.2. Fresh and dried canopy weights. Bar graphs representing the changes on (a,b) fresh and (c,d) dried canopy weights of (a,c) Ispir and (b,d) TR43477 varieties under salt stress. Error bars indicates SD (“*”: p<0.05, “**”: p<0.01, “***”: p<0.001).

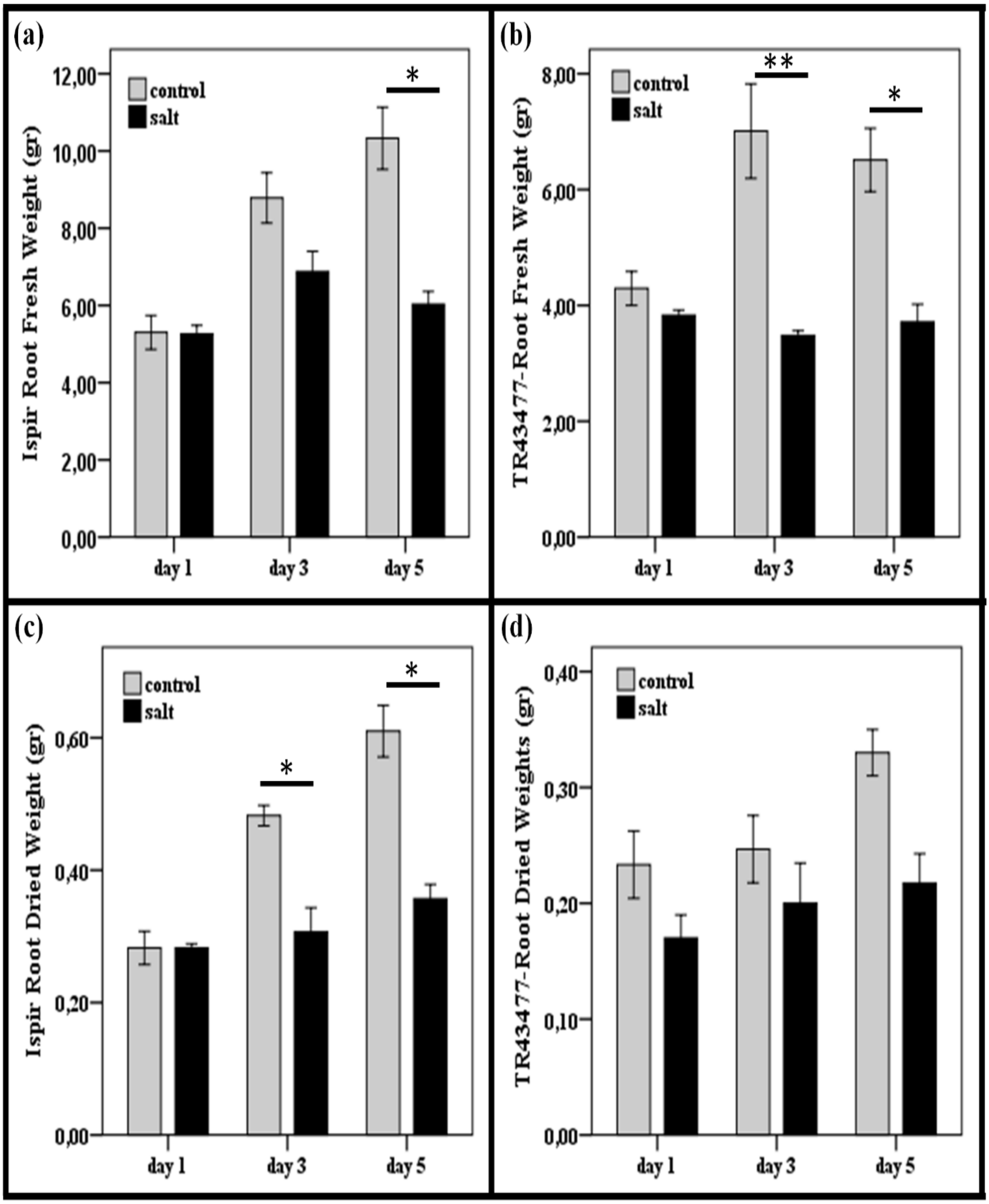


Figure 5.3. Fresh and dried root weights. Bar graphs representing the changes on (a,b) fresh and (c,d) dried root weights of (a,c) Ispir and (b,d) TR43477 varieties under salt stress. Error bars indicates SD (“*”: $p<0.05$, “**”: $p<0.01$).

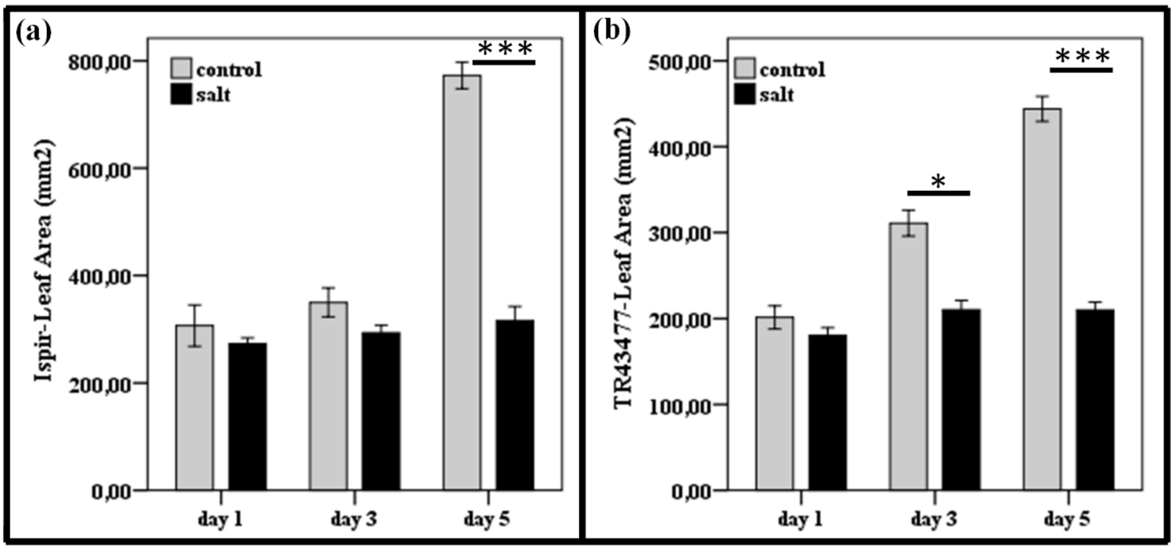


Figure 5.4. Leaf area measurements. Bar graphs representing the changes on leaf area of (a) Ispir and (b) TR43477 varieties under salt stress. (“*”: $p<0.05$, “***”: $p<0.001$).

5.1.3. Analysis of Changes in Water Content, Tissue Damage and Stomatal Conductance

Relative water content (RWC) is measured to analyze the amount of water retained in the leaf samples. RWC was expected to decrease as the salt concentration and duration of application increase [7]. The difference in the RWC of Ispir leaf samples were significant at the 3rd day of salt application (Figure 5.5a) and continued at the 5th days as expected. The TR43477 leaf samples demonstrated (Figure 5.5b) unexpected increase at the 3rd and 5th days which suspected to be due to the loss of leaf membrane integrity caused by tissue damages. The membrane injury index (MII) results (Figure 5.5c) have supported this observation. While MII was around 7% until the end of 5th day of treatment in Ispir, it reached around 15% in TR43477 at the same day of treatment. Maintenance of higher RWC during the first 3 days of stress treatment in Ispir leaves were also in agreement with the higher relative stomatal conductance capacity of this variety (Figure 5.5d) which reflected a successful functioning of stomatal gas exchange for sustainable growth. However, lack of stability in RWC and increased membrane damage in TR43477 concurred the rapid decline in the stomatal conductance observed even in the 1st day of salt treatment (Figure 5.5d).

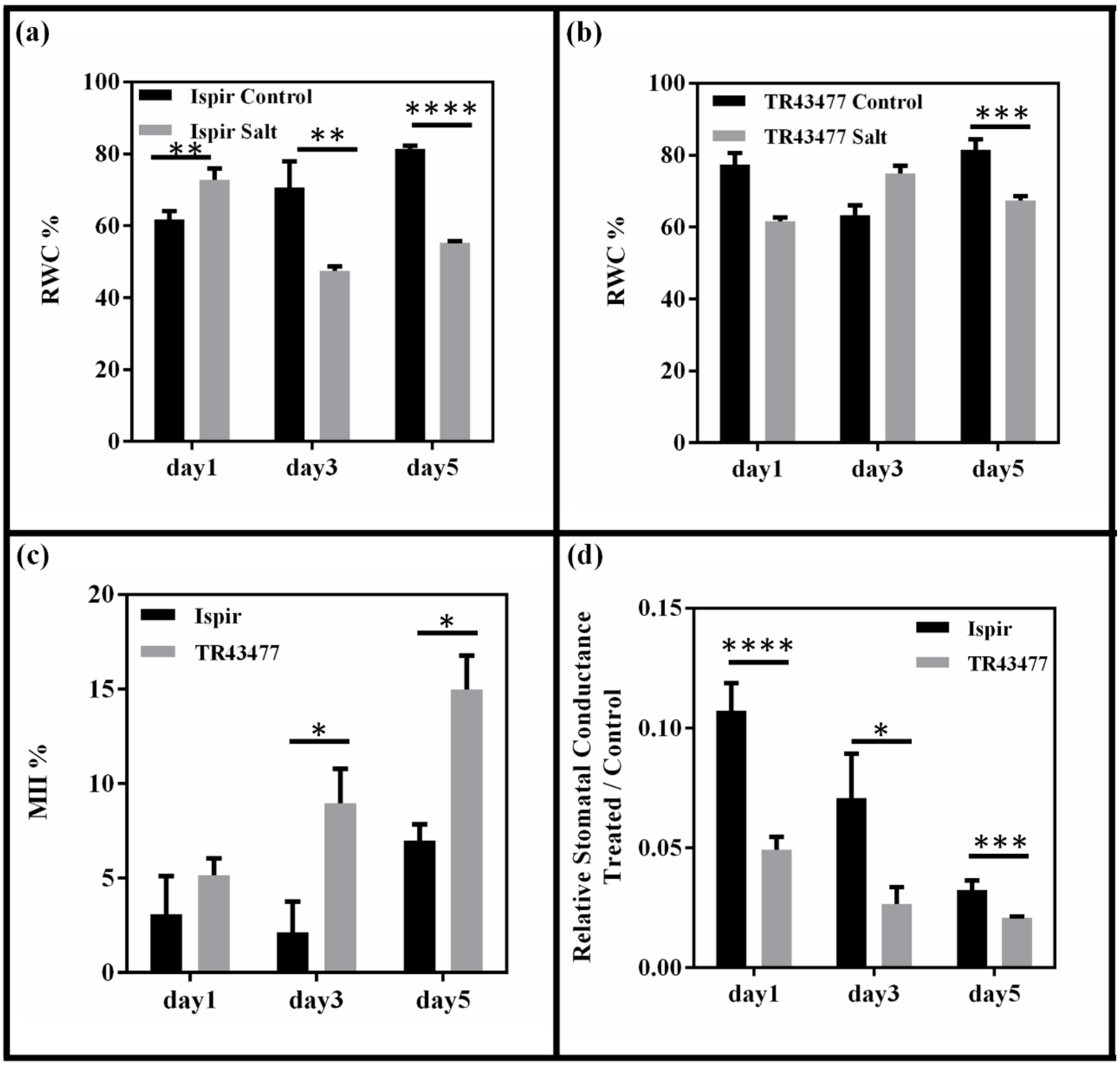


Figure 5.5. RWC, MII and relative stomatal closure graphs. Bar graphs representing the changes on (a,b) relative water content, (c) MII and (d) relative stomatal conductance of Ispir and TR43477 varieties under salt stress. Error bars indicates SD (“*”: $p<0.05$, “**”:$p<0.01$, “***”: $p<0.001$, “****”: $p<0.0001$).

5.1.4. Analysis of Ion Ratios

Elevated levels of NaCl stress create sodium (Na^+) and chloride (Cl^-) ion toxicity by interfering with cellular functions such as enzyme activities by competing with the uptake of nutrients such as K^+, and Ca^{+2} that acts as a secondary messenger in signal transduction pathways. Therefore management of Ca^{+2}/ Na^+ and K^+/ Na^+ cellular ratio is critical for salt tolerance in plants [50, 51].

In salt treatment, while roots and canopy of TR43477 have shown rapid intoxicity for elevated NaCl even at the 1st day of treatment with severe decline in K^+/ Na^+ (Figure 5.6b and d) and Ca^{+2}/ Na^+ (Figure 5.7b and d). In contrast, Ispir have demonstrated significantly better preservation of ionic ratios for the entire duration of treatment (Figure 5.6a, c and Figure 5.7a, c). The relative chloride concentrations of both Ispir and TR43477 presented no significant change starting on 3rd day of treatment in canopies (Figure 5.8a) or throughout the treatment periods (Figure 5.8b) in roots, however Cl- levels were generally higher in canopies in comparison to root tissues under salt stress.

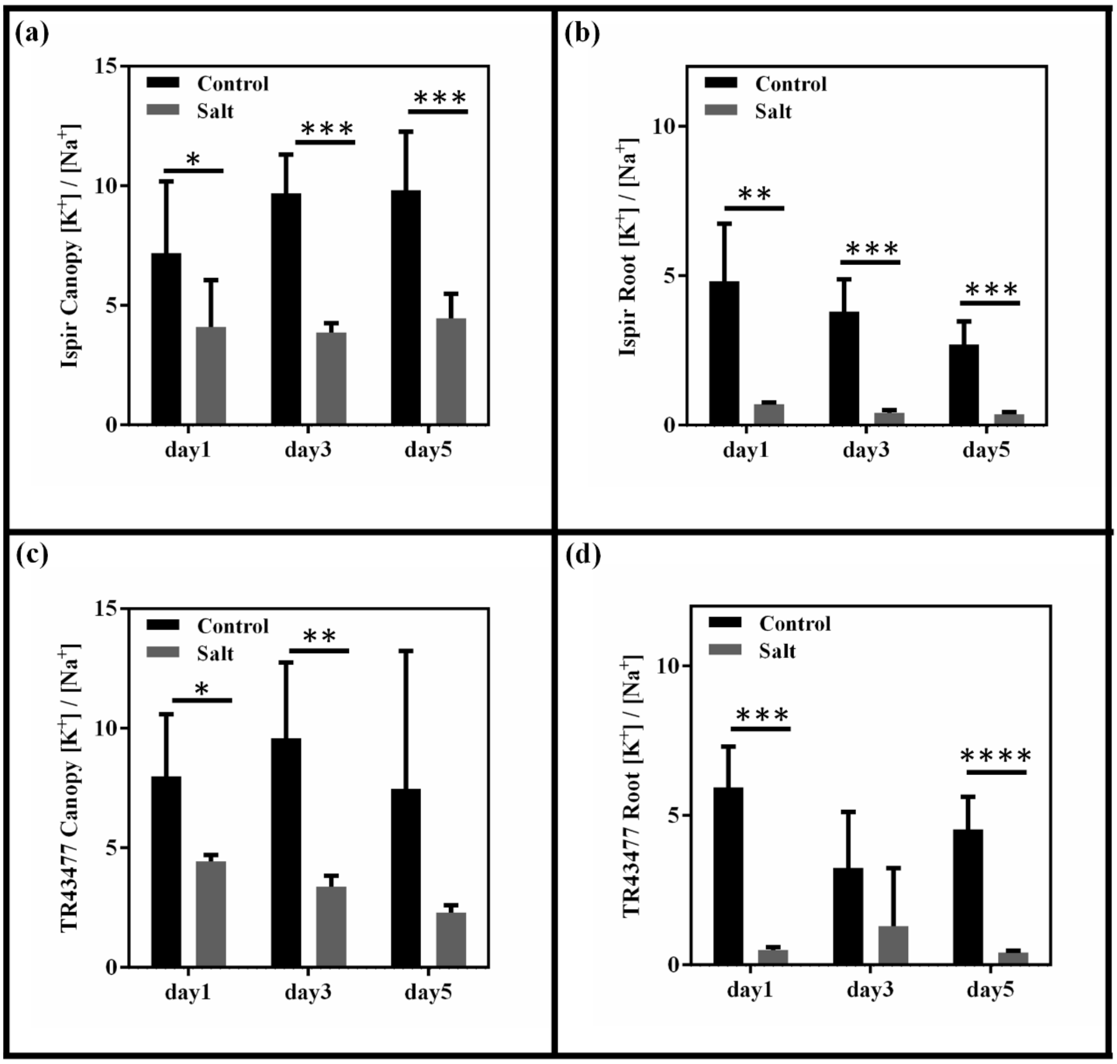


Figure 5.6. Potassium to sodium ion ratios. Bar graphs representing the changes on $[K^+]$ to $[Na^+]$ ratio in (a,c) canopy and (b,d) root samples of (a,b) Ispir and (c,d) TR43477 varieties under salt stress. Error bars indicates SD ("*": $p<0.05$, "**": $p<0.01$, "***": $p<0.001$, "****": $p<0.0001$).

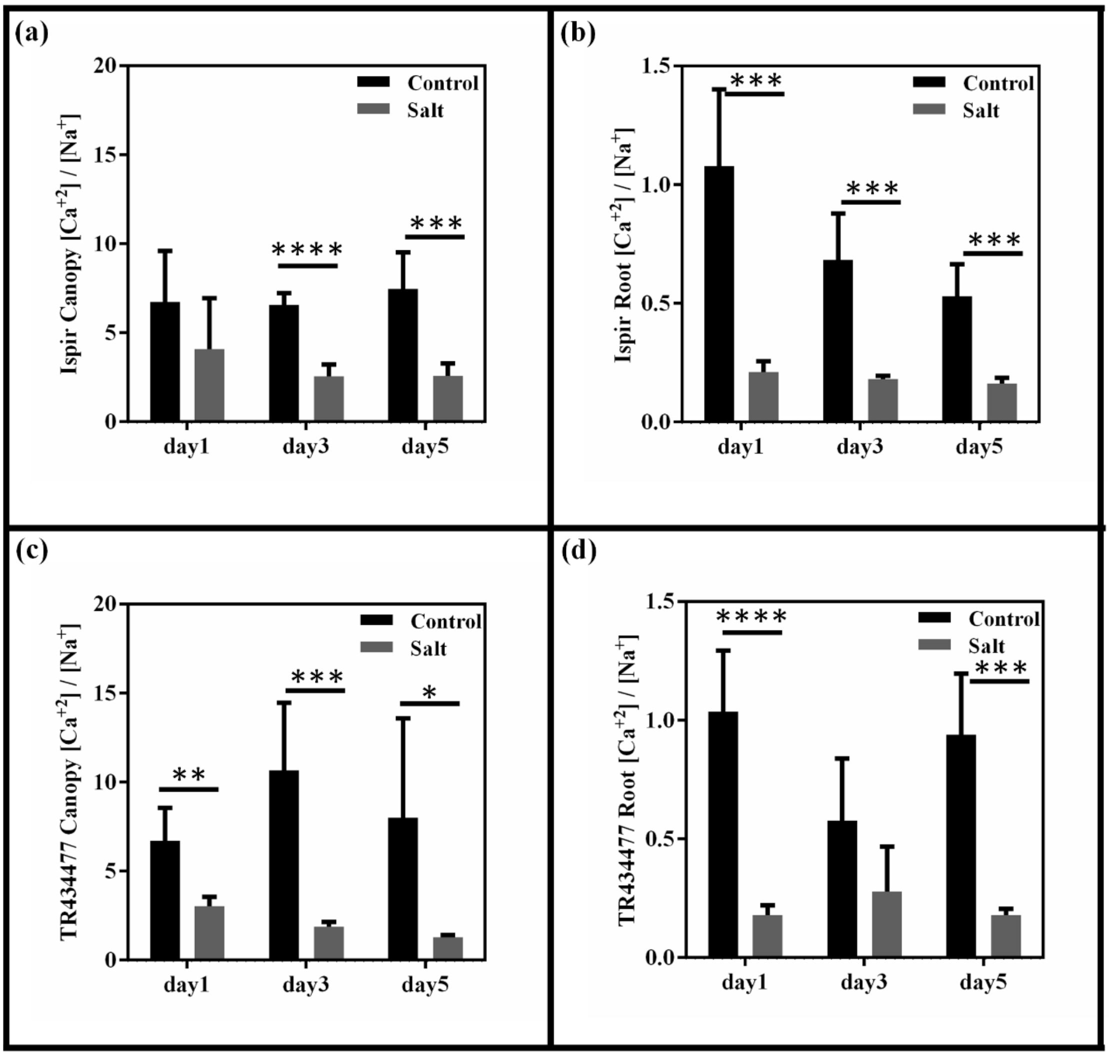


Figure 5.7. Calcium to sodium ion ratios. Bar graphs representing the changes on [Ca^{+2}] to [Na^{+}] ratio in (a,c) canopy and (b,d) root samples of (a,b) Ispir and (c,d) TR43477 varieties under salt stress. Error bars indicates SD (“*”: $p<0.05$, “**”: $p<0.01$, “***”: $p<0.001$, “****”: $p<0.0001$).

The overall changes in biomass, water content, membrane injury and ion concentrations demonstrated the most significant difference at the 3rd day of 125 mM salt application in both varieties. Thus the 3rd day was considered as the onset time of stress response.

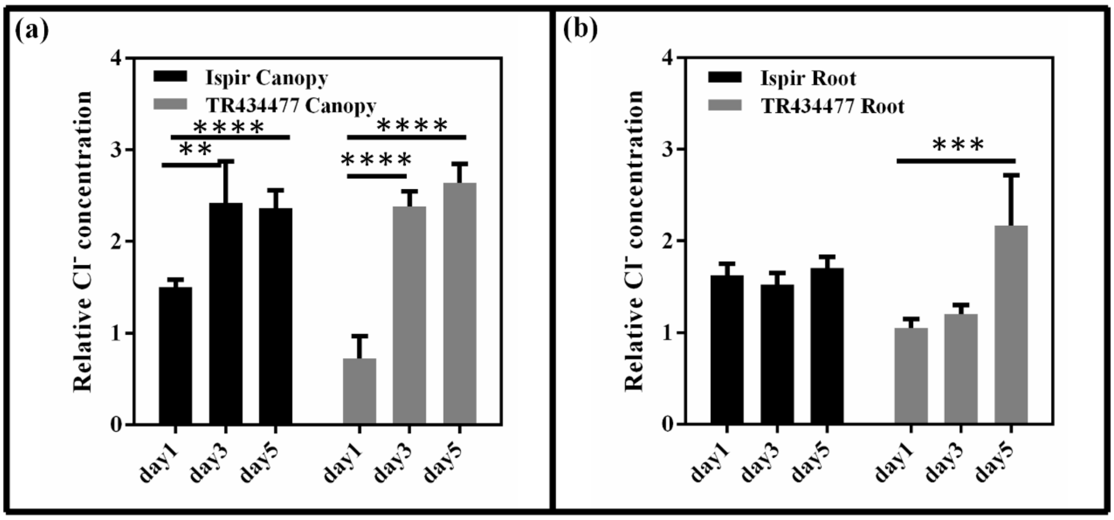


Figure 5.8. Relative chloride ion concentration. Bar graphs representing the changes on relative Cl^- concentration in (a) canopy and (b) root samples of Ispir and TR43477 varieties under salt stress normalized to their control each day. Error bars indicates SD ("**": $p<0.01$, "***": $p<0.001$, "****": $p<0.0001$).

5.2. Transcriptome Analysis of Common Bean

Total RNA extraction and cDNA library constructions were performed from root and leaf samples of both varieties grown 3 days at 125 mM NaCl treatment and control conditions described in Sections 4.2.2 and 4.2.3.

5.2.1. Sequencing Results and *De novo* Assembly

Illumina sequencing was performed for each total RNA pool extracted from the samples schematized in Figure 4.3 In total 10 subtranscriptomes (Table 5.1) containing more than a total of 266 million 90-bp long raw reads were generated. After quality trimming (Section 4.2.4) more than 255 million clean reads were remained which constituted over 23 gigabases (GBase).

Table 5.1. Sequencing output of leaf and root samples of Ispir and TR43477 varieties.

Sample Name	Total Raw Reads	Total Clean Reads	Total Clean Nucleotides (nt)	Q20 %	GC %
LC	25907332	25907332	2331659880	97.02%	46.02%
LS	27191626	27191626	2447246340	97.12%	46.19%
RC1	26481724	26481724	2383355160	96.91%	46.70%
RS1	27280462	27280462	2455241580	96.91%	47.08%
RC2	25998386	25998386	2339854740	96.94%	46.97%
RS2	25821046	25821046	2323894140	96.83%	47.30%
TLC	26928676	24379840	2194185600	98.82%	46.75%
TLS	27623716	25458348	2291251320	98.82%	46.33%
TRC	27255056	23699840	2132985600	98.65%	46.53%
TRS	26000000	23634116	2127070440	98.60%	46.78%
Average	26648802	25585272	2302674480	97.66%	46.67%
Total	266488024	255852720	23026744800	--	--

Clean reads from all subtranscriptomes were *de novo* assembled as described in section 4.2.4. The number of transcripts in each subtranscriptome, their mean (average) lengths and N50 values (weighted median of the sequence lengths) were presented in Table 5.2. Joint assembly of the subtranscriptomes were generated the *Phaseolus vulgaris* L. whole transcriptome which consisted of 73762 transcripts (all-unigenes) with a mean length of 930 bp (Table 5.2) ranging between 200 and 15549 bp. A total of 42503 transcripts (57.6%) were distinct singletons (unigenes) whereas 31259 (42.3%) were grouped into 10531 distinct clusters. The length of the transcripts were evenly distributed (Figure 5.9). In total 63671 transcripts were expressed in leaf samples whereas 71958 were expressed in root samples.

Table 5.2. Results of *de novo* assembly of leaf and root samples of Ispir and TR43477.

Sample Name	Total Number	Total Length(nt)	Mean Length(nt)	N50	Total Consensus Sequences	Distinct Clusters	Distinct Singletons
LC	57115	31768237	556	792	57115	13290	43825
LS	53781	33109773	616	940	53781	13496	40285
RC1	61642	35383270	574	841	61642	14686	46956
RS1	61176	32903809	538	763	61176	13211	47965
RC2	66299	36049902	544	792	66299	14960	51339
RS2	59156	31809079	538	763	59156	12526	46630
TLC	50527	33090899	655	1011	50527	13045	37482
TLS	48968	33951242	693	1097	48968	13161	35807
TRC	57602	34500939	599	908	57602	14636	42966
TRS	53915	33737664	626	970	53915	13281	40634
All	73762	68572394	930	1482	73762	31259	42503

5.2.2. Annotation and Functional Classification of Transcripts

It was revealed that, out of 73762 transcripts, 55433 (75.2%), 33395 (45.3%) and 36916 (50%) were shown significant (BLASTx *E*-value $\leq 10^{-5}$) similarity to the protein sequences present in the Nr, KEGG and Swiss-Prot databases, respectively (accessed August, 2014). Among them 28454 sequences were annotated with sequences in all three databases (Figure 5.10a). Almost half of the transcripts shorter that 500 bp had a match in Nr database whereas the annotation ratio increases dramatically to 98% as the transcript length increases over 1000 bp (Figure 5.10b). This has obviously indicated that the lengths of the transcripts were highly correlated with their probability to match with a sequence in a database. Among the Nr-annotated transcripts 87% showed significant homology to proteins of the legume species (Figure 5.11a). The similarity and *E*-value distributions implied that around 85% of the Nr-annotated transcripts showed more than 60% similarity (Figure 5.11b) to the proteins stored in Nr database with an *E*-value of less than 10^{-15} (Figure 5.11c).

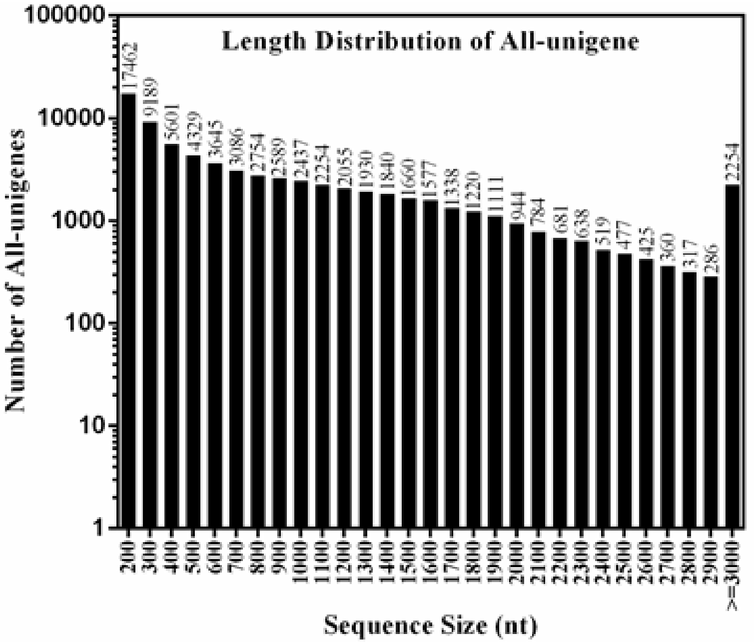


Figure 5.9. Length distribution of all-unigene between 200-3000 nt.

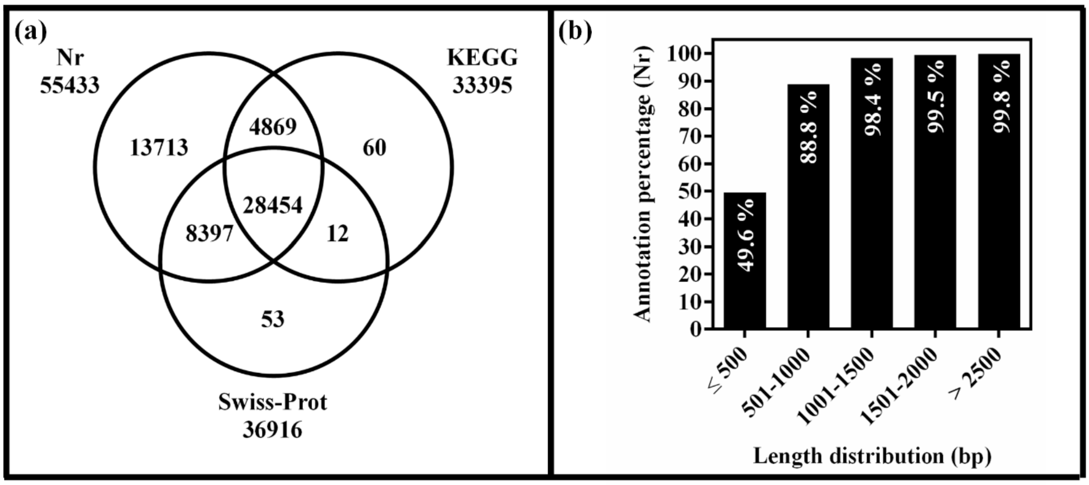


Figure 5.10. Database and length distribution of all-unigenes.(a) Distribution of annotated all-unigene in three different databases. (b) Histogram showing Nr annotation percentage of all unigenes based on their length distribution.

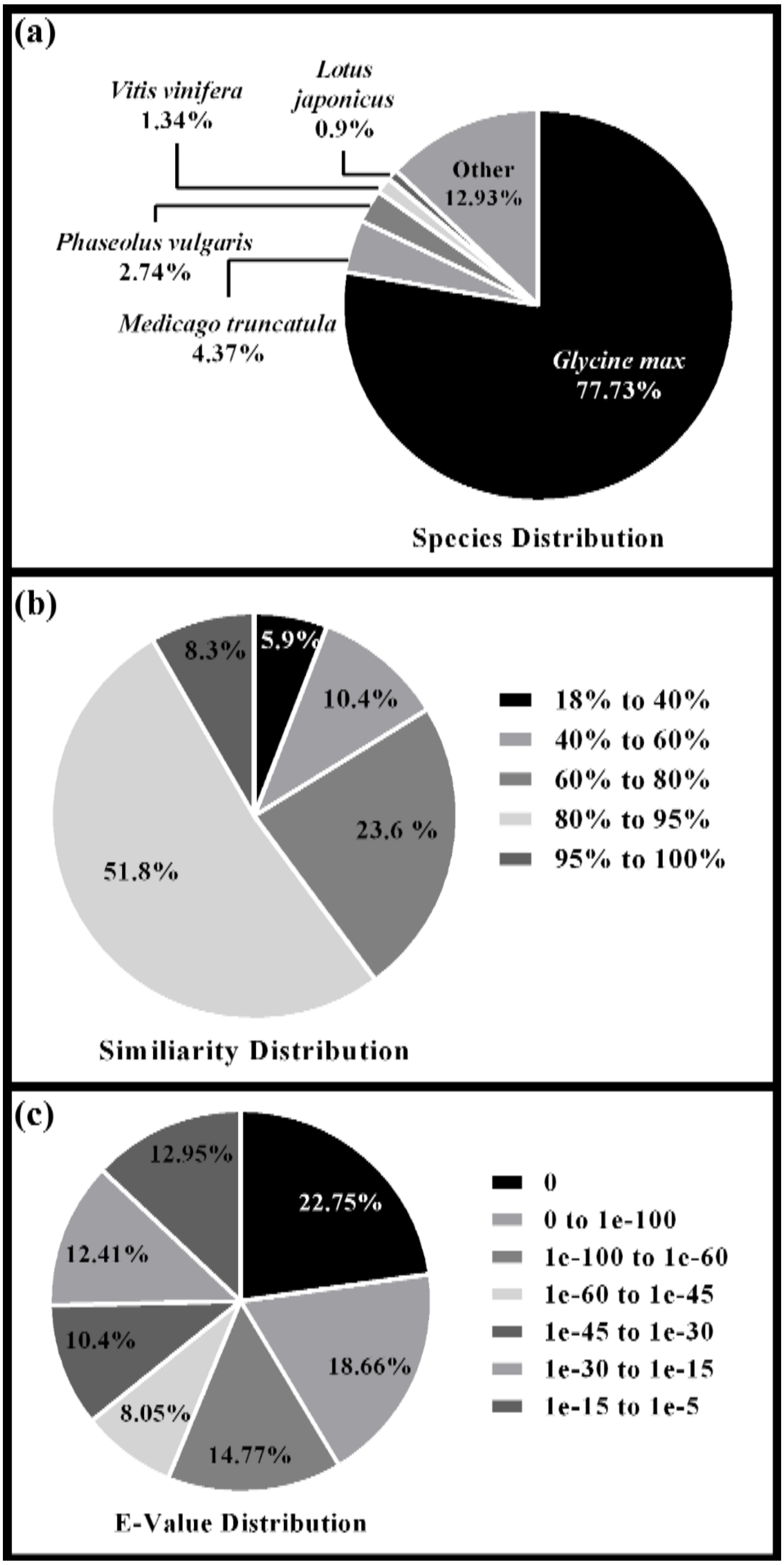


Figure 5.11. Distribution of all-unigenes within (a) species, (b) showing similarity to proteins in Nr database with their (c) *E*-values following BLASTx analysis.

5.2.3. Verification of RNA-seq Quantifications with qRT-PCR

Gene expression levels were determined with the calculation of RPKM levels during transcriptome analysis as described in Section 4.2.4. To assess the reliability of gene expression levels in transcriptome analysis, 43 transcripts were randomly selected and their expression levels were determined with qRT-PCRs. Categories of those selected transcripts were 17 from the non-regulated, 15 from the up-regulated and 11 from the downregulated groups. The results indicated a very high correlation (*r*=0.91) between the transcriptome analysis and qRT-PCR results (Figure 5.12) with melting curves implicating a single product amplification for all primer pairs.

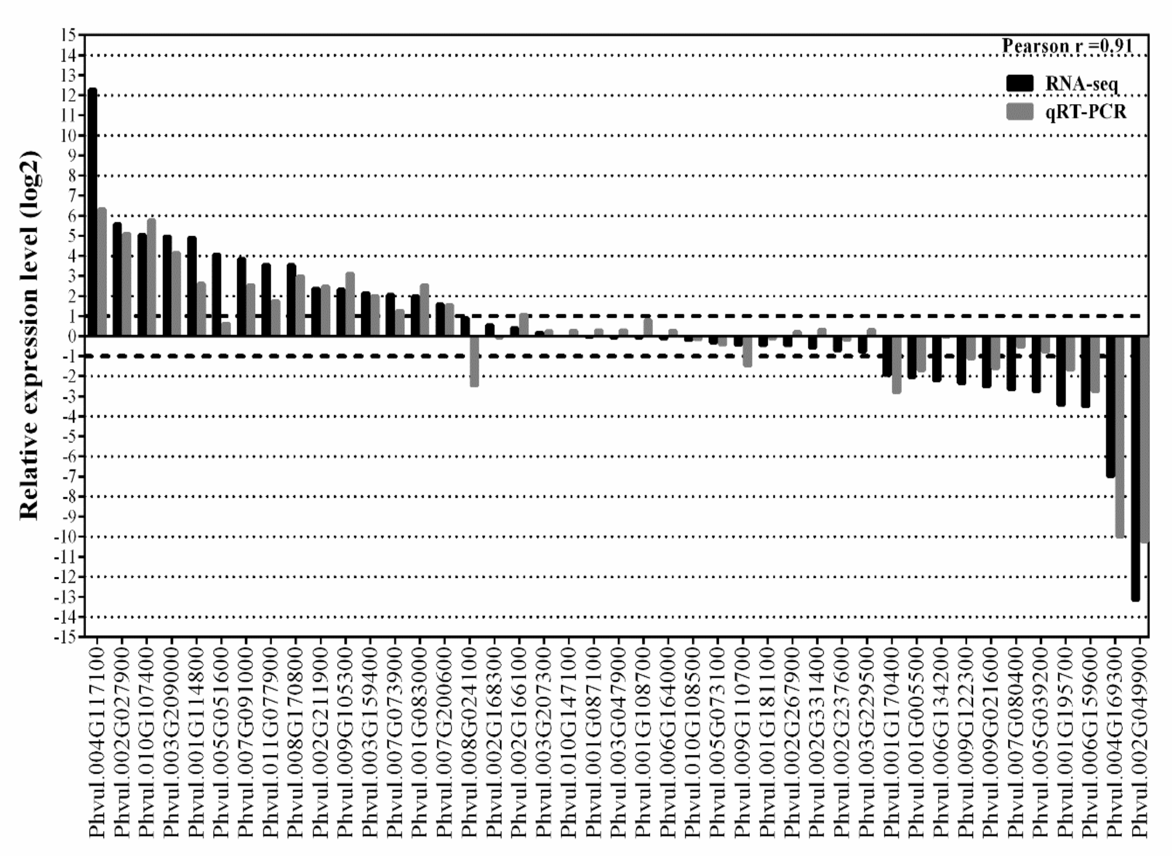


Figure 5.12. Comparison of relative expression levels between qRT-PCR and transcriptome analysis results under salt stress. Expression levels were normalized to the geometric average of Ct (threshold cycle) values of *Act-11* and *IDE* and their untreated controls.

5.2.4. Identification of the Differentially Expressed Genes (DEGs)

The transcripts which were up or down-regulated more than two fold with a false discovery rate (FDR) less than 10^{-3} were defined as differentially expressed. The number of DEGs uniquely up-regulated in leaf tissues were 938 and 1860 for Ispir and TR43477, respectively. However, these numbers dropped down to 258 and 871 in root tissues (Figure 5.13). When uniquely down regulated transcripts were analyzed although Ispir and TR43477 had similar number of transcripts in their leaves (1271 and 1407 respectively), in root tissues, Ispir had almost twice the number of transcripts down-regulated in comparison to TR43477 (1407 and 827 respectively) In total, when the data were analyzed irrespective of genotypes or tissue types a total of 133 up-regulated and 46 down-regulated transcripts were obtained during salt stress of common bean (Figure 5.13).

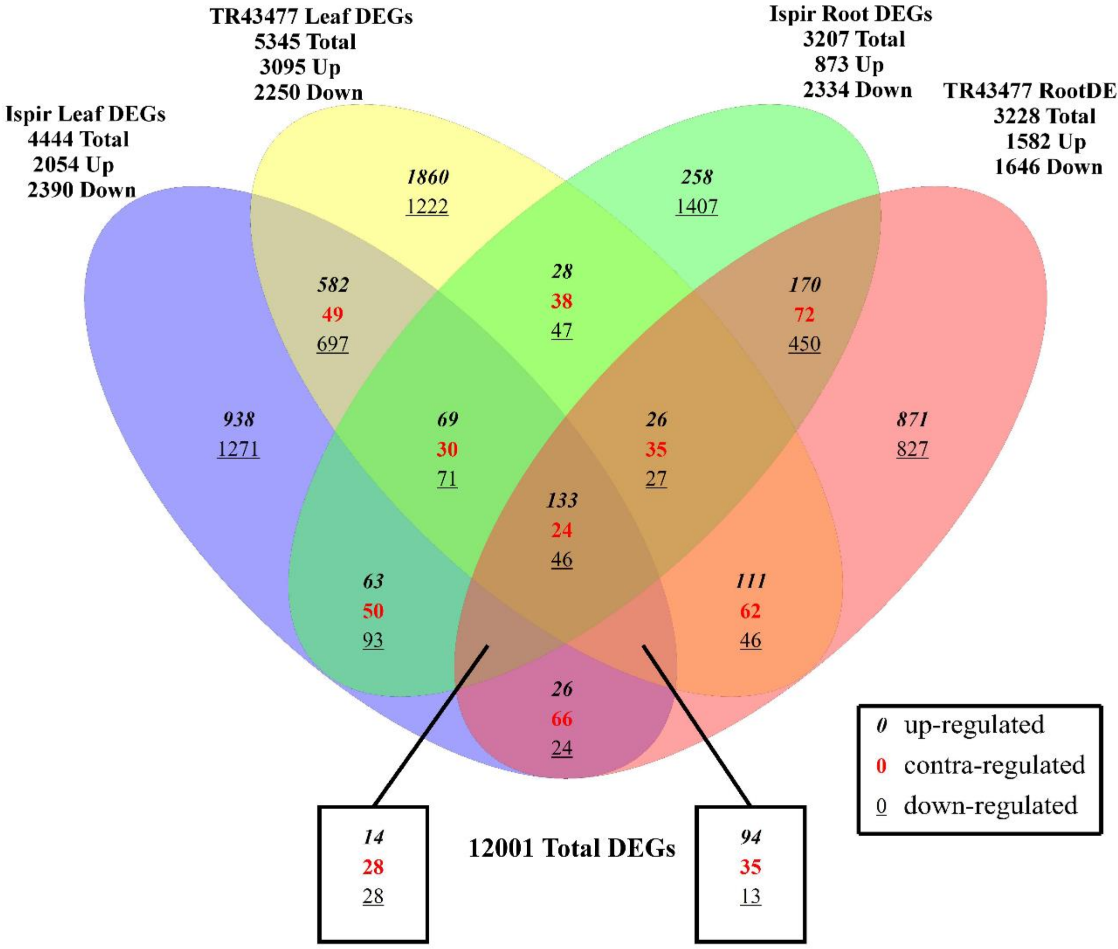


Figure 5.13. Venn diagram showing the number of DEGs regulated under salt stress.

5.2.5. Functional Classification of the Salt Responsive DEGs

The enriched terms from each GO, KEGG and COG databases were in agreement for all samples within upregulated and downregulated transcripts (Table B.1-24, Figure 5.14 and Figure 5.15).

The terms related to energy metabolism (lipid, amino acid and carbohydrate transport) (Figure 5.14 and Figure 5.15), oxidative stress (oxidation reduction process or response to oxidative stress, biosynthesis of secondary metabolites) and cell wall biogenesis were commonly enriched (Table B.1-9) within the DEGs of both varieties except for IRDW and TRUP groups. Terms related to ion and macromolecule transport (transmembrane transport, ABC transporters etc.) seemed to be enriched in all leaf samples of both varieties as well as within the upregulated transcripts of Ispir root samples (IRUP) (Table B.1-5, Table B.9-13 and Table B.17-21).

Transcription, translation and post translational modification related processes were exclusively enriched in root samples of both varieties (Table B.5-8 and Table B.21-24) and in the down-regulated transcripts of TR43477 leaf samples (Table B.8, Table B.16 and Table B.24). Intracellular trafficking, secretion and vesicular transport related processes also enriched in roots of both varieties as well (Figure 5.14, Figure 5.15).

The processes related to cell cycle / cell division, energy metabolism and transport were down-regulated significantly in all samples (Figure 5.14, Figure 5.15, Table B.18, Table B.20, Table B.22, Table B.24) whereas photosynthesis related transcripts (thylakoid and photosystem proteins) were exclusively down-regulated in TR43477 leaf samples (Table B.4, Table B.12).

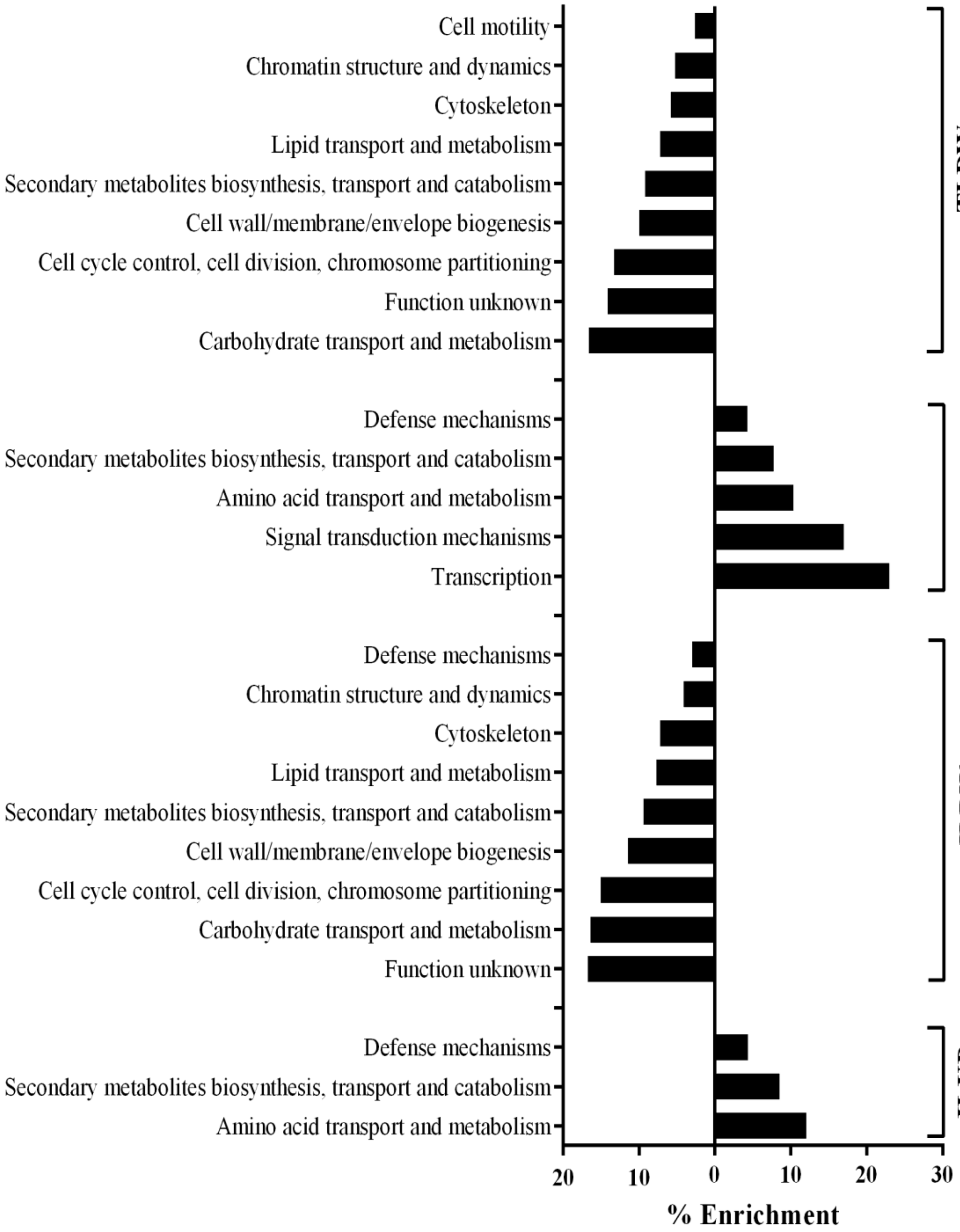


Figure 5.14. COG functional enrichment analysis of regulated transcripts identified in transcriptome analysis of leaf samples of Ispir and TR43477 varieties under salt stress. ILUP: Ispir leaf upregulated, ILDW: Ispir leaf down regulated, TLUP: TR43477 leaf upregulated and TLDW: TR43477 leaf downregulated transcripts.

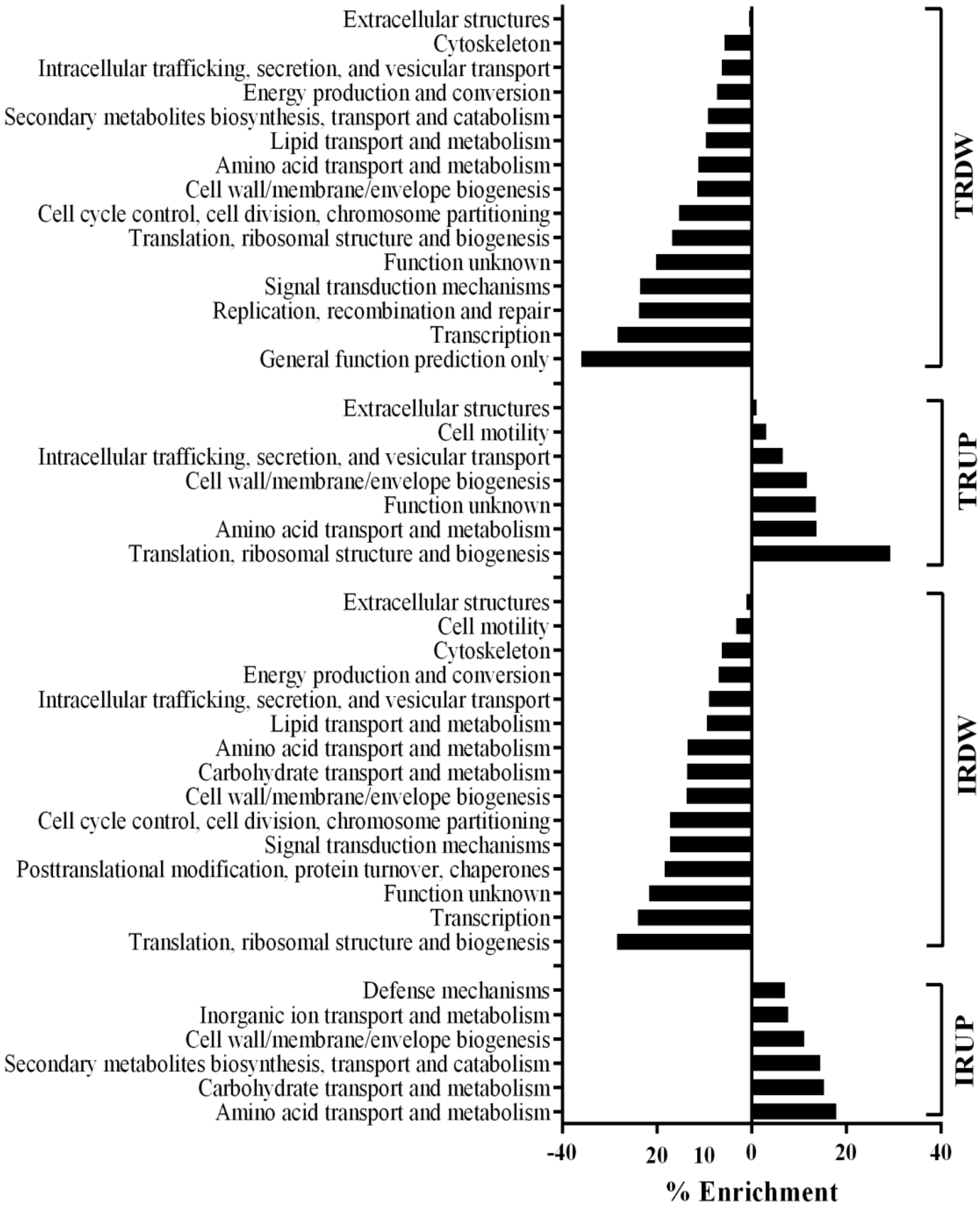


Figure 5.15. COG functional enrichment analysis of regulated transcripts identified in transcriptome analysis of root samples of Ispir and TR43477 varieties under salt stress. IRUP: Ispir root upregulated, IRDW: Ispir root down regulated, TRUP: TR43477 root upregulated and TRDW: TR43477 root downregulated transcripts.

5.3. Functional Characterization of Selected Transcripts

5.3.1. Cloning of Certain Salt Responsive Genes and Generation of Transgenic *A.thaliana* Overexpression Lines

The selected transcripts (Table 4.1) were amplified from the single stranded cDNAs of Ispir leaf or root samples by a two-step PCR reaction with att*B* primers containing gene specific overhangs as described in Section 4.4.2. (Figure 5.16). Gel purified fragments were cloned into the pDONR207 with BP reactions following the steps in Section 4.4.3 and Figure 4.4a. The mixes of BP reactions were transformed into *E. coli* DH5α and five colonies were selected and subjected to colony PCR (Section 4.4.5, Figure 5.17) for the confirmation before sequence verification. Verified clones in the entry vector were subcloned into pGWB412 expression vectors with LR reactions following the steps in Section 4.4.3 and Figure 4.4b. The plasmids isolated from one of the positive colonies were transformed to *A.tumafaciens* and transformations were confirmed again with colony PCR (Figure 5.18).

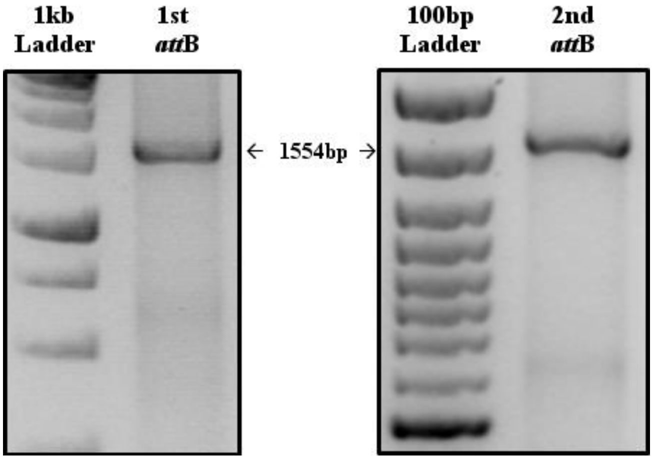


Figure 5.16. A representative image for two step PCR reaction using *TT12* (Phvul.011G104200.1) transcripts for *att*B addition.

The Arabidopsis plants were incubated with transformed *A.tumafaciens* suspension culture to apply floral dip method following the steps in Section 4.5.2 and Figure 4.5. The transformant lines were selected on Kanamycin containing MS medium. Success of floral dip transformations were also confirmed with PCR amplifications from gDNA (Figure 5.19) and/or with RT-PCR reactions (Figure 5.20). From Figure 5.17 to Figure 5.20- were representative images of *TT12* (Phvul.011G104200.1) cloning steps. The rest of the transcripts in Table 4.1 were subjected to the same procedure. However only the top five genes in the list could be cloned and successfully transformed to *A.thaliana*. The rest could not be cloned and/or transformed due to failure in various steps of the cloning procedure. For the 5 cloned genes (Table 5.3), the Figure 5.21 to Figure 5.24 represent the verification results of *in planta* transformation with RT-PCR (Figure 5.21 and Figure 5.22) from cDNAs or PCR from gDNA of transgenic line (Figure 5.23 and Figure 5.24) as described in Section 4.5.3. Each transformed plants were grown in soil to generate homozygous T3 lines following the steps detailed in Section 4.5.3 and subjected to phenotype analyses to assess their functional impact under salt stress conditions.

Table 5.3. The list of cloned candidates for cloning and functional characterization.

Gene ID (Transcriptome)	**Annotation**	**geneID (Phytozome.org)**	**LS vs. LC**	**RS vs. RS**	**TR-LS vs. TR-LC**	**TR-RS vs. TR-RC**
CL7815.Contig2_All	FAD-binding Berberine family protein (**CPRD2**)	Phvul.002G200600.1	Up	Up	Up	Down
Unigene24388_All	Uncharacterized protein (**Unc2**)	Phvul.002G211900.1	--	Up	--	Up
Unigene14206_All	Endo-1,3-beta-glucanase (**β-Glu**)	Phvul.011G077900.1	Up	Up	--	--
Unigene27640_All	Uncharacterized protein (**Unc4**)	Phvul.003G209000.1	Up	Up	Up	Up
Unigene4657_All	PREDICTED: protein TRANSPARENT TESTA 12-like (**TT12**)	Phvul.011G104200.1	Up	--	Up	--

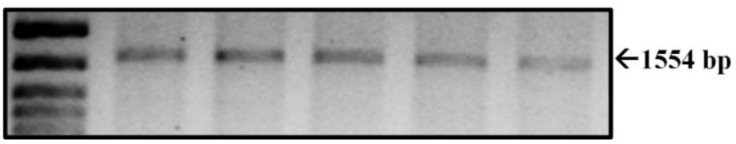


Figure 5.17. A representative image for confirmation of *TT12* (Phvul.011G104200.1) transformation by colony PCR after BP reaction (Col: Colony).

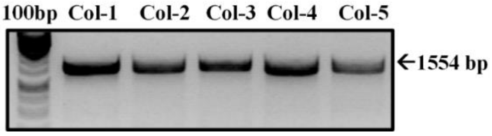


Figure 5.18. A representative image for confirmation of *TT12* (Phvul.011G104200.1) after *A.tumafaciens* transformation by colony PCR (Col: Colony).

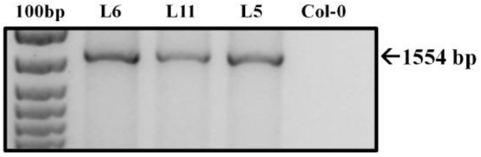


Figure 5.19. PCR verification of genomic integration of *TT12* (Phvul.011G104200.1) in transgenic *A.thaliana* lines with PCR using gDNA as template (Col-0: Wild type Arabidopsis).

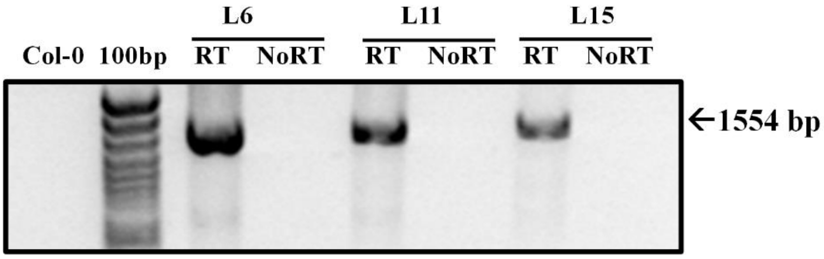


Figure 5.20. RT-PCR verification of *TT12* (Phvul.011G104200.1) expression in transgenic L6, L11 and L15 lines (Col-0: Wild type Arabidopsis).

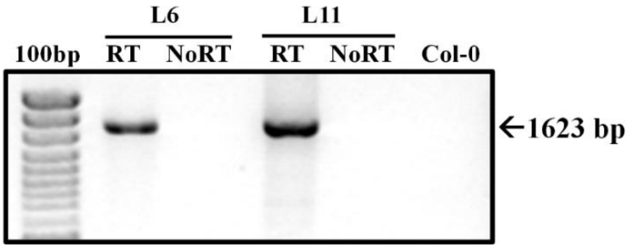


Figure 5.21. RT-PCR verification of *CPRD2* (Phvul.002G200600.1) expression in transgenic L6 and L11 lines.

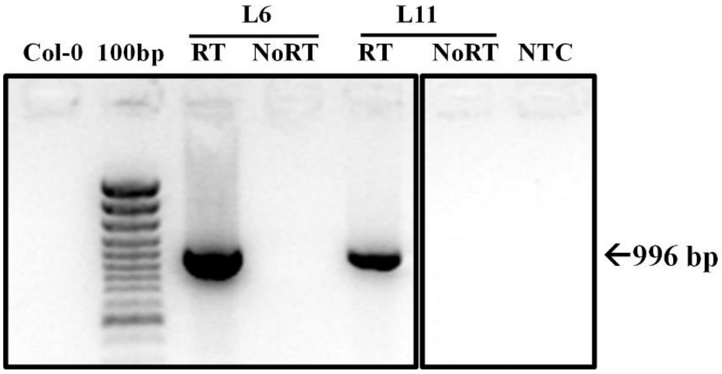


Figure 5.22. RT-PCR verification of *β-Glu* (Phvul.011G077900.1) expression in transgenic L6 and L11 lines.

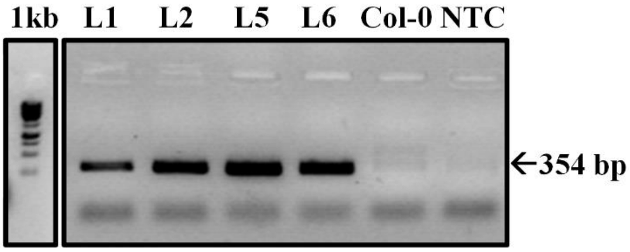


Figure 5.23. PCR verification of genomic integration of *Unc2* (Phvul.002G211900.1) in transgenic L1, L2, L5 and L6 lines using gDNA as template.

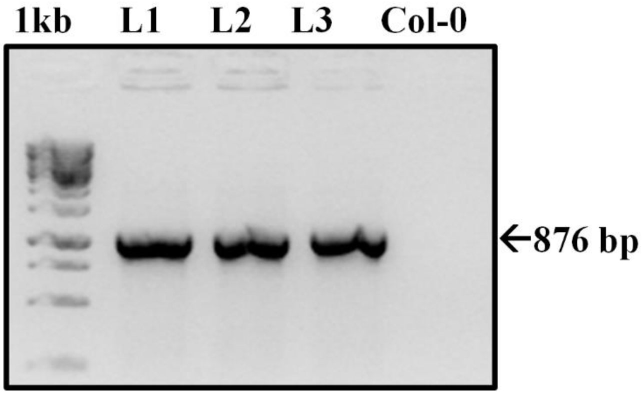
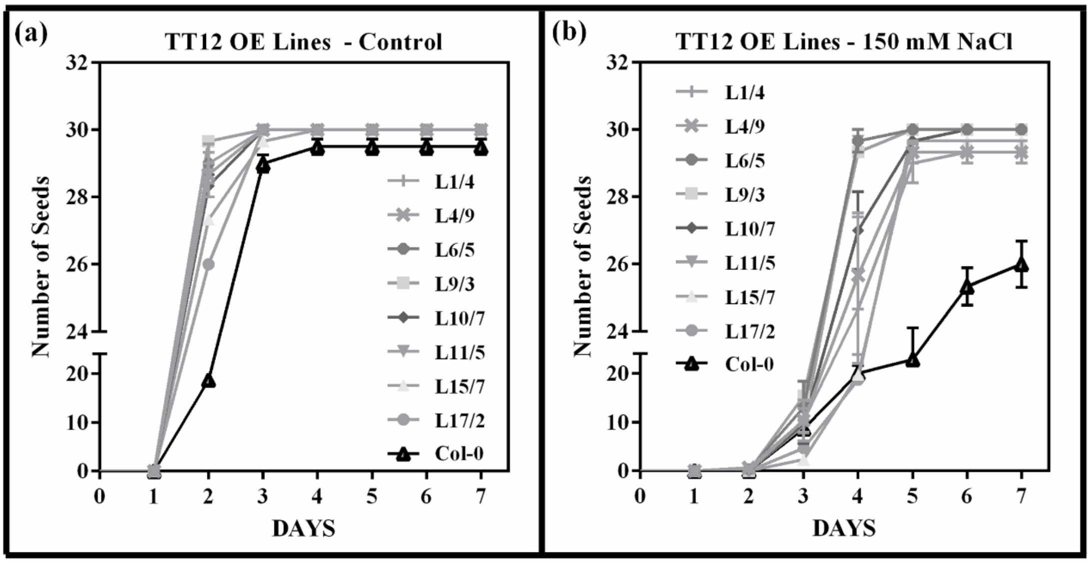


Figure 5.24. PCR verification of genomic integration of *Unc4* (Phvul.003G209000.1) in transgenic L1, L2 and L3 lines using gDNA as template.

5.3.2. Germination Assay of Transgenic Lines

Thirty seeds of each T3 transgenic lines were grown on 150 mM NaCl containing 0.5X MS medium and the number of successfully germinated seeds (Figure 4.6) were counted daily for seven days. The germination ratio reached to 100% for both Col-0 and all transgenic lines (Figure 5.25a, Figure 5.26a, Figure 5.27a, Figure 5.28a, Figure 5.29a and Figure 5.30a) at the 3rd day under control conditions. While all seeds of *TT12* transgenic lines germinated at the 5th day of salt treatment (Figure 5.25b).

The seeds of *CPRD2*, *β-glu* and *Unc2* lines showed variable number of germinating seeds until the 5th day. However, their germination ratio were always significantly higher than Col-0 (Figure 5.26b, Figure 5.27b and Figure 5.28b). The seeds of *Unc4* lines showed generally poorer performance in comparison to both Col-0 and to all other transgenic lines (Figure 5.29b) especially the germination rate and ratio of Unc4 L1/3 were even lower than Col-0 seed.



(a) TT12 OE Lines - Control

(b) TT12 OE Lines - 150 mM NaCl

Figure 5.25. Germination assay of *TT12* transgenic lines. (a) Control and (b) salt treated seeds. OE: overexpression.

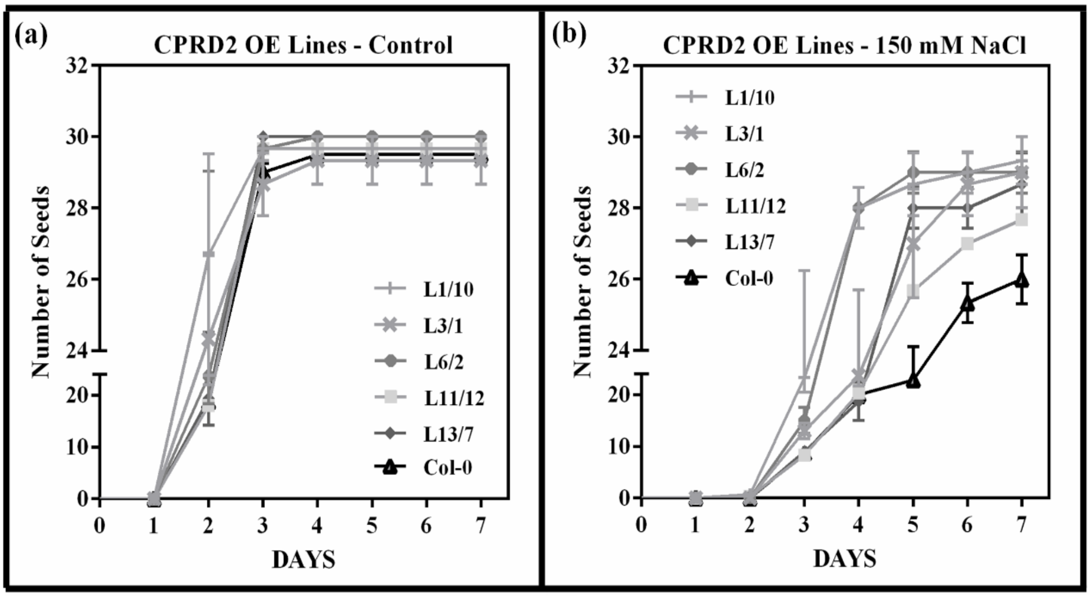


Figure 5.26. Germination assay of *CPRD2* transgenic lines. (a) Control and (b) salt treated seeds. OE: overexpression.

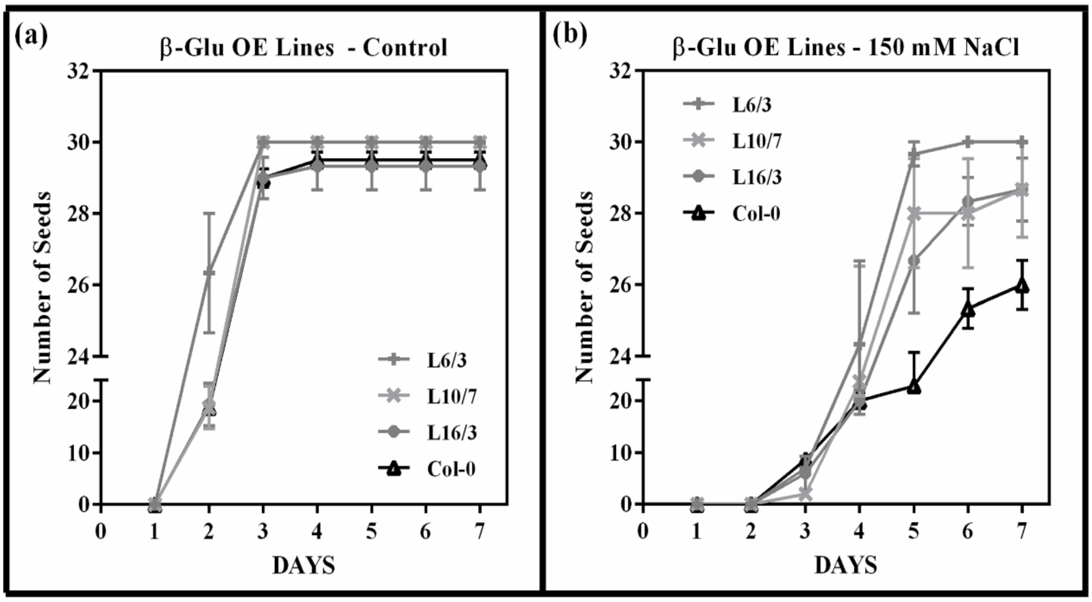


Figure 5.27. Germination assay of *β-Glu* transgenic lines. (a) Control and (b) salt treated seeds. OE: overexpression.

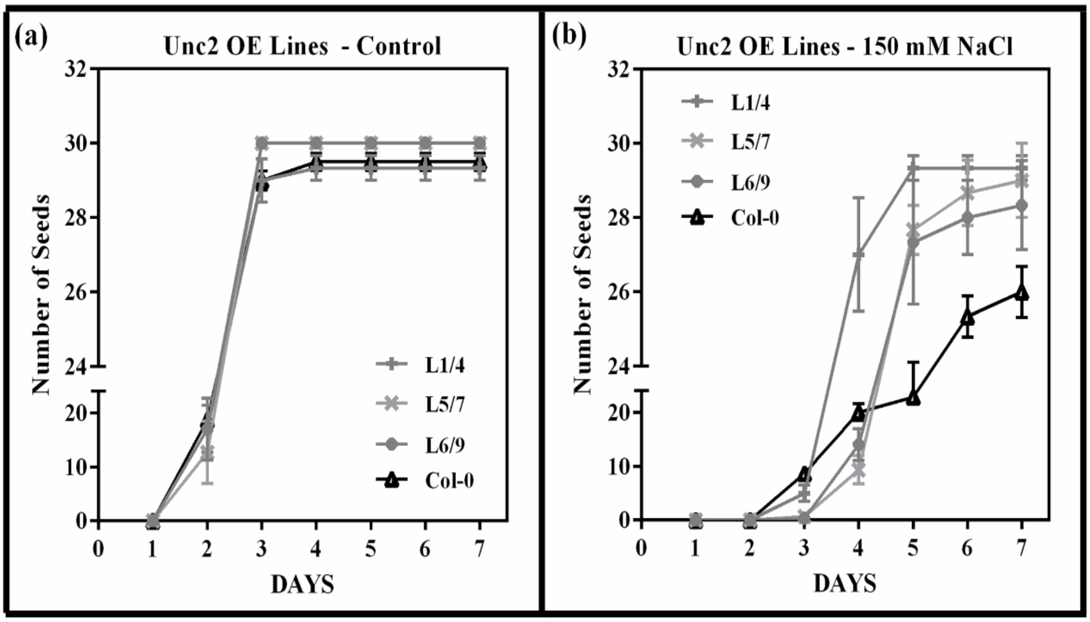


Figure 5.28. Germination assay of *Unc2* transgenic lines. (a) Control and (b) salt treated seeds. OE: overexpression.

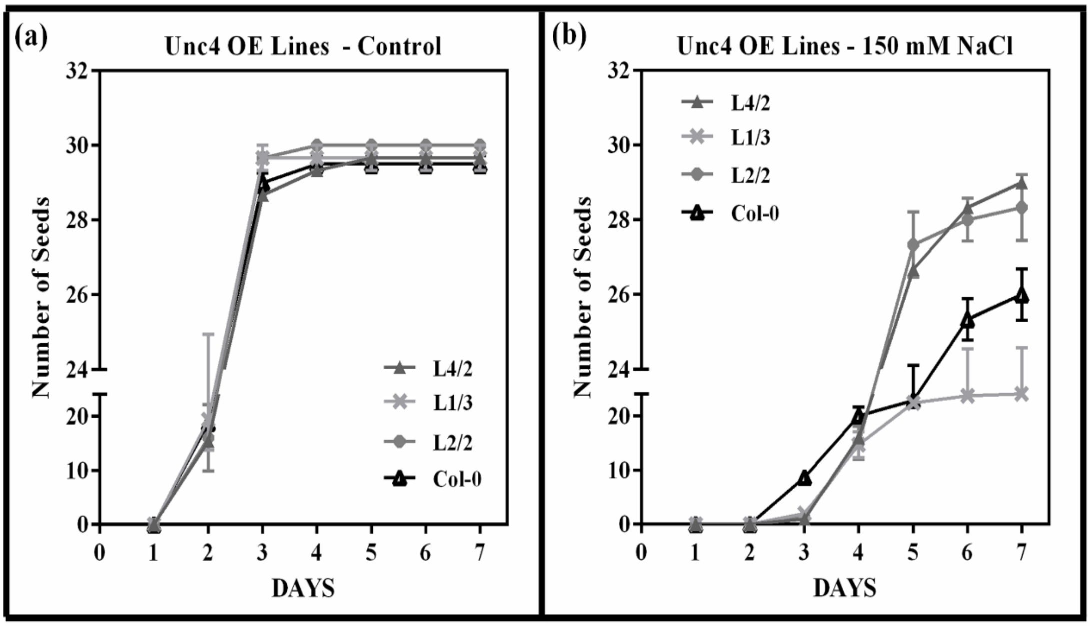


Figure 5.29. Germination assay of *Unc4* transgenic lines. (a) Control and (b) salt treated seeds. OE: overexpression.

5.3.3. Root elongation Assay of Transgenic Lines

Water, mineral uptake and foraging are largely determined by root system elongations and its inhibition is a rapid response to abiotic stress in plants [148]. Therefore, degrees of inhibition in root elongation was considered as a good indicator of tolerance to stress factors.

The seed of transgenic lines and Col-0 plants were grown on a straight line at vertical position in 150 mM salt containing half strength MS plates and after 14 days, the root sizes were measured. Root elongation rates were significantly higher than Col-0 plants in all *TT12*, *CPRD2*, *β-glu* and *Unc2* (Figure 5.30a-d) transgenic lines. However, similar to germination assay results the *Unc4* transgenic lines were shown no significant difference in elongation rate with Col-0 (Figure 5.30e) and again demonstrated very poor performance with respect to *TT12*, *CPRD2*, *β-glu* and *Unc2* transgenic lines.

Germination and root elongation assays have been considered as elimination steps of the high number of transgenic lines which were tested during the assays. These assays have been useful to select the specific lines for each transgenes (*TT12* L6/5, L11/5, L15/7, *CPRD2* L6/2, L1/10, *β-Glu* L6/3, L10/7, *Unc2* L5/7 and L6/9) and to be further tested with other physiological parameters as continued in the following sections.

5.3.4. Calculation of RWC of Transgenic Lines

Relative water content in plants considered as one of the best criterion reflecting the plant water status since it indicates the balance between absorbed water by roots and consumed water through transpiration in relation to change in cell volume. Therefore tolerance response in plants to stress is correlated with better maintenance of relative water content [149].

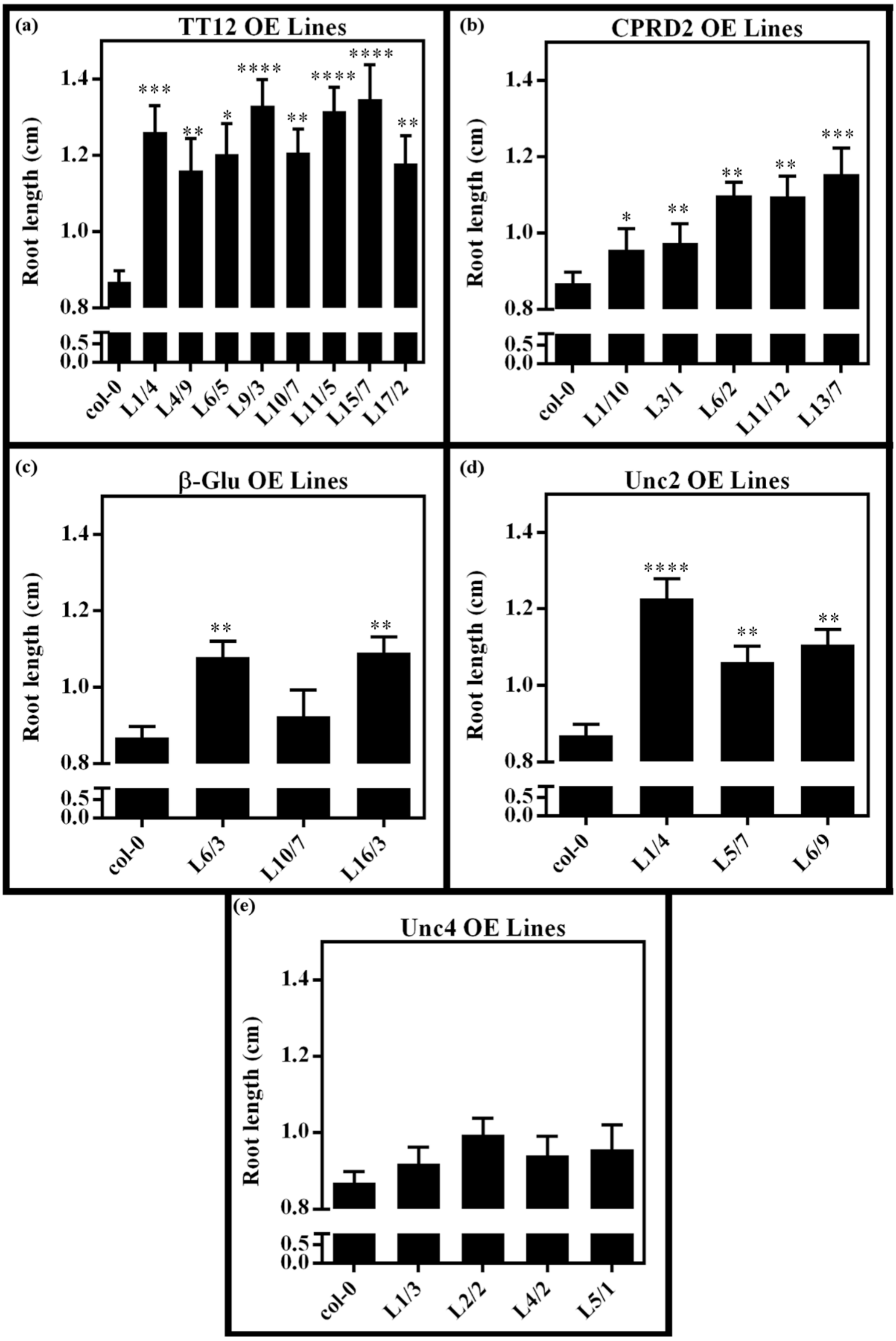


Figure 5.30. Root elongation assay of transgenic lines, over expressing (OE) (a) *TT12*, (b) *CPRD2*, (c) *β-Glu* (d) *Unc2* and (e) *Unc4* genes. Error bars indicates SEM (“*”: p<0.05, “**”:p<0.01, “***”: p<0.001, “****”: p<0.0001).

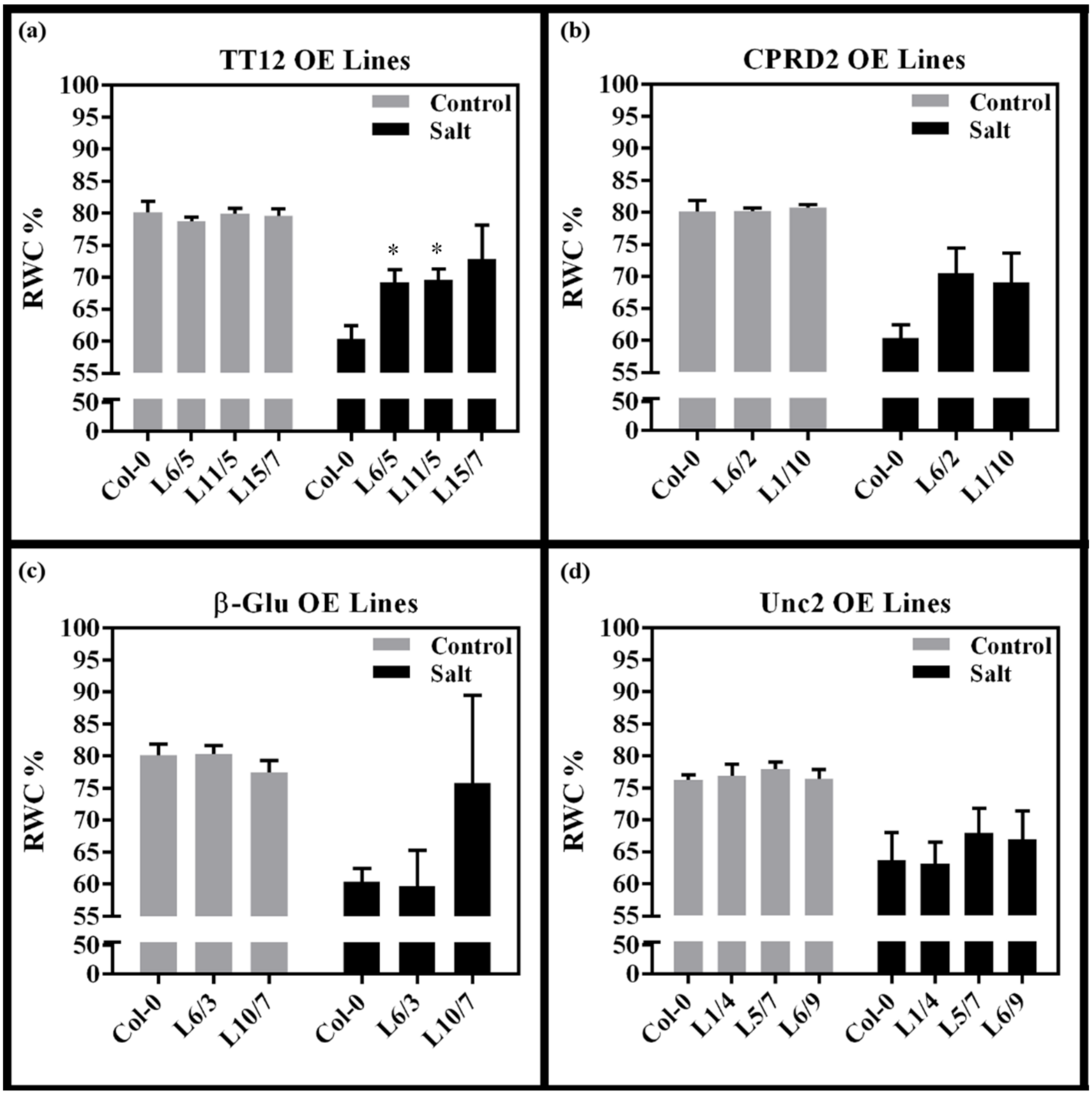


Figure 5.31. Relative water content (RWC) measurement of transgenic lines, over expressing (OE) (a) *TT12*, (b) *CPRD2*, (c) *β-Glu* and (d) *Unc2* genes. Error bars indicates SEM ("*": $p<0.05$).

The RWC of the Col-0 control plants and the selected transgenic lines grown in 150 mM NaCl treatment for 7 days in hydroponic system were calculated in control and stress conditions as described in Section 4.6.3.

The water retention capacity of both Col-0 and all the transgenic lines in control conditions have shown a base line of 80% RWC however in salt stress while the RWC of Col-0 plants declined to 60%, the *TT12* and *CPRD2* transgenic lines RWC values were around 72-75%

(Figure 5.31a, b) suggesting significant water retention better than *β-Glu* and *Unc2* lines which demonstrated no significant difference in RWC (Figure 5.33c, d).

5.3.5. Proline Content Measurement of Transgenic Lines

One of the well documented response to osmotic stress factors (salinity, drought and cold) in plants is the accumulation of compatible osmolytes such as proline which is thought to protect cells against stress damage by reducing the cellular water potential and thus maintaining turgor pressure high enough to sustain growth. Additionally, they show anti-oxidant potential to ROS damages [150]. Therefore, elevated levels of proline has been accepted as plants adaptation to the stress conditions.

Proline content of transgenic lines and Col-0 plants were measured under control and stress conditions following the steps in Section 4.6.3. The leaf tissues of *TT12* transgenic lines accumulated significantly higher levels of proline compared to the Col-0 plants. However, in *CPRD2, β-Glu* and *Unc2* transgenic lines, proline content was stable.

5.3.6. qRT-PCR Analysis of Stress-associated Genes in Transgenic Lines.

Analyzed physiological parameters have indicated certain improvements to salt stress tolerance in transgenic lines. To probe the underlying molecular mechanisms, four functional genes (*RAB18, RD29B, P5CS1* and *P5CS2*) and three transcription factors (*DREB2A, MYB2* and *RD26*) were selected to determine their expression level in transgenic lines. The upregulated expression of these genes have been highly correlated with salt stress tolerance in many studies [151, 152]. Among the functional genes, *RAB18* and *RD29B* belong to LEA (late embryogenesis abundant) class, low molecular weight hydrophilic proteins and their accumulation on certain part of tissues was suspected to provide water retention during stress [28]. The *P5CS1* and *P5CS2* has role in osmolyte biosynthesis [65] by increasing the solute content of cells and they were implicated in osmoprotection.

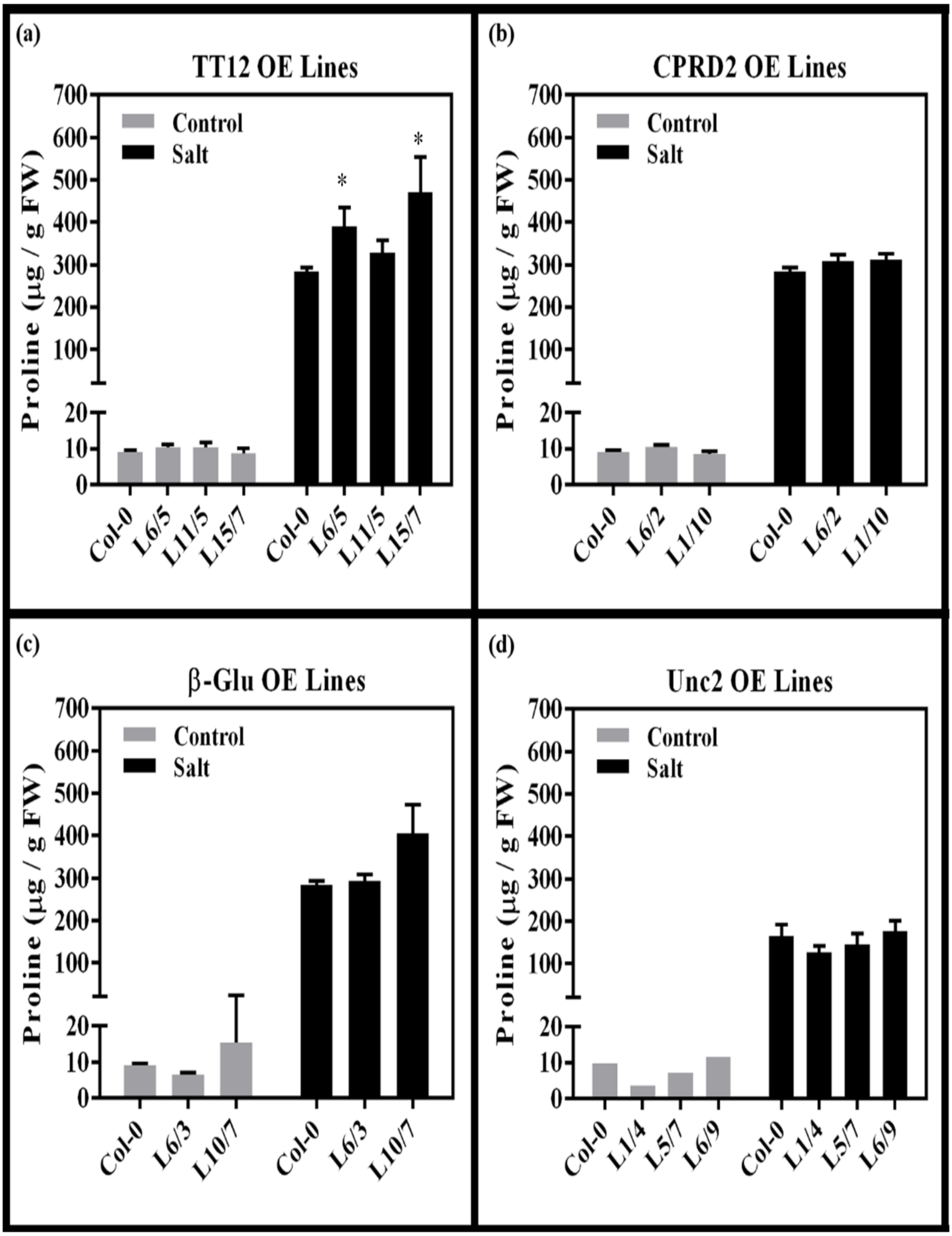


Figure 5.32. Proline content measurement of transgenic lines, over expressing (OE) (a) *TT12*, (b) *CPRD2*, (c) *β-Glu* and (d) *Unc2* genes. Error bars indicates SEM ("*": $p<0.05$).

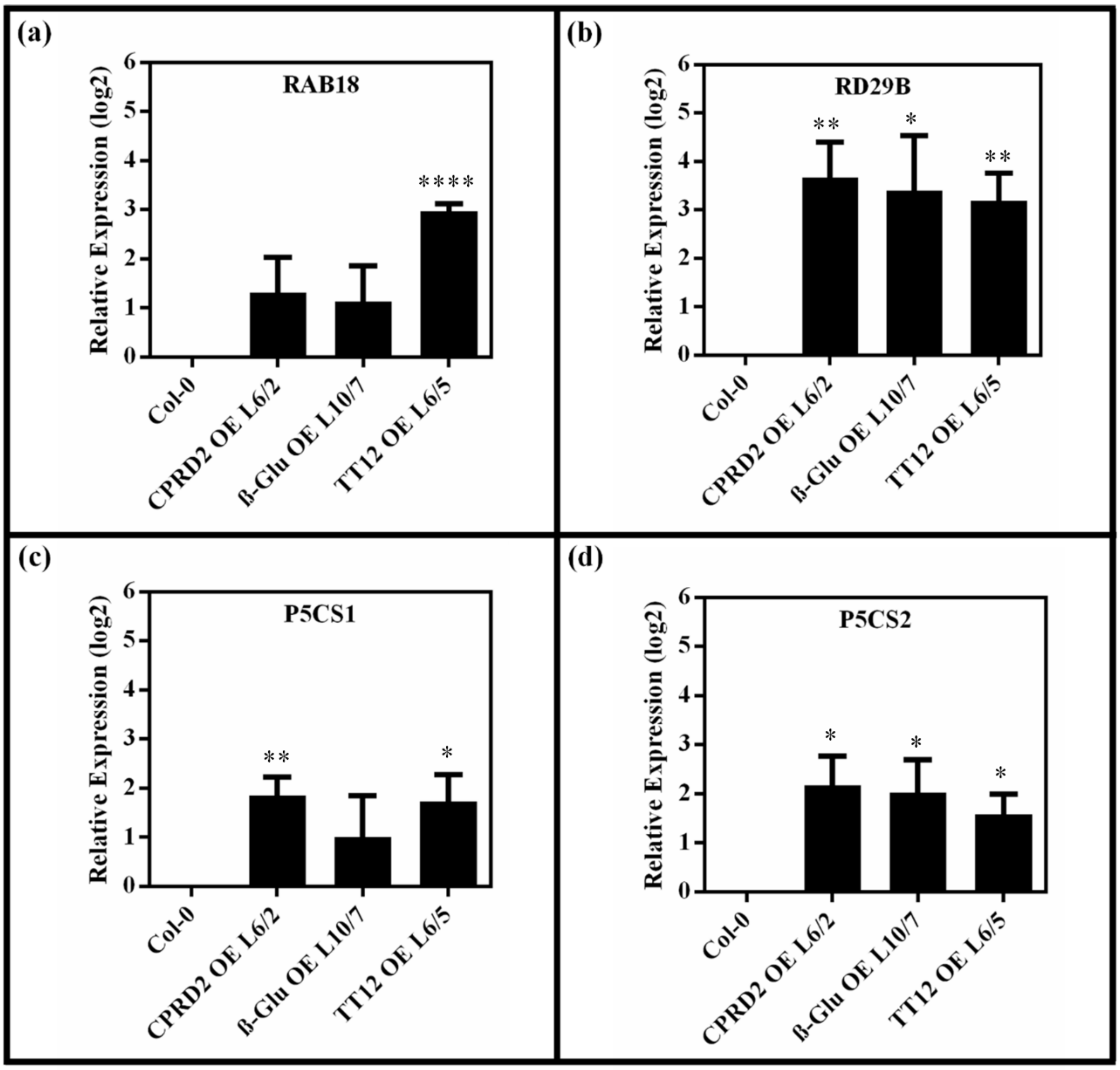


Figure 5.33. qRT-PCR analysis of (a) *RAB18*, (b) *RD29b*, (c) *P5CS1* and (d) *P5CS1* functional genes selected transgenic lines, over expressing (OE) *TT12, CPRD2* and *β-Glu* genes. Expression levels were normalized to Ct (threshold cycle) value of *Act-11* and their untreated controls. Error bars indicates SEM (“*”: p<0.05, “**”:p<0.01, “****”: p<0.0001).

Expression levels of these functional genes were significantly higher in *CPRD2, β-Glu* and *TT12* transgenic lines compared to the wild type Col-0 plants. (Figure 5.33). Especially the *RD29B* (Figure 5.33b) was consistently and significantly upregulated in all transgenic lines. Several studies [30, 31, 153] including our transcriptome analysis [154] have reported the significance of AP2/ERF (ABA-independent pathway), MYB and NAC (ABA-dependent pathway) transcription factor (TF) families in stress. The *DREB2A*, *MYB2* and

RD26 belong to these TF families respectively. The *DREB2A* (Figure 5.34a) was significantly upregulated in all transgenic lines whereas the expression level of *RD26* (Figure 5.34b) seemed to be not regulated under salt stress. The *MYB2* expression was also significantly high in most transgenic lines except the *TT12* lines (Figure 5.34c).

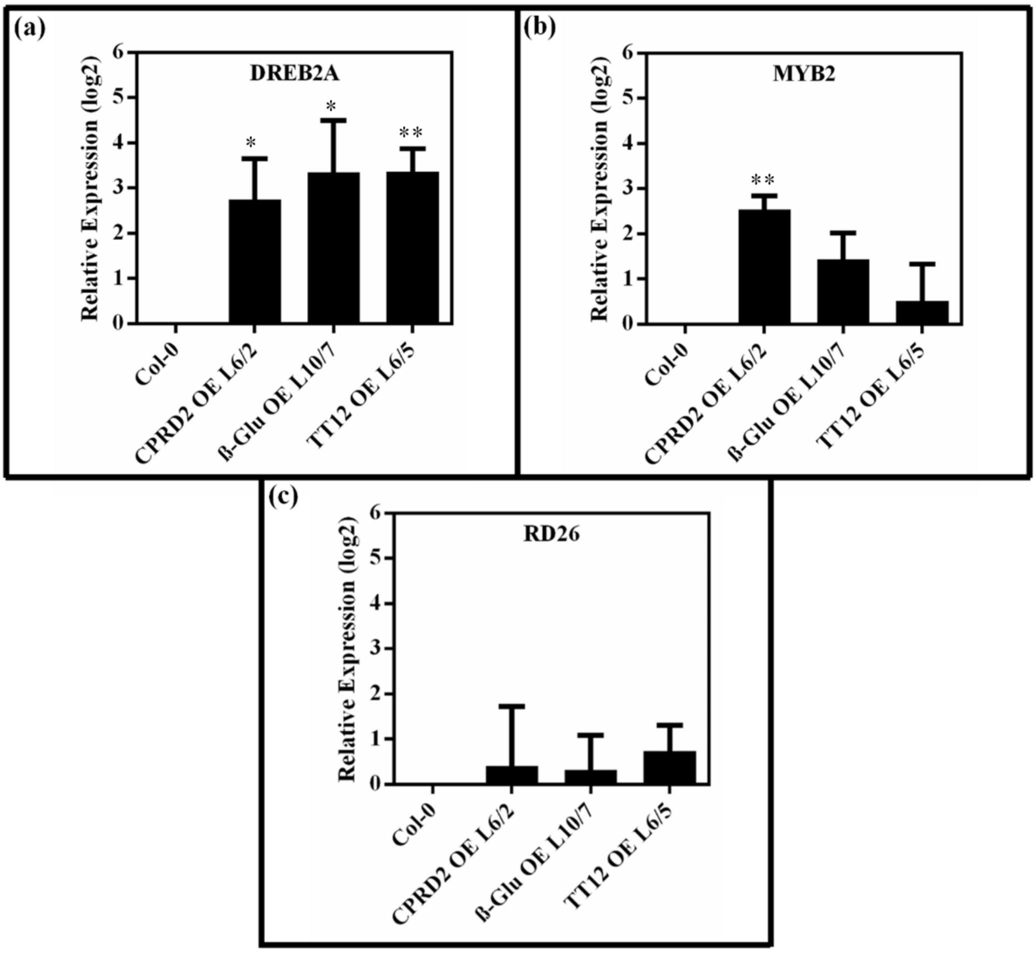


Figure 5.34. qRT-PCR analysis of (a) *DREB2A*, (b) *RMYB2* and (c) *RD26* functional genes selected transgenic lines, over expressing (OE) *TT12*, *CPRD2* and *β-Glu* genes. Expression levels were normalized to Ct (threshold cycle) value of *Act-11* and their untreated controls. Error bars indicates SEM (“*”: $p<0.05$, “**”:$p<0.01$).

6. DISCUSSION & CONCLUSION

Plants have to cope with several abiotic stress factors which inhibits normal functioning and well-being of them [1]. Soil salinity is one of the main abiotic stress factors affecting agricultural productivity in 50% of irrigated areas in the world [9]. Salinity majorly effects various physiological and metabolic processes and severely inhibits plant growth and productivity [10, 155]. Plants can be classified roughly as halophytes and glycophytes according to adaptive evolution based on salt stress. Most of the major crop species including common bean (*Phaseolus vulgaris* L.) are classified as glycophytes [68]. Common bean is an important legume which provides 30% of the protein diet in developing countries (FAO: http://faostat.fao.org/faostat). Several common bean varieties have been classified as tolerant or susceptible to salt stress by phenotypical measures [72] and this genetic diversity within the species offer a valuable opportunity to identify the key elements which has role in salt tolerance. Thus, in this study we selected two common bean varieties which were classified as salt tolerant (Ispir) [71] and salt susceptible (TR43477) [72] to analyze their transcriptomes under salt stress in order to reveal the differentially expressed genes and mechanisms that play role in salinity tolerance.

6.1. Physiological Assays and Transcriptome Analysis of Ispir and TR43477 Varieties

In recent years several transcriptome analysis reports were published regarding the effects of salinity on legume species such as Medicago [93, 156], Glycine [157], Cicer [101, 158] and Millettia [102]. However, for common bean the limited studies were mostly regarding nitrogen effect on nodulation [159], drought stress [99], aluminum treatment [160] and general identification of transcriptome [96]. Therefore, our published large-scale comprehensive transcriptome analysis performed on salt tolerant variety, Ispir, with two different tissues under salt stress is still the only available transcriptome study regarding salt stress response in common bean [154]. Our results have generated 73762 all-unigenes (Table 5.2) which were comparable to previous studies [96-98] and among the all-unigenes, 12001 (16.3%) were differentially regulated more than 2 fold upon salt stress (Figure 5.13). The reliability of the results was demonstrated with high percentage of (%75.2) Nr annotations as well as with high correlation (*r*=0.91) between qRT-PCR results and RPKM values (Figure 5.12). Functional annotations of common bean transcriptome revealed by GO, KEGG and COG analyses also shared the highly represented terms obtained in previous legume transcriptome studies regarding abiotic stress factors [102, 161, 162]. However, the number of DEGs found in our study was quite higher than previous studies in legumes [91, 93] most probably due to sudden application of salt stress to the final concentration in soil conditions in those studies. Whereas in our study, gradual increase in salt under hydroponic conditions acclimatized the plants and most probably allowed detection of DEGs regulated upon salt responses rather than osmotic shock responses [128]. Additionally, the disparity may also stem from the differences in experimental approach, sample collection times as well as the differences between assembly software.

Classification of DEGs based on their functional categories annotated in databases to identify the prominent metabolic processes revealed that major processes like macromolecule metabolism as well as the processes working against oxidative stress and production of secondary metabolites (osmoprotectants like sugars, amines) were effected during salt stress in leaf and root tissues of both genotypes (Table B.1-9, Figure 5.14, Figure 5.15). With the support of similar results from other studies [163] it can be speculated that plants may require to eliminate water and nutrient deficits by regulating osmotic potential via osmoprotectants and increasing the synthesis of macromolecules [16, 164]. Increased metabolic activities cause the generation of reactive oxygen species [165, 166]. Thus, elevated gene expressions playing role in scavenging (oxidation-reduction processes) pathways (Table B.1, Table B.5 and Table B.8) may reflect attempts to eliminate free oxygen radicals. Cell wall biogenesis related processes were also enriched in both leaf and root tissues of each genotype (Table B.1-9). This finding was also expected since tolerance to water deficit caused by osmotic and ionic imbalance requires cell structure integrity via reorganization of membrane and cell wall structures [167].

Biomass in plants is a reflection of canopy and root growth which are dictated by cell division and cellular expansion [166]. Processes related to cell cycle and cell division were enriched within down-regulated transcripts (Table B.18, Table B.20, Table B.22 and Table B.24. Figure 5.14 and Figure 5.15) of common bean which suggested a general reduction in growth rate under salt stress. Photosynthesis is another contributing activity to plant growth.

The decline in photosynthesis rate may be caused by lack of CO_2 due to stomatal closure [168, 169], oxidative stress or due to the alterations in photosynthetic metabolism [170]. Photosynthetic activities and proteins found in photosystem and thylakoid space were identified to be down regulated in leaf tissues of TR43477 variety exclusively (Table B.4 and Table B.12).

Salt ions taken up by roots concentrates in vacuoles of tissues and creates toxic effects by inhibiting enzyme activities and cause nutrient deficiencies by replacement of potassium ions in cytoplasm [50]. It was observed that ion transport related terms were highly enriched in upregulated transcripts of Ispir root tissues exclusively (Table B.5).

Prior to transcriptome studies, several physiological parameters were analyzed to determine the salt stress onset time of Ispir and TR43477 varieties. These analyses demonstrated that, the onset of major differences in their morphological responses started to appear after the 3rd day of 125 mM NaCl application. Most of the differential gene expressions and their functionally annotated processes were also in agreement with the observations in morphological responses. Significant decline in canopy and root biomass (Figure 5.2 and Figure 5.3) was observed in both varieties indicating a strong inhibitory effect of salt stress on growth rate. Whereas the conservation of better leaf area, RWC and ionic balance (Figure 5.4-Figure 5.8) with low membrane injury and higher stomatal conductance (Figure 5.5c, d) in Ispir have indicated its success in better management of adverse effects of osmotic stress around the root zone and increased the water availability to shoots and leaves. [171].

6.2. Functional Studies of Selected Transcripts

We have selected several candidate transcripts for functional characterization via generation of transgenic overexpression Arabidopsis lines based on their classification in transcriptome analysis and expression patterns across the tissues and genotypes (Table 4.1 and Table 4.2). Among the selected 13 transcripts we were able to clone and generate homozygous transgenic lines overexpressing only 5 of them. Two of these were novel genes (*Unc2* and *Unc4*) whereas the rest were proposed to play role in oxidative stress regulation

(*CPRD2*), transportation of secondary metabolites (*TT12*) and hydrolase activity in energy metabolism (*β-Glu*) according to the Blastx analysis in public databases (Table 4.2).

Biosynthesis and transportation of secondary metabolites (prolines, flavonoids, alkaloids etc.) has a critical role as osmoprotectant in plant tolerance to osmotic stress [172]. The *TT12,* is a MATE (multidrug and toxic compound extrusion) family protein and plays role in the sequestration of flavonoids to the vacuoles [111]. The *CPRD2* has an FAD binding berberine domain and shown to be upregulated in osmotic stress and pathogen attack [116, 117]. Another candidate gene *β-Glu* (Endo-1,3-beta-glucanase) belongs to glycosyl hydrolase superfamily and reported to play role in hydrolysis of beta-glucans. Beta glucans are known to have important role in cell wall biogenesis and was reported to be upregulated under salt stress in several studies [121-123].

During functional characterization of these genes, our results have indicated certain degree of improvement in salt tolerance of selected overexpression lines of *TT12, CPRD2, β-Glu* and *Unc2* genes, whereas significance of their performance were not consistent for all of the phenotypic assays, except the *TT12* lines. All the overexpression lines were significantly better in their capacity to germinate and maintenance of root elongation under salt stress (Figure 5.25 - Figure 5.30). However the *TT12* and *CPRD2* lines have demonstrated highly significant performance in terms of ability to retain higher water content only. Furthermore, the maintenance of cell turgor by accumulation of proline osmolytes was observed in *TT12* lines exceptionally. (Figure 5.31 and Figure 5.32). These may be due to differences in activation of response pathways caused by overexpression of different transgenes in each line.

Plants perceive various types of stresses throughout their lives. Their constitutive basal defense mechanism and rapid response to these stress factors lead them to activate a complex signaling cascades varying from one stress to another [68]. Accumulated studies have mainly concentrated on understanding plant responses to individual stress factors ([173] and references therein). However, plants face with simultaneous stresses and the responses were expected to lead to a much more complex scenario in nature [174]. Despite the lack of understanding whether simultaneous stresses are rather antagonistic, synergistic or additive, studies working on multiple stress factors have indicated the possibility to predispose the plant to respond faster to the stress factors. This effect was suspected to make the plants more resistant to additional challenges [175]. This has generated the concept of cross-tolerance which is suspected to be the result of cross- talk between different signal pathways that share common components and outputs to minimize energy cost and increase flexibility of signaling network [176, 177].

Involvement of abscisic acid (ABA) phytohormone [178, 179] and certain transcription factor (TFs) families such as MYB, NAC, bZIP and AP2/ERF together with their activated downstream functional genes in the execution of tolerance responses to abiotic stresses including salinity, were well documented [180-182]. There is a classification of response pathways as ABA–dependent and ABA independent with respect to the binding of TFs to various *cis*-elements of functional genes However, the presence of cross talk [38] and activation of both pathways simultaneously especially in osmotic stresses (such as salinity and drought) were also documented [24].

In an attempt to understand the effects of transgenes on the expression of osmotic stress related TFs and downstream functional genes, our results have suggested that the over expression of *TT12* may lead the plants to use ABA-independent pathways majorly since the expression levels of *RD26* (NAC member) [37] and *MYB2* (MYB member) [30] which play role in ABA-dependent regulatory system were not changed. This suggestion may also be supported by the significant increase in the expression level of *DREB2A* (AP2/ERF) (Figure 5.34) [31] which orchestrates downstream genes via ABA-independent manner. However, it can be speculated that the *CPRD2* overexpressing lines were employing both ABA-dependent and ABA-independent pathways successfully based on the significantly elevated expression levels of both the *DREB2A* and *MYB2* TFs in these lines (Figure 5.34). Although the LEA family member dehydrin proteins *Rab18* and *RD29B* are targets of both *bZIP* and *NAC* TFs and thus considered to be regulated by ABA-dependent pathway, [38] the presence of several ABA binding ABRE elements in the *RD29B* suggested a control of this gene especially by both ABA dependent/independent manner and thus implicated different functional roles for both proteins as osmolytes in stress response [24, 28, 183]. Observation of highly significant levels of both *RD29B* and *Rab18* expression in the *TT12* line and lack of significant expression in *Rab18* especially in *CPRD2* and *β-Glu* lines may suggest that responses generated by the *TT12* overexpression were a coherent activity of both dehydrin proteins and interactive regulation by both pathways [38, 174]. However, responses to *CPRD2* and *β-Glu* overexpression were not contributed by *Rab18* activities.

Biosynthesis and accumulation of secondary metabolites (prolines, flavonoids, alkaloids etc.) has a critical role in plant tolerances [172]. Especially the proline is an important osmoprotectant and ROS scavenger which was implicated in responses to osmotic stress [184]. The biosynthesis of proline are tightly regulated by *P5CS* genes [185, 186]. The ABA-dependent expression of *P5CS1* was mainly demonstrated to be responsible for proline accumulation and highly associated with salt/drought tolerances. However, *P5CS2* was mostly associated with cell division activities and housekeeping functions [187]. In our study, although the expression level of both *P5CS1* and *P5CS2* were elevated in the *TT12*, *CPRD2* and *β-Glu* lines whereas significant expression of the *P5SC1* only was observed in the *TT12* and *CPRD2* lines. Similar to our findings, in studies with salt induced gene *LcSAIN1* transgenic Arabidopsis and rice lines the expression level of *P5SC1* was observed highly significant. However, *P5CS2* levels were similar to non-stressed plants in the same study [65]. Interestingly, the proline metabolite intermediates were also associated with the synthesis of dehydrins [61]. Therefore, it may be speculated that the *TT12* and *CPRD2* transgenic lines cope with salinity by inducing osmolyte and osmoprotectant accumulations and alleviating ROS damages based on the elevated levels of *P5CS* and dehydrin gene expression (*RD29B* and *Rab18*) and proline assay results (Figure 5.32).

In conclusion, our results provided a comprehensive analysis of common bean transcriptome under salt stress highlighting the major differences in gene expression profile in two different tissues of tolerant and susceptible varieties. Determination of differential gene expression under stress conditions provided a distinctive pool of upregulated salt responsive genes as candidates. Five of them were successfully characterized by overexpression in transgenic Arabidopsis lines. For both *TT12* and *CPRD2* genes of common bean, a positive regulatory role may be suggested in improvement of salt tolerance under the control of both ABA dependent/independent pathways through expression of *P5SC1* and *RD29B* genes and *DREB2A* and *MYB2* transcription factors. Although our findings regarding the salt tolerance associated *TT12* and *CPRD2* genes of common bean may be applicable in breeding programs, we recommend further studies of their timely and spatially regulated expressions under other stress conditions (drought, chilling, exogenous ABA etc.), using overexpression and/or mutant lines of leguminous species to concisely explain their role in stress tolerance mechanisms.

APPENDIX A: LIST OF PRIMERS

Table A.1. Primers used for cloning of selected genes with Gateway system.

Primer Name	Public Database ID	Sequence Sense / Antisense	Annealing Temp. (°C)
pv_c_TT12_Fwd	Phvul.011G104200	AAAAAGCAGGCTTCATGGATTCTCCGTCGGCG	58/72
pv_c_TT12_Rev		AAGAAAGCTGGGTCTCAGTTCTTCAGAAGTGGC	
pv_c_CPRD2_Fwd	Phvul.002G200600	AAAAAGCAGGCTTCATGAAGTGCCTAAGCTCC	56/72
pv_c_CPRD2_Rev		AAGAAAGCTGGGTCTTAATATTTTTCTTCCTTCTGC	
pv_c_Unc2_Fwd	Phvul.002G211900	AAAAAAGCAGGCTTCATGGCCTCAGCCAAATCCTT	55/72
pv_c_Unc2_Rev		CAAGAAAGCTGGGTCTTAAGGGCTAGAGGTAGAAG	
pv_c_beta-glucanase_Fwd	Phvul.011G077900	AAAAAAGCAGGCTTCATGTCTGCCTTATTGCTGCT	57/72
pv_c_beta-glucanase_Rev		CAAGAAAGCTGGGTCTCAATTGAAACTGAGTTGGT	
pv_c_Unc4_Fwd	Phvul.003G209000	AAAAAAGCAGGCTTCATGGATGGTAGGGGAGGCTG	59/72
pv_c_Unc4_Rev		CAAGAAAGCTGGGTCTCAGTAACCCAATTTCAAGC	
attB1_F universal primer	-	GGGGACAAGTTTGTACAAAAAAGCAGGCT	54/72
attB1_R universal primer		GGGGACCACTTTGTACAAGAAAGCTGGGTC	

Table A.2. Primers designed to sequence clones for verification.

Primer Name	**Public Database ID / Vector Name**	**Sequence Sense / Antisense**	**Annealing Temp. (°C)**
TT12_seq_F1	Phvul.011G104200	GTTGGGTATGGGAAGTGC	56.1
TT12_seq_R1		CACCCAACTAAACACCAGA	55.2
TT12_seq_F2		CTCTCGATTCCCTTTCCA	53.9
TT12_seq_R2		CCCAGACAAAACAGGTTGA	55.2
CPRD2_seq_F1	Phvul.002G200600	TCCCTTTGTTATCCTTGACC	56.4
CPRD2_seq_R1		CCCAAAACAGATCCTCACCCAT	62.1
CPRD2_seq_F2		AGAGTTGGGTTTGGTCAG	53.9
CPRD2_seq_R2		AGATCGGTGTGAGAAAGG	53.9
Seq_Pri_b_gluc_R	Phvul.011G077900	GTG GTG TCT ATT GCT GTG	53.9
Seq_Pri_b_gluc_F		CAC AGC AAT AGA CAC CAC	53.9
Seq_pri_Unc_4_R	Phvul.003G209000	AAC AAC ACC CTG CCA TCA	53.9
Seq_pri_Unc_4_F		TGA TGG CAG GGT GTT GTT	53.9
pDONR207-F	pDONR207	TCGCGTTAACGCTAGCATGGATCTC	62
pDONR207-R		GTAACATCAGAGATTTTGAGACAC	51

Table A.3. Primers designed for verification of *in planta* expression of selected genes.

Primer Name	**Public Database ID**	**Sequence Sense / Antisense**	**Amplicon Size (bp)**	**Annealing Temp. (°C)**
Pv_011G104200_F	Phvul.011G104200	ATGGATTCTCCGTCGGCG	1554	53
Pv_011G104200_R		TCAGTTCTTCAGAAGTGGCTCCTT		
Pv_002G200600_F	Phvul.002G200600	ATGAAGTGCCTAAGCTCCTA	1623	42
Pv_002G200600_R		TTAATATTTTTCTTCCTTCTGCA		
Pv_002G211900_F	Phvul.002G211900	ATGGCCTCAGCCAAATCC	354	51
Pv_002G211900_R		TTAAGGGCTAGAGGTAGAAGGTG		
Pv_011G077900_F	Phvul.011G077900	ATGTCTGCCTTATTGCTGCT	999	45
Pv_011G077900_R		TCAATTGAAACTGAGTTGGTATTT		
Pv_003G209000_F	Phvul.003G209000	ATGGATGGTAGGGGAGGCT	876	48
Pv_003G209000_R		TCAGTAACCCAATTTCAAGCTAAG		

Table A.4. Primers designed for qPCR verification of transcriptome analysis.

Gene ID in *de novo* transcriptome	**Public Database ID**	**Sequence Sense / Antisense**	**Amplicon Size (bp)**	**Annealing Temp. (°C)**
Cl1850.Contig1	Phvul.002G237600.1	TTGGTGTGGGATGAGGCACAGG	177	60
		ACAACGGATGAACTCCACGAAGG		
Cl214.Contig3	Phvul.008G024100.1	GTTTTCTCTCGTCCAATTCT	146	50
		TACACTCTATCCCTTTCCCT		
Cl5000.Contig2	Phvul.010G147100.1	TGGAGCCAAGAAGCTGTGCAATC	100	60
		GGCCATGTCAACCCTGCTGTGA		
Cl7616.Contig3	Phvul.002G166100.1	CCTACCTCCCCACACAGATT	124	60
		ATGCCTTCAAATTCCTCAACC		
Cl8124.Contig1	Phvul.002G331400.1	TGCCCGAGTTTCTTCCTCTGGACTT	186	58
		AGGAGGAGACGGCACAATGGAGA		
Cl8128.Contig1	Phvul.005G073100.1	CCCAAAACAGAGGCACAACA	185	60
		GGAAAAGAGACAACGGCGAA		
Unigene1387	Phvul.003G047900.1	CTCTTGTGACCAACCTCT	174	54.5
		CTTTTCCATTCACCCAACC		
Unigene14991	Phvul.001G170400.1	TTCTTTGCCGTTGCTCTT	113	56.2
		GGCTGTATCTTGTGGTGT		
Unigene15046	Phvul.010G107400.1	ATGAAGTCAGAAAGGAGG	246	52
		GCTGCTGAAGTTTGTTAG		
Unigene15454	Phvul.005G039200.1	CTTAACTCCTTTTGTCACTC	148	54.5
		ATTCTTCACTCTGTCTCC		
Unigene16608	Phvul.002G049900.1	GGAATAGGGAGGGGTTGGA	164	57
		GGAAGTTTGAGATGGACTGG		
Unigene17621	Phvul.004G169300.1	ACTGTGCAGGGGGATAAA	173	57
		AGAGGAGGAGCATGTGAA		

Table A.4. Primers designed for qPCR verification of transcriptome analysis (cont.).

Gene ID in *de novo* transcriptome	Public Database ID	Sequence Sense / Antisense	Amplicon Size (bp)	Annealing Temp. (°C)
Unigene18722	Phvul.001G083000.1	CTCCTTTATCGCCTTCCT ACTTCCGCATTACCAACA	230	54
Unigene18877	Phvul.002G168300.1	TTGTTGTCGTGATCCTTGCG TTCCTCTCCCAATCCTCCTCC	143	60
Unigene19047	Phvul.003G229500.1	GCTAGCTGTTCCATTTACGCAGAGT AGCTGCCGTAGAGTTTGATTGCACC	100	60
Unigene19559	Phvul.009G110700.1	TACCAAACCCTCGTGATCCC CGCACATATTCTTCACATCCT	163	60
Unigene22119	Phvul.007G080400.1	CTTAGTCTCCTCATGCTT CCTTCTACCTTCTTCTCC	108	53
Unigene22480	Phvul.011G077900.1	TTCACCACTGCTTGCTTT GCCTTATTGCTGCTTCTT	122	52
Unigene23432	Phvul.007G091000.1	TCCACAACAACCACCTTC AATCTCCAAATACCCTTCC	237	54
Unigene29681	Phvul.006G164000.1	TGAGGAAGAGGATGGGGAG AGCGTGAGTTGATGGTGA	139	57.5
Unigene29873	Phvul.001G181100.1	GCAGCTCCCAACCACTGACTAC CCATCCAACCAAAGATCAACGCCCA	186	58
Unigene31674	Phvul.004G117100.1	GCCTTCTCTTTTACCTTCT ACACCACCATAATCCTCA	102	53
Unigene32754	Phvul.002G267900.1	GAATTCTTCAGCCAGACA ACTACAACAAACTCGCCA	220	53
Unigene37863	Phvul.005G051600.1	AACCATGCCTTCACCAGCTTCAAAT AGGTTGTGGGAGAAGAAGATGTGGA	107	60

Table A.4. Primers designed for qPCR verification of transcriptome analysis (cont.).

Gene ID in *de novo* transcriptome	**Public Database ID**	**Sequence Sense / Antisense**	**Amplicon Size (bp)**	**Annealing Temp. (°C)**
Unigene4034	Phvul.003G159400.2	CCTTAGTATCCCAACTTCCA AGAGCCATTACAACCAACA	225	55
Unigene4220	Phvul.001G108700.1	GAGAAGGAAGCCAGAGAA CAACAAGAACCTCAACCA	190	54.5
Unigene43067	Phvul.001G195700.1	TCTTGCCTTGATCTTCGG AGGTTTGAATAGAGGATGTG	172	53
Unigene4622	Phvul.008G170800.1	TGATCCCATTGCAAATCC TCCCCCCATAAAACCAAC	151	53
Unigene49735	Phvul.001G087100.1	CCACTCCTGGCACTACGAAC GTAACTGTGGATGGACATGATTT	106	60
Unigene5066	Phvul.009G105300.1	CTCCACCTTTTCCACCAAC CTTCCCACTACTCCTATTCC	157	56
Unigene5243	Phvul.006G159600.1	GCTATGGTTCCAGCTTTT AGTTATTGGGGTTGGGTT	120	54
Unigene53502	Phvul.001G114800.1	AGATGTTCAGAGATTGCGT TGGGTGGTTATGGAGAGG	202	56
Unigene623	Phvul.002G027900.1	ACTCCAACAAACTCGAAACA CACATACCACTCGGACCA	234	55
Unigene7698	Phvul.001G005500.1	AAACACCACACAAATCCC CACGTAGCCCAAACTTTC	210	54
Unigene779	Phvul.006G134200.1	ATGTGGAAGTTAAGGAGG GGTTTCTGGTTTTCTCTG	141	52
Unigene7890	Phvul.009G021600.1	TAAGTTGAGGGAAGAGGAG TGTTTAAGGATGAGGCGT	146	54.5

Table A.4. Primers designed for qPCR verification of transcriptome analysis (cont.).

Gene ID in *de novo* transcriptome	**Public Database ID**	**Sequence Sense / Antisense**	**Amplicon Size (bp)**	**Annealing Temp. (°C)**
Unigene8178	Phvul.003G209000.1	GCAACAAAGGAAGAGTCA CTCAAAACCAGAACCTCTAA	154	51
Unigene8254	Phvul.009G122300.1	GAATAGGTCACTTGGCGA TATAACACCCTCCACCAC	114	53
Unigene8342	Phvul.002G211900.1	CAGTGTTGGGTTTGAAGG CTTTCTTGTGATTGTGAGC	151	52
Unigene8562	Phvul.003G207300.1	GGAGGTGAAGAAAGAGAGGAG AGGAGGAGACGGCACAATGGAGA	152	60
Unigene8629	Phvul.010G108500.1	GGAGGCAGAGAAGGCAAA ACCAACCAAAGTGCCAAA	186	57.5
Unigene8812	Phvul.007G200600.1	TTGTCTCTCCTCCAATGT GAGGCCACTGATTTATTCT	202	54
Unigene8946	Phvul.007G073900.1	CAGAGTCAAGCATCAAGT GCAATAATCCCAGCTACAT	163	54
Cl442.Contig3 (*Act*11)	Phvul.008G011000.1	TGCATACGTTGGTGATGAGG AGCCTTGGGGTTAAGAGGAG	190	58
Unigene29213 (*IDE*)	Phvul.001G133200.1	GCAACCAACCTTTCATCAGC AGAAATGCCTCAACCCTTTG	156	56

Table A.5. Primers designed for qPCR analysis of selected salt responsive Arabidopsis genes.

Primer Name	**TAIR ID**	**Sequence Sense / Antisense**	**Amplicon Size (bp)**	**Annealing Temp. (°C)**
AtACTIN2-S	AT5G09810.1	TGCTGACCGTATGAGCAAAG	109	57
AtACTIN2-AS		GATTGATCCTCCGATCCAGA		
AtP5CS1-S	AT2G39800.1	TAGCACCCGAAGAGCCCCAT	88	59
AtP5CS1-AS		TTTCAGTTCCAACGCCAGTAGA		
AtP5CS2-S	AT3G55610.1	AAGCACTCGCAGAGCCCCCT	265	62
AtP5CS2-AS		AGCTCTAGCGACAGAAGAGCGGC		
AtMYB2-S	AT2G47190.1	AACGTCTTCGAATTCTCCGGCTGA	109	63
AtMYB2-AS		ATCGTTGAACTCTCCGAAACCCGT		
AtDREB2A-S	AT5G05410.1	AAACCTGTCAGCAACAACAGCAGG	153	63
AtDREB2A-AS		TTAAGCCTGCAAACACATCGTCGC		
AtRAB18-S	AT5G66400.1	CCACGAGAAGAAGGGGATGAT	126	57
AtRAB18-AS		CGAATGCGACTGCGTTACAA		
AtRD26-S	AT4G27410.3	GCACGAGTATCGCTTAATAGAACA	155	57
AtRD26-AS		CGACACAACACCCAATCATC		
AtRD29B-S	AT5G26742.2	GCAAGCAGAAGAACCAATCA	152	56
AtRD29B-AS		CTTTGGATGCTCCCTTCTCA		

APPENDIX B: RESULTS OF ENRICHMENT ANALYSES

Table B.1. GO enrichment analysis of up-regulated genes in Ispir leaf samples.

GO Term Biological Process	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
oxidation-reduction process	GO:0055114	13.41	6.92	2.86E-09	1.55E-05
response to abscisic acid	GO:0009737	4.17	1.58	6.87E-06	7.17E-03
response to stimulus	GO:0050896	2.53	0.73	1.90E-05	1.29E-02
oxylipin biosynthetic process	GO:0031408	1.49	0.27	2.37E-05	1.42E-02
tricyclic triterpenoid biosynthetic process	GO:0010263	0.45	0	3.93E-05	1.42E-02
thalianol metabolic process	GO:0080003	0.45	0	3.93E-05	1.42E-02
GO Term Molecular Function	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
iron ion binding	GO:0005506	4.62	1.52	1.34E-07	3.64E-04
heme binding	GO:0020037	3.28	1.33	1.70E-04	4.59E-02
sugar:proton symporter activity	GO:0005351	1.49	0.3	6.10E-05	2.06E-02
linoleate 13S-lipoxygenase activity	GO:0016165	1.34	0.18	7.95E-06	7.17E-03
zeaxanthin epoxidase activity	GO:0052662	0.6	0.01	4.24E-06	7.17E-03
zeaxanthin epoxidase [overall] activity	GO:0009540	0.6	0.01	7.54E-06	7.17E-03
flavonoid 3'-monooxygenase activity	GO:0016711	0.45	0	1.59E-05	1.23E-02
arabidiol synthase activity	GO:0034075	0.45	0	3.93E-05	1.42E-02
marneral synthase activity	GO:0034074	0.45	0	3.93E-05	1.42E-02
beta-amyrin synthase activity	GO:0042300	0.45	0	3.93E-05	1.42E-02
thalianol synthase activity	GO:0051746	0.45	0	3.93E-05	1.42E-02
lupeol synthase activity	GO:0042299	0.45	0.01	7.76E-05	2.33E-02
naringenin 3-dioxygenase activity	GO:0045486	0.45	0.01	7.76E-05	2.33E-02
alpha-1,4-glucosidase activity	GO:0004558	0.45	0.01	1.34E-04	3.82E-02

Table B.2. GO enrichment analysis of down-regulated genes in Ispir leaf samples.

GO Term Biological Process	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
oxidation-reduction process	GO:0055114	12.27	6.91	1.36E-08	1.05E-05
carbohydrate metabolic process	GO:0005975	4.47	1.32	2.23E-10	3.02E-07
regulation of meristem growth	GO:0010075	2.98	0.58	8.16E-11	1.47E-07
plant-type cell wall organization	GO:0009664	2.64	0.53	1.86E-09	1.68E-06
lipid metabolic process	GO:0006629	2.18	0.75	6.75E-05	1.14E-02
transmembrane receptor protein tyrosine kinase signaling pathway	GO:0007169	2.06	0.44	1.94E-07	9.54E-05
stamen development	GO:0048443	1.95	0.26	1.13E-09	1.22E-06
sterol biosynthetic process	GO:0016126	1.95	0.45	1.24E-06	5.17E-04
stomatal complex morphogenesis	GO:0010103	1.83	0.56	6.26E-05	1.13E-02
anthocyanin accumulation in tissues in response to UV light	GO:0043481	1.72	0.4	5.09E-06	1.84E-03
cell proliferation	GO:0008283	1.72	0.59	3.54E-04	4.17E-02
brassinosteroid biosynthetic process	GO:0016132	1.61	0.36	8.99E-06	2.52E-03
microtubule-based movement	GO:0007018	1.61	0.4	2.74E-05	5.68E-03
response to karrikin	GO:0080167	1.61	0.54	4.50E-04	4.97E-02
response to molecule of bacterial origin	GO:0002237	1.49	0.27	2.13E-06	8.23E-04
response to brassinosteroid	GO:0009741	1.38	0.28	1.29E-05	3.04E-03
multidimensional cell growth	GO:0009825	1.38	0.4	3.81E-04	4.39E-02
meristem development	GO:0048507	1.26	0.13	6.48E-08	3.90E-05
embryo sac development	GO:0009553	1.15	0.24	1.08E-04	1.53E-02
oxylipin biosynthetic process	GO:0031408	1.15	0.27	2.05E-04	2.58E-02
gibberellic acid mediated signaling pathway	GO:0009740	1.03	0.13	5.82E-06	1.97E-03
regulation of cell size	GO:0008361	1.03	0.14	9.77E-06	2.52E-03
regulation of G2/M transition of mitotic cell cycle	GO:0010389	1.03	0.19	9.70E-05	1.42E-02
acetyl-CoA metabolic process	GO:0006084	1.03	0.21	1.61E-04	2.17E-02
petal development	GO:0048441	0.92	0.05	1.27E-07	6.90E-05
gibberellin biosynthetic process	GO:0009686	0.8	0.1	7.31E-05	1.20E-02
cell fate specification	GO:0001708	0.69	0.05	1.58E-05	3.56E-03
cellulose microfibril organization	GO:0010215	0.57	0.04	9.27E-05	1.42E-02
aspartate family amino acid biosynthetic process	GO:0009067	0.34	0.01	2.91E-04	3.57E-02
GO Term Molecular Function	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
oxidoreductase activity	GO:0016491	3.67	1.69	6.76E-05	1.14E-02
carbohydrate binding	GO:0030246	2.06	0.57	6.28E-06	2.00E-03
microtubule motor activity	GO:0003777	1.49	0.38	6.27E-05	1.13E-02
hydrolase activity, acting on ester bonds	GO:0016788	1.26	0.27	5.68E-05	1.10E-02
linoleate 13S-lipoxygenase activity	GO:0016165	1.15	0.18	9.50E-06	2.52E-03
beta-galactosidase activity	GO:0004565	1.03	0.16	1.99E-05	4.30E-03
nutrient reservoir activity	GO:0045735	0.8	0.11	8.32E-05	1.32E-02
steroid 22-alpha hydroxylase activity	GO:0010012	0.34	0	8.84E-06	2.52E-03
homoserine dehydrogenase activity	GO:0004412	0.34	0.01	1.69E-04	2.17E-02
aspartate kinase activity	GO:0004072	0.34	0.01	1.69E-04	2.17E-02
alcohol dehydrogenase activity, zinc-dependent	GO:0004024	0.23	0	4.28E-04	4.82E-02

Table B.3. GO enrichment analysis of up-regulated genes in TR43477 leaf samples.

GO Term Biological Process	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
transmembrane transport	GO:0055085	3.91	2.25	1.17E-04	4.52E-02
response to chitin	GO:0010200	2.06	0.9	7.67E-05	4.15E-02
protein import into nucleus	GO:0006606	1.42	0.42	7.50E-06	6.76E-03
response to karrikin	GO:0080167	1.42	0.53	1.42E-04	4.88E-02
jasmonic acid biosynthetic process	GO:0009695	1.28	0.42	6.91E-05	4.15E-02
protein targeting to mitochondrion	GO:0006626	1.07	0.32	1.08E-04	4.52E-02
defense response by callose deposition	GO:0052542	0.78	0.18	9.76E-05	4.52E-02
detection of mechanical stimulus	GO:0050982	0.21	0	1.44E-04	4.88E-02
GO Term Molecular Function	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
Photinus-luciferin 4-monooxygenase (ATP-hydrolyzing) activity	GO:0047077	0.71	0.04	8.28E-09	2.24E-05
4-coumarate-CoA ligase activity	GO:0016207	0.64	0.04	4.13E-08	7.46E-05
fatty-acyl-CoA synthase activity	GO:0004321	0.57	0.01	3.76E-09	2.04E-05
o-succinylbenzoate-CoA ligase activity	GO:0008756	0.57	0.03	3.16E-07	3.51E-04
AMP binding	GO:0016208	0.43	0.04	1.09E-04	4.52E-02
precorrin-2 dehydrogenase activity	GO:0043115	0.21	0	3.70E-05	2.50E-02
uroporphyrin-III C-methyltransferase activity	GO:0004851	0.21	0	3.70E-05	2.50E-02

Table B.4. GO enrichment analysis of down-regulated genes in TR43477 leaf samples.

GO Term Biological Process	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
rRNA processing	GO:0006364	3.41	0.84	1.68E-09	1.82E-06
pentose-phosphate shunt	GO:0006098	2.93	0.76	6.94E-08	3.76E-05
thylakoid membrane organization	GO:0010027	2.8	0.86	1.90E-06	5.40E-04
isopentenyl diphosphate biosynthetic process, methylerythritol 4-phosphate pathway	GO:0019288	2.8	0.93	6.44E-06	1.52E-03
starch biosynthetic process	GO:0019252	2.68	0.77	1.10E-06	3.72E-04
nucleosome assembly	GO:0006334	2.44	0.25	3.72E-13	1.01E-09
response to blue light	GO:0009637	2.32	0.36	7.51E-10	1.02E-06
photosystem II assembly	GO:0010207	2.32	0.57	8.12E-07	2.93E-04
maltose metabolic process	GO:0000023	2.32	0.59	1.29E-06	4.10E-04
response to far red light	GO:0010218	2.07	0.33	1.01E-08	9.14E-06
response to red light	GO:0010114	2.07	0.37	3.74E-08	2.53E-05
chlorophyll biosynthetic process	GO:0015995	2.07	0.46	7.07E-07	2.73E-04
cell proliferation	GO:0008283	2.07	0.58	1.47E-05	2.83E-03
negative regulation of catalytic activity	GO:0043086	1.95	0.33	6.47E-08	3.76E-05
chloroplast relocation	GO:0009902	1.95	0.4	6.43E-07	2.68E-04
stomatal complex morphogenesis	GO:0010103	1.95	0.56	3.03E-05	4.83E-03
photosynthesis	GO:0015979	1.83	0.46	1.28E-05	2.56E-03
positive regulation of catalytic activity	GO:0043085	1.71	0.42	1.90E-05	3.45E-03
regulation of protein dephosphorylation	GO:0035304	1.59	0.34	9.59E-06	2.08E-03
mRNA modification	GO:0016556	1.59	0.46	2.11E-04	2.71E-02
DNA replication initiation	GO:0006270	1.46	0.27	6.45E-06	1.52E-03
regulation of DNA replication	GO:0006275	1.46	0.38	1.36E-04	1.83E-02
plastid organization	GO:0009657	1.34	0.12	2.93E-08	2.26E-05
photosynthetic electron transport in photosystem I	GO:0009773	1.34	0.14	7.94E-08	3.90E-05
photosynthesis, light reaction	GO:0019684	1.1	0.18	3.16E-05	4.89E-03
divalent metal ion transport	GO:0070838	1.1	0.21	9.34E-05	1.30E-02
cellular cation homeostasis	GO:0030003	1.1	0.25	3.78E-04	4.06E-02
glycine catabolic process	GO:0006546	0.98	0.17	1.40E-04	1.85E-02
photosynthesis, light harvesting	GO:0009765	0.85	0.06	2.83E-06	7.30E-04
photosynthetic electron transport in photosystem II	GO:0009772	0.61	0.03	1.91E-05	3.45E-03
NAD(P)H dehydrogenase complex assembly	GO:0010275	0.49	0.01	9.37E-06	2.08E-03
trichome differentiation	GO:0010026	0.49	0.03	2.15E-04	2.71E-02
transpiration	GO:0010148	0.49	0.03	2.77E-04	3.26E-02
regulation of cell adhesion	GO:0030155	0.37	0.01	3.82E-04	4.06E-02
regulation of organ morphogenesis	GO:2000027	0.37	0.01	3.82E-04	4.06E-02

Table B.4. GO enrichment analysis of up-regulated genes in TR43477 leaf samples (cont.).

GO Term **Molecular Function**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
protein heterodimerization activity	GO:0046982	2.07	0.49	1.65E-06	4.96E-04
serine-type endopeptidase activity	GO:0004252	1.59	0.42	7.57E-05	1.08E-02
carboxylic ester hydrolase activity	GO:0004091	1.34	0.27	2.60E-05	4.54E-03
enzyme inhibitor activity	GO:0004857	0.85	0.15	3.90E-04	4.06E-02
aminomethyltransferase activity	GO:0004047	0.49	0.01	2.72E-05	4.61E-03
isoflavone 7-O-glucosyltransferase activity	GO:0050004	0.37	0	2.90E-05	4.75E-03
nicotinamidase activity	GO:0008936	0.37	0.01	2.42E-04	2.98E-02
DNA photolyase activity	GO:0003913	0.37	0.01	3.82E-04	4.06E-02
phosphoethanolamine N-methyltransferase activity	GO:0000234	0.37	0.01	3.82E-04	4.06E-02

Table B.5. GO enrichment analysis of up-regulated genes in Ispir root samples.

GO Term **Biological Process**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
oxidation-reduction process	GO:0055114	17.53	6.97	7.02E-07	1.27E-03
response to hypoxia	GO:0001666	3.61	0.21	3.09E-07	1.27E-03
inorganic anion transport	GO:0015698	1.55	0.01	5.29E-06	2.86E-03
regulation of cellular metabolic process	GO:0031323	1.55	0.04	1.03E-04	4.17E-02
GO Term **Molecular Function**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
iron ion binding	GO:0005506	7.22	1.55	2.82E-06	2.39E-03
heme binding	GO:0020037	6.7	1.34	3.09E-06	2.39E-03
electron carrier activity	GO:0009055	5.67	1.39	1.08E-04	4.17E-02
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	GO:0016705	4.64	0.53	1.41E-06	1.53E-03
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors	GO:0016706	4.12	0.35	6.75E-07	1.27E-03
monooxygenase activity	GO:0004497	3.61	0.51	7.75E-05	3.50E-02
organic phosphonate transmembrane-transporting ATPase activity	GO:0015416	2.58	0.17	2.61E-05	1.29E-02
structural constituent of cell wall	GO:0005199	2.06	0.03	9.90E-07	1.34E-03
O-methyltransferase activity	GO:0008171	2.06	0.05	3.60E-06	2.44E-03
pyruvate decarboxylase activity	GO:0004737	1.55	0.01	5.29E-06	2.86E-03

Table B.6. GO enrichment analysis of down-regulated genes in Ispir root samples.

GO Term **Biological Process**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
translation	GO:0006412	11.27	2.28	1.63E-30	4.42E-27
response to cadmium ion	GO:0046686	5.7	3.01	1.18E-04	2.78E-02
response to oxidative stress	GO:0006979	2.78	0.99	6.01E-05	1.63E-02
response to chitin	GO:0010200	2.5	0.91	1.88E-04	4.08E-02
RNA methylation	GO:0001510	2.36	0.75	5.66E-05	1.61E-02
obsolete GTP catabolic process	GO:0006184	2.36	0.84	2.19E-04	4.55E-02
single-organism process	GO:0044699	1.81	0.46	5.22E-05	1.57E-02
translational elongation	GO:0006414	1.67	0.39	4.93E-05	1.57E-02
microtubule-based process	GO:0007017	0.97	0.15	1.61E-04	3.63E-02
leucine catabolic process	GO:0006552	0.83	0.05	5.27E-06	2.20E-03
GO Term **Molecular Function**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
structural constituent of ribosome	GO:0003735	11.27	2.24	5.17E-31	2.80E-27
peroxidase activity	GO:0004601	2.09	0.5	7.10E-06	2.74E-03
structural constituent of cytoskeleton	GO:0005200	1.25	0.23	7.59E-05	1.96E-02
asparaginase activity	GO:0004067	0.56	0.01	1.63E-05	5.87E-03
trans-cinnamate 4-monooxygenase activity	GO:0016710	0.42	0	4.82E-05	1.57E-02

Table B.7. GO enrichment analysis of up-regulated genes in TR43477 root samples.

GO Term **Biological Process**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
translation	GO:0006412	21.4	2.19	4.12E-72	2.20E-68
RNA methylation	GO:0001510	6.06	0.71	4.12E-19	3.19E-16
obsolete GTP catabolic process	GO:0006184	4.92	0.82	1.67E-12	6.96E-10
translational elongation	GO:0006414	4.17	0.37	6.96E-16	3.76E-13
ribosome biogenesis	GO:0042254	2.84	0.39	8.90E-09	3.01E-06
cell wall modification	GO:0042545	2.65	0.4	8.17E-08	2.60E-05
plant-type cell wall organization	GO:0009664	2.46	0.55	1.42E-05	2.96E-03
maintenance of DNA methylation	GO:0010216	0.76	0.05	2.50E-04	4.51E-02
GO Term **Molecular Function**	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
structural constituent of ribosome	GO:0003735	21.21	2.15	8.14E-72	2.20E-68
GTP binding	GO:0005525	5.11	1.65	4.93E-07	1.33E-04
RNA binding	GO:0003723	5.11	2.3	1.51E-04	2.81E-02
GTPase activity	GO:0003924	4.92	0.93	2.37E-11	8.54E-09
translation elongation factor activity	GO:0003746	3.41	0.32	8.51E-13	3.84E-10
rRNA binding	GO:0019843	1.7	0.2	2.70E-06	6.63E-04
structural constituent of cytoskeleton	GO:0005200	1.52	0.23	5.19E-05	1.04E-02

Table B.8. GO enrichment analysis of down-regulated genes in TR43477 root samples.

GO Term Biological Process	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
oxidation-reduction process	GO:0055114	14.42	6.95	9.62E-08	8.67E-05
protein phosphorylation	GO:0006468	12.5	4.7	2.95E-10	7.99E-07
phosphorylation	GO:0016310	7.93	2.56	2.30E-08	3.11E-05
nitrate transport	GO:0015706	2.88	0.45	7.60E-07	5.88E-04
response to nitrate	GO:0010167	2.88	0.47	1.15E-06	7.81E-04
chitin catabolic process	GO:0006032	0.96	0.05	1.18E-04	4.25E-02
GO Term Molecular Function	**GO ID**	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
protein serine/threonine kinase activity	GO:0004674	10.58	4.08	1.70E-08	3.07E-05
protein kinase activity	GO:0004672	7.69	1.63	1.76E-12	9.51E-09
heme binding	GO:0020037	5.29	1.33	9.12E-08	8.67E-05
peroxidase activity	GO:0004601	2.64	0.5	1.43E-05	7.75E-03
amino acid binding	GO:0016597	1.44	0.17	1.09E-04	4.22E-02
phospholipase C activity	GO:0004629	0.96	0.02	4.36E-06	2.62E-03
malate dehydrogenase (decarboxylating) (NAD+) activity	GO:0004471	0.72	0.01	5.16E-05	2.33E-02
oxaloacetate decarboxylase activity	GO:0008948	0.72	0.01	5.16E-05	2.33E-02
oxidoreductase activity, acting on NAD(P)H, NAD(P) as acceptor	GO:0016652	0.72	0.01	7.68E-05	3.20E-02

Table B.9. KEGG pathway enrichment analysis of up-regulated genes in Ispir leaf samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Biosynthesis of secondary metabolites	26.23	16.56	2.01E-12	2.57E-10
Plant-pathogen interaction	13.47	9.57	1.96E-04	2.91E-03
Plant hormone signal transduction	11.83	8.79	1.98E-03	1.58E-02
Phenylpropanoid biosynthesis	5.04	2.89	5.72E-04	6.10E-03
ABC transporters	3.28	1.72	1.56E-03	1.33E-02
Flavonoid biosynthesis	4.8	1.37	5.45E-11	3.49E-09
Stilbenoid, diarylheptanoid and gingerol biosynthesis	3.16	1.2	1.48E-05	3.16E-04
Cysteine and methionine metabolism	2.58	1.16	7.99E-04	7.86E-03
Zeatin biosynthesis	3.28	1.11	1.39E-06	4.46E-05
Terpenoid backbone biosynthesis	3.04	1.07	6.67E-06	1.71E-04
Glutathione metabolism	2.11	1	3.88E-03	2.92E-02
Limonene and pinene degradation	2.46	0.97	2.04E-04	2.91E-03
Carotenoid biosynthesis	3.04	0.91	4.58E-07	1.96E-05
Alanine, aspartate and glutamate metabolism	1.87	0.88	5.72E-03	4.07E-02
alpha-Linolenic acid metabolism	2.11	0.87	9.02E-04	8.24E-03
Flavone and flavonol biosynthesis	2.11	0.7	8.23E-05	1.50E-03
Diterpenoid biosynthesis	1.76	0.57	2.49E-04	3.19E-03
Linoleic acid metabolism	1.64	0.55	5.42E-04	6.10E-03

Table B.10. KEGG pathway enrichment analysis of down-regulated genes in Ispir leaf samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Metabolic pathways	48.44	36.64	4.89E-13	1.66E-11
Biosynthesis of secondary metabolites	23.62	16.64	6.58E-08	7.66E-07
Plant hormone signal transduction	14.46	8.66	1.17E-08	1.93E-07
Endocytosis	9.92	6.66	1.59E-04	1.13E-03
Glycerophospholipid metabolism	10.36	6.36	4.68E-06	4.60E-05
Ether lipid metabolism	9.82	5.21	2.34E-08	3.23E-07
Pentose and glucuronate interconversions	4.64	2.39	7.12E-05	5.69E-04
Flavonoid biosynthesis	2.8	1.44	1.79E-03	9.94E-03
Galactose metabolism	3.24	1.34	2.48E-05	2.12E-04
Stilbenoid, diarylheptanoid and gingerol biosynthesis	2.8	1.21	1.53E-04	1.13E-03
Limonene and pinene degradation	2.05	0.98	3.38E-03	1.73E-02
Carotenoid biosynthesis	3.99	0.86	5.17E-13	1.66E-11
Other glycan degradation	5.61	0.75	1.01E-25	1.29E-23
Ascorbate and aldarate metabolism	1.94	0.85	1.78E-03	9.94E-03
DNA replication	1.83	0.81	2.46E-03	1.31E-02
Flavone and flavonol biosynthesis	1.83	0.71	6.53E-04	4.18E-03
Sphingolipid metabolism	2.37	0.58	1.50E-07	1.60E-06
Cutin, suberine and wax biosynthesis	1.62	0.58	6.90E-04	4.20E-03
Linoleic acid metabolism	2.37	0.51	2.52E-08	3.23E-07
Glycosaminoglycan degradation	2.59	0.4	1.87E-11	4.00E-10
Photosynthesis	2.91	0.36	1.34E-14	8.55E-13
Isoflavonoid biosynthesis	1.29	0.37	4.08E-04	2.75E-03
Glycosphingolipid biosynthesis - ganglio series	2.27	0.26	3.52E-12	9.02E-11
Brassinosteroid biosynthesis	0.97	0.31	3.91E-03	1.92E-02
Sesquiterpenoid and triterpenoid biosynthesis	1.4	0.29	1.03E-05	9.42E-05
Photosynthesis - antenna proteins	1.29	0.11	1.20E-08	1.93E-07

Table B.11. KEGG pathway enrichment analysis of up-regulated genes in TR43477 leaf samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Biosynthesis of secondary metabolites	22.32	16.6	2.00E-07	3.65E-06
Plant-pathogen interaction	13.08	9.52	3.78E-05	5.38E-04
Plant hormone signal transduction	11.82	8.73	1.73E-04	1.85E-03
Phenylpropanoid biosynthesis	6.66	2.74	2.20E-12	2.81E-10
ABC transporters	3.68	1.67	2.08E-06	3.33E-05
Flavonoid biosynthesis	3.52	1.38	9.09E-08	1.94E-06
Phenylalanine metabolism	3.68	1.32	4.86E-09	2.07E-07
Arginine and proline metabolism	2.35	1.23	1.27E-03	1.16E-02
Stilbenoid, diarylheptanoid and gingerol biosynthesis	3.6	1.13	1.63E-10	1.04E-08
Zeatin biosynthesis	2.51	1.11	5.93E-05	7.59E-04
Glutathione metabolism	1.88	0.99	3.96E-03	2.98E-02
Limonene and pinene degradation	2.19	0.95	1.25E-04	1.46E-03
Flavone and flavonol biosynthesis	2.27	0.66	9.01E-08	1.94E-06
Cutin, suberine and wax biosynthesis	1.33	0.58	2.54E-03	2.17E-02
Ubiquinone and other terpenoid-quinone biosynthesis	2.11	0.53	1.67E-08	5.35E-07
Diterpenoid biosynthesis	1.41	0.57	8.40E-04	8.27E-03
Indole alkaloid biosynthesis	0.47	0.09	2.81E-03	2.25E-02

Table B.12. KEGG pathway enrichment analysis of down-regulated genes in TR43477 leaf samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Metabolic pathways	46.61	36.75	3.43E-09	1.10E-07
Biosynthesis of secondary metabolites	20.09	16.81	7.34E-03	4.83E-02
Starch and sucrose metabolism	6.89	4.42	8.11E-04	9.00E-03
Pentose and glucuronate interconversions	4.13	2.42	2.14E-03	1.83E-02
Stilbenoid, diarylheptanoid and gingerol biosynthesis	2.64	1.22	8.44E-04	9.00E-03
Limonene and pinene degradation	2.07	0.98	3.95E-03	3.16E-02
Carotenoid biosynthesis	3.1	0.91	1.89E-07	4.84E-06
Other glycan degradation	4.13	0.82	1.06E-13	4.53E-12
DNA replication	2.18	0.8	1.68E-04	2.68E-03
Nitrogen metabolism	1.72	0.68	1.73E-03	1.58E-02
Sphingolipid metabolism	1.49	0.62	4.63E-03	3.42E-02
SNARE interactions in vesicular transport	1.95	0.57	3.10E-05	5.67E-04
Diterpenoid biosynthesis	1.38	0.58	7.54E-03	4.83E-02
Linoleic acid metabolism	1.95	0.53	1.54E-05	3.28E-04
Glycosaminoglycan degradation	1.38	0.46	1.25E-03	1.23E-02
Photosynthesis	4.82	0.29	8.20E-32	1.05E-29
Isoflavonoid biosynthesis	1.38	0.37	2.32E-04	2.97E-03
Sulfur metabolism	1.03	0.34	4.81E-03	3.42E-02
Glycosphingolipid biosynthesis - ganglio series	1.26	0.31	1.97E-04	2.81E-03
Photosynthesis - antenna proteins	2.87	0.05	1.20E-27	7.70E-26

Table B.13. KEGG pathway enrichment analysis of up-regulated genes in Ispir root samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Metabolic pathways	52.48	36.85	1.79E-10	5.73E-09
Biosynthesis of secondary metabolites	32.67	16.64	5.21E-15	3.33E-13
Ether lipid metabolism	9.16	5.34	1.27E-03	1.19E-02
Starch and sucrose metabolism	7.67	4.46	3.04E-03	2.29E-02
Phenylpropanoid biosynthesis	7.92	2.88	5.57E-07	1.02E-05
Pentose and glucuronate interconversions	5.2	2.44	1.30E-03	1.19E-02
ABC transporters	5.94	1.71	3.19E-07	6.80E-06
Flavonoid biosynthesis	7.43	1.39	6.81E-13	2.91E-11
Phenylalanine metabolism	3.47	1.42	2.64E-03	2.25E-02
Stilbenoid, diarylheptanoid and gingerol biosynthesis	7.92	1.15	1.72E-16	2.20E-14
Zeatin biosynthesis	3.47	1.15	3.81E-04	4.47E-03
Limonene and pinene degradation	5.2	0.95	1.34E-09	3.42E-08
Alanine, aspartate and glutamate metabolism	2.97	0.88	3.84E-04	4.47E-03
Tyrosine metabolism	2.72	0.79	5.69E-04	6.07E-03
Flavone and flavonol biosynthesis	2.97	0.71	5.91E-05	8.41E-04
Nitrogen metabolism	2.23	0.7	2.84E-03	2.27E-02
Diterpenoid biosynthesis	3.22	0.57	1.31E-06	2.10E-05
Benzoxazinoid biosynthesis	1.24	0.27	6.01E-03	4.27E-02

Table B.14. KEGG pathway enrichment analysis of down-regulated genes in Ispir root samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
RNA transport	14.44	7.34	4.14E-14	2.65E-12
Endocytosis	9.5	6.67	5.34E-04	9.77E-03
Glycerophospholipid metabolism	9.99	6.37	1.23E-05	3.14E-04
Ribosome	17.71	5.28	3.02E-42	3.87E-40
Ether lipid metabolism	9.4	5.21	9.30E-08	3.97E-06
Phagosome	3.66	1.91	2.81E-04	6.00E-03
Photosynthesis	1.78	0.41	1.08E-06	3.45E-05
Photosynthesis - antenna proteins	0.69	0.14	1.02E-03	1.63E-02

Table B.15. KEGG pathway enrichment analysis of up-regulated genes in TR43477 root samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Ribosome	23.69	5.24	3.64E-59	4.65E-57
Ether lipid metabolism	8.54	5.3	2.55E-04	1.63E-02

Table B.16. KEGG pathway enrichment analysis of down-regulated genes in TR43477 root samples.

KEGG Pathway	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*Value**
Plant-pathogen interaction	25.46	9.23	2.12E-32	2.71E-30
Plant hormone signal transduction	12.96	8.79	2.90E-04	1.24E-02
Ascorbate and aldarate metabolism	2.29	0.85	8.86E-04	2.84E-02
Glucosinolate biosynthesis	1.22	0.17	4.64E-05	2.97E-03

Table B.17. COG functional enrichment analysis of up-regulated genes in Ispir leaf samples.

COG Term	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
Amino acid transport and metabolism	12.05	7.55	2.42E-06	3.02E-05
Secondary metabolites biosynthesis, transport and catabolism	8.48	4.91	6.75E-06	5.62E-05
Defense mechanisms	4.35	1.46	1.10E-08	2.75E-07

Table B.18. COG functional enrichment analysis of down-regulated genes in Ispir leaf samples.

COG Term	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
Function unknown	16.72	10.15	6.60E-10	8.25E-09
Carbohydrate transport and metabolism	16.41	10.04	1.70E-09	1.42E-08
Cell cycle control, cell division, chromosome partitioning	15.07	8.61	1.21E-10	3.04E-09
Cell wall/membrane/envelope biogenesis	11.46	6.76	1.24E-07	5.15E-07
Secondary metabolites biosynthesis, transport and catabolism	9.39	4.86	7.94E-09	3.97E-08
Lipid transport and metabolism	7.74	4.97	2.00E-04	6.25E-04
Cytoskeleton	7.22	3.28	4.31E-09	2.69E-08
Chromatin structure and dynamics	4.13	1.73	1.58E-06	5.66E-06
Defense mechanisms	2.99	1.51	7.71E-04	2.14E-03

Table B.19. COG functional enrichment analysis of up-regulated genes in TR43477 leaf samples.

COG Term	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
Transcription	22.98	19.45	8.20E-04	5.13E-03
Signal transduction mechanisms	16.97	14.44	5.64E-03	2.82E-02
Amino acid transport and metabolism	10.33	7.56	1.74E-04	1.45E-03
Secondary metabolites biosynthesis, transport and catabolism	7.71	4.87	6.13E-06	7.67E-05
Defense mechanisms	4.31	1.39	6.24E-13	1.56E-11

Table B.20. COG functional enrichment analysis of down-regulated genes in TR43477 leaf samples.

COG Term	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
Carbohydrate transport and metabolism	16.58	10.05	2.20E-09	2.75E-08
Function unknown	14.16	10.27	1.88E-04	7.84E-04
Cell cycle control, cell division, chromosome partitioning	13.28	8.71	4.62E-06	2.89E-05
Cell wall/membrane/envelope biogenesis	9.99	6.83	3.04E-04	1.09E-03
Secondary metabolites biosynthesis, transport and catabolism	9.22	4.88	6.87E-08	5.72E-07
Lipid transport and metabolism	7.24	5	2.51E-03	7.83E-03
Cytoskeleton	5.82	3.35	1.51E-04	7.54E-04
Chromatin structure and dynamics	5.27	1.69	5.33E-11	1.33E-09
Cell motility	2.63	1.4	3.83E-03	1.06E-02

Table B.21. COG functional enrichment analysis of up-regulated genes in Ispir root samples.

COG Term	% in DEGs	% in Rest	*p*-Value	Adj *p*-Value
Amino acid transport and metabolism	17.87	7.54	8.61E-12	1.08E-10
Carbohydrate transport and metabolism	15.22	10.23	1.09E-03	5.75E-03
Secondary metabolites biosynthesis, transport and catabolism	14.49	4.87	1.69E-13	4.22E-12
Cell wall/membrane/envelope biogenesis	11.11	6.88	1.15E-03	5.75E-03
Inorganic ion transport and metabolism	7.73	4.64	4.12E-03	1.72E-02
Defense mechanisms	7	1.48	2.35E-11	1.96E-10

Table B.22. COG functional enrichment analysis of down-regulated genes in Ispir root samples.

COG Term	% in DEGs	% in Rest	*p*-Value	Adj *p*-Value
Translation, ribosomal structure and biogenesis	28.38	12.43	2.50E-41	6.25E-40
Transcription	24.06	19.46	1.80E-04	3.75E-04
Function unknown	21.62	9.88	4.95E-28	6.19E-27
Posttranslational modification, protein turnover, chaperones	18.42	12.53	5.83E-08	1.62E-07
Signal transduction mechanisms	17.29	14.46	6.91E-03	1.23E-02
Cell cycle control, cell division, chromosome partitioning	17.2	8.48	8.70E-19	7.25E-18
Cell wall/membrane/envelope biogenesis	13.72	6.62	1.08E-15	6.73E-15
Carbohydrate transport and metabolism	13.63	10.15	2.73E-04	5.25E-04
Amino acid transport and metabolism	13.53	7.45	1.87E-11	9.35E-11
Lipid transport and metabolism	9.49	4.87	9.70E-10	3.03E-09
Intracellular trafficking, secretion, and vesicular transport	9.02	4.24	3.61E-11	1.50E-10
Energy production and conversion	6.95	5.2	9.34E-03	1.56E-02
Cytoskeleton	6.3	3.31	1.50E-06	3.76E-06
Cell motility	3.2	1.37	1.53E-05	3.47E-05
Extracellular structures	1.13	0.06	1.69E-10	6.02E-10

Table B.23. COG functional enrichment analysis of up-regulated genes in TR43477 root samples.

COG Term	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
Translation, ribosomal structure and biogenesis	29.29	12.61	2.80E-33	6.99E-32
Amino acid transport and metabolism	13.68	7.52	5.40E-09	6.75E-08
Function unknown	13.55	10.32	3.08E-03	1.28E-02
Cell wall/membrane/envelope biogenesis	11.61	6.79	1.11E-06	6.92E-06
Intracellular trafficking, secretion, and vesicular transport	6.58	4.39	3.79E-03	1.35E-02
Cell motility	3.1	1.39	4.45E-04	2.23E-03
Extracellular structures	1.03	0.07	8.03E-07	6.69E-06

Table B.24. COG functional enrichment analysis of down-regulated genes in TR43477 root samples.

COG Term	**% in DEGs**	**% in Rest**	***p*-Value**	**Adj *p*-Value**
General function prediction only	35.94	29.91	1.24E-03	3.41E-03
Transcription	28.3	19.45	2.86E-07	2.14E-06
Replication, recombination and repair	23.78	17.12	3.72E-05	1.33E-04
Signal transduction mechanisms	23.61	14.36	4.24E-09	5.29E-08
Function unknown	20.14	10.18	2.11E-12	5.28E-11
Translation, ribosomal structure and biogenesis	16.84	13.08	6.25E-03	1.30E-02
Cell cycle control, cell division, chromosome partitioning	15.28	8.72	3.43E-07	2.14E-06
Cell wall/membrane/envelope biogenesis	11.46	6.84	4.46E-05	1.39E-04
Amino acid transport and metabolism	11.28	7.64	1.36E-03	3.41E-03
Lipid transport and metabolism	9.72	4.97	2.81E-06	1.41E-05
Secondary metabolites biosynthesis, transport and catabolism	9.2	4.94	1.96E-05	8.16E-05
Energy production and conversion	7.29	5.23	2.19E-02	3.95E-02
Intracellular trafficking, secretion, and vesicular transport	6.25	4.42	2.71E-02	4.52E-02
Cytoskeleton	5.73	3.39	3.24E-03	7.36E-03
Extracellular structures	0.52	0.09	2.21E-02	3.95E-02

REFERENCES

1. Jones, H. G. and M. B. Jones, *Introduction: Some Terminology and Common Mechanisms*, Cambridge University Press, New York, 1989.

2. Grime, J. P., "Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory", *American Naturalist*, Vol. 11, No. 982, pp. 1169-1194, 1977.

3. Parihar, P., S. Singh, R. Singh, and V. P. Singh, "Effect of Salinity Stress on Plants and Its Tolerance Strategies", *Environmental Science and Pollution Research*, Vol. 22, No. 6, pp. 4056-4075, 2015.

4. Tilman, D., C. Balzer, J. Hill, and B. L. Befort, "Global Food Demand and the Sustainable Intensification of Agriculture", *Proceedings of the National Academy of Sciences*, Vol. 108, No. 50, pp. 20260-20264, 2011.

5. OECD/FAO, *Agricultural Outlook 2012*, OECD Publishing, Paris, 2012.

6. Hide, J. C., *Diagnosis and Improvement of Saline and Alkali Soils*, U.S. Dept. of Agriculture, Washington, D.C., 1954.

7. Gama, P. B. S., S. Inanaga, K. Tanaka, and R. Nakazawa, "Physiological Response of Common Bean (*Phaseolus Vulgaris* L.) Seedlings to Salinity Stress", *African Journal of Biotechnology*, Vol. 6, No. 2, pp. 79-88, 2007.

8. Tuteja, N., "Mechanisms of High Salinity Tolerance in Plants", *Methods in Enzymology*, Vol. 428, No. 1, pp. 419-38, 2007.

9. Zhu, J. K., "Plant Salt Tolerance", *Trends in Plant Science*, Vol. 6, No. 2, pp. 66-71, 2001.

10. Mahajan, S. and N. Tuteja, "Cold, Salinity and Drought Stresses: An Overview", *Archives Biochemistry Biophysics*, Vol. 444, No. 2, pp. 139-58, 2005.

11. Hallett, S. H., D. M. Ozden, C. A. Keay, A. Koral, S. Keskin, and R. I. Bradley, "A Land Information System for Turkey - a Key to the Country's Sustainable Development", *Journal of Arid Environments,* Vol. 54, No. 3, pp. 513-525, 2003.

12. Munns, R., "Comparative Physiology of Salt and Water Stress", *Plant Cell and Environment,* Vol. 25, No. 2, pp. 239-250, 2002.

13. Greenway, H. and R. Munns, "Mechanisms of Salt Tolerance in Non-Halophytes", *Annual Review of Plant Physiology and Plant Molecular Biology*, Vol. 31, No. 1, pp. 149-190, 1980.

14. Rengasamy, P., "World Salinization with Emphasis on Australia", *Journal of Experimental Botany,* Vol. 57, No. 5, pp. 1017-23, 2006.

15. Higbie, S. M., F. Wang, j. M. Srtewart, T. M. Sterling, W. C. Lindemann, E. Hughs, and J. Zhang, "Physiological Response to Salt (Nacl) Stress in Selected Cultivated Tetraploid Cottons", *International Journal of Agronomy*, Vol. 2010, No. 1, pp. 1-12, 2010.

16. Munns, R. and M. Tester, "Mechanisms of Salinity Tolerance", *Annual Review of Plant Biology*, Vol. 59, No. 1, pp. 651-81, 2008.

17. Wignarajah, K., "Growth Response of *Phaseolus Vulgaris* to Varying Salinity Regimes", *Environmental and Experimental Botany,* Vol. 30, No. 2, pp. 141-147, 1990.

18. Lockhart, J. A., "An Analysis of Irreversible Plant Cell Elongation", *Journal of Theoretical Biology*, Vol. 8, No. 2, pp. 264-75, 1965.

19. Slabu, C., C. Zörb, D. Steffens, and S. Schubert, "Is Salt Stress of Faba Bean (*Vicia Faba*) Caused by Na^+ or Cl^- Toxicity?", *Journal of Plant Nutrition and Soil Science*, Vol. 172, No. 5, pp. 644-651, 2009.

20. Stoeva, N. and M. Kaymakanova, "Effect of Salt Stress on the Growth and Photosynthesis Rate of Bean Plants (*Phaseolus Vulgaris* L.)", *Journal of Central European Agriculture*, Vol. 9, No. 1, pp. 385-392, 2008.

21. Gomes-Filho, E., C. Lima, J. H. Costa, and A. C. M. da Silva, "Cowpea Ribonuclease: Properties and Effect of Nacl-Salinity on Its Activation During Seed Germination and Seedling Establishment", *Plant Cell Reports*, Vol. 27, No. 1, pp. 147-157, 2008.

22. Dantas, B. F., L. S. Ribeiro, and C. A. Aragão, "Germination, Initial Growth and Cotyledon Protein Content of Bean Cultivars under Salinity Stress", *Revista Brasileira de Sementes*, Vol. 29, No. 2, pp. 106-110, 2007.

23. Khan, M. A. and Y. Rizvi, "Effect of Salinity, Temperature, and Growth Regulators on the Germination and Early Seedling Growth of Atriplex Griffithii Var. Stocksii", *Canadian Journal of Botany*, Vol. 72, No. 4, pp. 475-479, 1994.

24. Yoshida, T. and J. Mogami, "Aba-Dependent and Aba-Independent Signaling in Response to Osmotic Stress in Plants", *Current Opinion in Plant Biology*, Vol. 21, No. 1, pp. 133-139, 2014.

25. Ishitani, M., L. Xiong, B. Stevenson, and J. K. Zhu, "Genetic Analysis of Osmotic and Cold Stress Signal Transduction in Arabidopsis: Interactions and Convergence of Abscisic Acid-Dependent and Abscisic Acid-Independent Pathways", *The Plant Cell*, Vol. 9, No. 11, pp. 1935-1949, 1997.

26. Xiong, L., M. Ishitani, H. Lee, and J. K. Zhu, "*Hos5*-a Negative Regulator of Osmotic Stress-Induced Gene Expression in Arabidopsis Thaliana", *The Plant Journal*, Vol. 19, No. 5, pp. 569-578, 1999.

27. Grill, E. and A. Himmelbach, "Aba Signal Transduction", *Current Opinion in Plant Biology*, Vol. 1, No. 5, pp. 412-418, 1998.

28. Uno, Y., T. Furihata, H. Abe, R. Yoshida, K. Shinozaki, and K. Yamaguchi-Shinozaki, "Arabidopsis Basic Leucine Zipper Transcription Factors Involved in an Abscisic Acid-Dependent Signal Transduction Pathway under Drought and High-Salinity Conditions", *Proceedings of the National Academy of Sciences* Vol. 97, No. 21, pp. 11632-11637, 2000.

29. Liao, Y., H.-F. F. Zou, W. Wei, Y.-J. J. Hao, A.-G. G. Tian, J. Huang, Y.-F. F. Liu, J.-S. S. Zhang, and S.-Y. Y. Chen, "Soybean *Gmbzip44*, *Gmbzip62* and *Gmbzip78* Genes

Function as Negative Regulator of Aba Signaling and Confer Salt and Freezing Tolerance in Transgenic Arabidopsis", *Planta*, Vol. 228, No. 2, pp. 225-240, 2008.

30. Abe, H., T. Urao, T. Ito, M. Seki, and K. Shinozaki, "Arabidopsis *Atmyc2* (Bhlh) and *Atmyb2* (Myb) Function as Transcriptional Activators in Abscisic Acid Signaling", *The Plant Cell*, Vol. 15, No. 1, pp. 63-78, 2003.

31. Sakuma, Y., K. Maruyama, Y. Osakabe, and F. Qin, "Functional Analysis of an Arabidopsis Transcription Factor, *Dreb2a*, Involved in Drought-Responsive Gene Expression", *The Plant Cell*, Vol. 18, No. 5, pp. 1292-1309, 2006.

32. Kim, J. S., J. Mizoi, T. Yoshida, and Y. Fujita, "An Abre Promoter Sequence Is Involved in Osmotic Stress-Responsive Expression of the Dreb2a Gene, Which Encodes a Transcription Factor Regulating Drought-Inducible Genes in Arabidopsis", *Plant and Cell Physiology*, Vol. 52, No. 12, pp. 2136-2146, 2011.

33. Lee, S., J. Kang, H. J. Park, M. D. Kim, and M. S. Bae, "*Dreb2c* Interacts with *Abf2*, a Bzip Protein Regulating Abscisic Acid-Responsive Gene Expression, and Its Overexpression Affects Abscisic Acid Sensitivity", *Plant Physiology,* Vol. 152, No. 2, pp. 716-727, 2010.

34. Elfving, N., C. Davoine, R. Benlloch, J. Blomberg, K. Brännström, D. Müller, A. Nilsson, M. Ulfstedt, H. Ronne, and G. Wingsle, "The Arabidopsis Thaliana Med25 Mediator Subunit Integrates Environmental Cues to Control Plant Development", *Proceedings of the National Academy of Sciences*, Vol. 108, No. 20, pp. 8245-8250, 2011.

35. Kiyosue, T., K. Yamaguchi-Shinozaki, and K. Shinozaki, "Cloning of Cdnas for Genes That Are Early-Responsive to Dehydration Stress (Erds) in *Arabidopsis Thaliana* L.: Identification of Three Erds as Hsp Cognate Genes", *Plant Molecular Biology,* Vol. 25, No. 5, pp. 791-798, 1994.

36. Nakashima, K., T. Kiyosue, K. Yamaguchi-Shinozaki, and K. Shinozaki, "A Nuclear Gene, Erd1, Encoding a Chloroplast-Targeted Clp Protease Regulatory Subunit Homolog Is Not Only Induced by Water Stress but Also Developmentally up-

Regulated During Senescence in *Arabidopsis Thaliana*", *The Plant Journal*, Vol. 12, No. 4, pp. 851-861, 1997.

37. Fujita, M., Y. Fujita, K. Maruyama, and M. Seki, "A Dehydration-Induced Nac Protein, *Rd26*, Is Involved in a Novel Aba-Dependent Stress-Signaling Pathway", *The Plant Journal*, Vol. 39, No. 6, pp. 863-76, 2004.

38. Agarwal, P. K. and B. Jha, "Transcription Factors in Plants and Aba Dependent and Independent Abiotic Stress Signalling", *Biologia Plantarum*, Vol. 54, No. 2, pp. 201-212, 2010.

39. Shinozaki, K., "Gene Networks Involved in Drought Stress Response and Tolerance", *Journal of Experimental Botany*, Vol. 58, No. 2, pp. 221-227, 2007.

40. Yamaguchi-Shinozaki, K. and K. Shinozaki, "The Plant Hormone Abscisic Acid Mediates the Drought-Induced Expression but Not the Seed-Specific Expression of *Rd22*, a Gene Responsive to Dehydration Stress in Arabidopsis Thaliana", *Molecular & General Genetics*, Vol. 238, No. 1-2, pp. 17-25, 1993.

41. Yamaguchi-Shinozaki, K. and K. Shinozaki, "A Novel Cis-Acting Element in an Arabidopsis Gene Is Involved in Responsiveness to Drought, Low-Temperature, or High-Salt Stress", *The Plant Cell*, Vol. 6, No. 2, pp. 251-264, 1994.

42. Wang, Q., Y. Guan, Y. Wu, H. Chen, F. Chen, and C. Chu, "Overexpression of a Rice *Osdreb1f* Gene Increases Salt, Drought, and Low Temperature Tolerance in Both Arabidopsis and Rice", *Plant Molecular Biology*, Vol. 67, No. 6, pp. 589-602, 2008.

43. Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki, and K. Shinozaki, "Two Transcription Factors, *Dreb1* and *Dreb2*, with an Erebp/Ap2 DNA Binding Domain Separate Two Cellular Signal Transduction Pathways in Drought- and Low-Temperature-Responsive Gene Expression, Respectively, in Arabidopsis", *The Plant Cell*, Vol. 10, No. 8, pp. 1391-1406, 1998.

44. Leung, J., S. Merlot, and J. Giraudat, "The Arabidopsis Abscisic Acid-Insensitive2 (*Abi2*) and *Abi1* Genes Encode Homologous Protein Phosphatases 2c Involved in Abscisic Acid Signal Transduction", *The Plant Cell*, Vol. 9, No. 5, pp. 759-771, 1997.

45. Fujita, Y., T. Yoshida, and K. Yamaguchi-Shinozaki, "Pivotal Role of the Areb/Abf-Snrk2 Pathway in Abre-Mediated Transcription in Response to Osmotic Stress in Plants", *Physiologia Plantarum*, Vol. 147, No. 1, pp. 15-27, 2013.

46. Rodríguez, M., E. Canales, and O. Borrás-Hidalgo, "Molecular Aspects of Abiotic Stress in Plants", *Biotecnología Aplicada*, Vol. 22, No. 1, pp. 1-10, 2005.

47. Gupta, B. and B. Huang, "Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization", *International Journal of Genomics*, Vol. 2014, No. 1, pp. 1-18, 2014.

48. Jian-Kang, Z., "Regulation of Ion Homeostasis under Salt Stress", *Current Opinion in Plant Biology*, Vol. 6, No. 5, pp. 441-445, 2003.

49. Touchette, B. W., G. A. Smith, K. L. Rhodes, and M. Poole, "Tolerance and Avoidance: Two Contrasting Physiological Responses to Salt Stress in Mature Marsh Halophytes *Juncus Roemerianus* Scheele and *Spartina Alterniflora* Loisel ", *Journal of Experimental Marine Biology and Ecology*, Vol. 380, No. 1-2, pp. 106-112, 2009.

50. Tester, M. and R. Davenport, "Na^+ Tolerance and Na^+ Transport in Higher Plants", *Annals of Botany*, Vol. 91, No. 5, pp. 503-527, 2003.

51. Silva, P. and H. Geros, "Regulation by Salt of Vacuolar H^+-Atpase and H^+-Pyrophosphatase Activities and Na^+/H^+ Exchange", *Plant Signaling & Behavior*, Vol. 4, No. 8, pp. 718-726, 2009.

52. Ahmad, M. S. A., M. Ashraf, and Q. Ali, "Soil Salinity as a Selection Pressure Is a Key Determinant for the Evolution of Salt Tolerance in Blue Panicgrass (*Panicum Antidotale* Retz.)", *Flora - Morphology, Distribution, Functional Ecology of Plants*, Vol. 205, No. 1, pp. 37-45, 2010.

53. Kerepesi, I. and G. Galiba, "Osmotic and Salt Stress-Induced Alteration in Soluble Carbohydrate Content in Wheat Seedlings", *Crop Science*, Vol. 40, No. 2, pp. 482-487, 2000.

54. Saxena, S. C., H. Kaur, P. Verma, and B. P. Petla, "Osmoprotectants: Potential for Crop Improvement under Adverse Conditions", *Plant Acclimation to Environmental Stress*, Vol. 2012, No. 1, pp. 197-232, 2013.

55. Md. Anamul, H., B. Mst. Nasrin Akhter, O. Eiji, A. Katsumi, N. Yoshimasa, S. Yasuaki, and M. Yoshiyuki, "Exogenous Proline and Glycinebetaine Increase Nacl-Induced Ascorbate–Glutathione Cycle Enzyme Activities, and Proline Improves Salt Tolerance More Than Glycinebetaine in Tobacco Bright Yellow-2 Suspension-Cultured Cells", *Journal of Plant Physiology*, Vol. 164, No. 11, pp. 1457-1468, 2007.

56. El-Shintinawy, F. and M. N. El-Shourbagy, "Alleviation of Changes in Protein Metabolism in Nacl-Stressed Wheat Seedlings by Thiamine", *Biologia Plantarum*, Vol. 44, No. 4, pp. 541-545, 2001.

57. Ben Ahmed, C., B. Ben Rouina, S. Sensoy, M. Boukhriss, and F. Ben Abdullah, "Exogenous Proline Effects on Photosynthetic Performance and Antioxidant Defense System of Young Olive Tree", *Journal of Agricultural and Food Chemistry*, Vol. 58, No. 7, pp. 4216-4222, 2010.

58. Matysik, J., B. Bhalu, and P. Mohanty, "Molecular Mechanisms of Quenching of Reactive Oxygen Species by Proline under Stress in Plants", *Current Science*, Vol. 82, No. 5, pp. 525-532, 2002.

59. Md Anamul, H., B. Mst Nasrin Akhter, N. Yoshimasa, S. Yasuaki, and M. Yoshiyuki, "Proline and Glycinebetaine Enhance Antioxidant Defense and Methylglyoxal Detoxification Systems and Reduce Nacl-Induced Damage in Cultured Tobacco Cells", *Journal of Plant Physiology*, Vol. 165, No. 8, pp. 813-824, 2008.

60. Xavier, R. and O. F. Lim, "Role of Exogenous Proline in Ameliorating Salt Stress at Early Stage in Two Rice Cultivars", *Journal of Stress Physiology & Biochemistry*, Vol. 7, No. 4, pp. 157-174, 2011.

61. Iyer, S. and A. Caplan, "Products of Proline Catabolism Can Induce Osmotically Regulated Genes in Rice", *Plant Physiology*, Vol. 116, No. 1, pp. 203-211, 1998.

62. Sairam, R. K. and A. Tyagi, "Physiology and Molecular Biology of Salinity Stress Tolerance in Plants", *Current Science*, Vol. 86, No. 3, pp. 407-421, 2004.

63. Planchet, E., O. Rannou, and C. Ricoult, "Unraveling the Involvement of Aba in the Water Deficit-Induced Modulation of Nitrogen Metabolism in Medicago Truncatula Seedlings", *Plant Signaling & Behavior*, Vol. 6, No. 7, pp. 1074-1076, 2011.

64. Zengrong, H., Z. Long, C. Dandan, L. Mingxiang, L. Zhaopu, S. Hongbo, and L. Xiaohua, "Salt Stress Encourages Proline Accumulation by Regulating Proline Biosynthesis and Degradation in Jerusalem Artichoke Plantlets", *PLoS One*, Vol. 8, No. 4, pp. e62085, 2013.

65. Li, X., S. Hou, Q. Gao, P. Zhao, S. Chen, D. Qi, B. H. Lee, L. Cheng, and G. Liu, "*Lcsain1*, a Novel Salt-Induced Gene from Sheepgrass, Confers Salt Stress Tolerance in Transgenic Arabidopsis and Rice", *Plant and Cell Physiology*, Vol. 54, No. 7, pp. 1172-85, 2013.

66. Groß, F., J. Durner, and F. Gaupels, "Nitric Oxide, Antioxidants and Prooxidants in Plant Defence Responses", *Frontiers in Plant Science*, Vol. 4, No. 419, pp. 1-15, 2013.

67. Broughton, W. J., G. Hernandez, M. Blair, S. Beebe, P. Gepts, and J. Vanderleyden, "Beans (*Phaseolus* Spp.) - Model Food Legumes", *Plant and Soil*, Vol. 252, No. 1, pp. 55-128, 2003.

68. Chinnusamy, V., A. Jagendorf, and J. K. Zhu, "Understanding and Improving Salt Tolerance in Plants", *Crop Science*, Vol. 45, No. 2, pp. 437-448, 2005.

69. Madakbas, S. Y. and S. Ellialtioglu, "Taze Fasulyede Dayaniklilik Islahinin Kavrami, Mekanizmasi Ve Kalitimi", *Hasad Bitkisel Üretim*, Vol. 21, No. 244, pp. 80-86, 2005.

70. Cemek, B., M. Guler, and H. Arslan, "Bafra Ovası Sağ Sahil Sulama Alanındaki Tuzluluk Dağılımının Coğrafi Bilgi Sistemleri (Cbs)Kullanılarak Belirlenmesi", *Atatürk Üniv. Ziraat Fak. Derg.*, Vol. 37, No. 1, pp. 63-72, 2006.

71. Gulduren, S. and E. Elkoca, "Salinity Tolerance at Germination Stage of Some Bean (*Phaseolus Vulgaris* L.) Genotypes Collected from North East Anatolia Region and

Coruh Valley", *Journal of Agricultural Faculty of Ataturk University*, Vol. 43, No. 1, pp. 29-41, 2012.

72. Dasgan, H. Y. and S. Koc, "Evaluation of Salt Tolerance in Common Bean Genotypes by Ion Regulation and Searching for Screening Parameters", *Journal of Food Agriculture & Environment*, Vol. 7, No. 2, pp. 363-372, 2009.

73. Sodha, N. and R. Karan, "Omics Study for Abiotic Stress Responses in Plants", *Advances in Plants & Agriculture Research*, Vol. 2, No. 1, pp. 1-7, 2015.

74. Schmutz, J., S. Cannon, J. Schlueter, J. Ma, T. Mitros, W. Nelson, D. Hyten, Q. Song, J. Thelen, J. Cheng, D. Xu, U. Hellsten, G. May, Y. Yu, T. Sakurai, T. Umezawa, M. Bhattacharyya, D. Sandhu, B. Valliyodan, E. Lindquist, M. Peto, D. Grant, S. Shu, D. Goodstein, K. Barry, M. Futrell-Griggs, B. Abernathy, J. Du, Z. Tian, L. Zhu, N. Gill, T. Joshi, M. Libault, A. Sethuraman, X.-C. Zhang, K. Shinozaki, H. Nguyen, R. Wing, P. Cregan, J. Specht, J. Grimwood, D. Rokhsar, G. Stacey, R. Shoemaker, and S. Jackson, "Genome Sequence of the Palaeopolyploid Soybean", *Nature*, Vol. 463, No. 7278, pp. 178-183, 2010.

75. Sato, S., Y. Nakamura, T. Kaneko, E. Asamizu, T. Kato, M. Nakao, S. Sasamoto, A. Watanabe, A. Ono, K. Kawashima, T. Fujishiro, M. Katoh, M. Kohara, Y. Kishida, C. Minami, S. Nakayama, N. Nakazaki, Y. Shimizu, S. Shinpo, C. Takahashi, T. Wada, M. Yamada, N. Ohmido, M. Hayashi, K. Fukui, T. Baba, T. Nakamichi, H. Mori, and S. Tabata, "Genome Structure of the Legume, *Lotus Japonicus*", *DNA Research*, Vol. 15, No. 4, pp. 227-239, 2008.

76. Young, N., F. Debellé, G. Oldroyd, R. Geurts, S. Cannon, M. Udvardi, V. Benedito, K. Mayer, J. Gouzy, H. Schoof, Y. Van de Peer, S. Proost, D. Cook, B. Meyers, M. Spannagl, F. Cheung, S. De Mita, V. Krishnakumar, H. Gundlach, S. Zhou, J. Mudge, A. Bharti, J. Murray, M. Naoumkina, B. Rosen, K. Silverstein, H. Tang, S. Rombauts, P. Zhao, P. Zhou, V. Barbe, P. Bardou, M. Bechner, A. Bellec, A. Berger, H. Bergès, S. Bidwell, T. Bisseling, N. Choisne, A. Couloux, R. Denny, S. Deshpande, X. Dai, J. Doyle, A.-M. Dudez, A. Farmer, S. Fouteau, C. Franken, C. Gibelin, J. Gish, S. Goldstein, A. González, P. Green, A. Hallab, M. Hartog, A. Hua, S. Humphray, D.-H. Jeong, Y. Jing, A. Jöcker, S. Kenton, D.-J. Kim, K. Klee, H. Lai, C. Lang, S. Lin, S.

Macmil, G. Magdelenat, L. Matthews, J. McCorrison, E. Monaghan, J.-H. Mun, F. Najar, C. Nicholson, C. Noirot, M. O'Bleness, C. Paule, J. Poulain, F. Prion, B. Qin, C. Qu, E. Retzel, C. Riddle, E. Sallet, S. Samain, N. Samson, I. Sanders, O. Saurat, C. Scarpelli, T. Schiex, B. Segurens, A. Severin, D. Sherrier, R. Shi, S. Sims, S. Singer, S. Sinharoy, L. Sterck, A. Viollet, and B.-B. Wang, "The Medicago Genome Provides Insight into the Evolution of Rhizobial Symbioses", *Nature*, Vol. 480, No. 7378, pp. 520-524, 2011.

77. Varshney, R., W. Chen, Y. Li, A. Bharti, R. Saxena, J. Schlueter, M. Donoghue, S. Azam, G. Fan, A. Whaley, A. Farmer, J. Sheridan, A. Iwata, R. Tuteja, R. Penmetsa, W. Wu, H. Upadhyaya, S.-P. Yang, T. Shah, K. Saxena, T. Michael, W. McCombie, B. Yang, G. Zhang, H. Yang, J. Wang, C. Spillane, D. Cook, G. May, X. Xu, and S. Jackson, "Draft Genome Sequence of Pigeonpea (*Cajanus Cajan*), an Orphan Legume Crop of Resource-Poor Farmers", *Nature Biotechnology*, Vol. 30, No. 1, pp. 83-89, 2012.

78. Varshney, R., C. Song, R. Saxena, S. Azam, S. Yu, A. Sharpe, S. Cannon, J. Baek, B. Rosen, B. Tar'an, T. Millan, X. Zhang, L. Ramsay, A. Iwata, Y. Wang, W. Nelson, A. Farmer, P. Gaur, C. Soderlund, R. Penmetsa, C. Xu, A. Bharti, W. He, P. Winter, S. Zhao, J. Hane, N. Carrasquilla-Garcia, J. Condie, H. Upadhyaya, M.-C. Luo, M. Thudi, C. L. Gowda, N. Singh, J. Lichtenzveig, K. Gali, J. Rubio, N. Nadarajan, J. Dolezel, K. Bansal, X. Xu, D. Edwards, G. Zhang, G. Kahl, J. Gil, K. Singh, S. Datta, S. Jackson, J. Wang, and D. Cook, "Draft Genome Sequence of Chickpea (*Cicer Arietinum*) Provides a Resource for Trait Improvement", *Nature Biotechnology*, Vol. 31, No. 3, pp. 240-246, 2013.

79. Schmutz, J., P. E. McClean, S. Mamidi, G. A. Wu, S. B. Cannon, J. Grimwood, J. Jenkins, S. Shu, Q. Song, C. Chavarro, M. Torres-Torres, V. Geffroy, S. M. Moghaddam, D. Gao, B. Abernathy, K. Barry, M. Blair, M. A. Brick, M. Chovatia, P. Gepts, D. M. Goodstein, M. Gonzales, U. Hellsten, D. L. Hyten, G. Jia, J. D. Kelly, D. Kudrna, R. Lee, M. M. S. Richard, P. N. Miklas, J. M. Osorno, J. Rodrigues, V. Thareau, C. A. Urrea, M. Wang, Y. Yu, M. Zhang, R. A. Wing, P. B. Cregan, D. S. Rokhsar, and S. A. Jackson, "A Reference Genome for Common Bean and Genome-

Wide Analysis of Dual Domestications", *Nature Genetics*, Vol. 46, No. 7, pp. 707-713, 2014.

80. Jackson, S. A., A. Iwata, S. H. Lee, and J. Schmutz, "Sequencing Crop Genomes: Approaches and Applications", *New Phytologist*, Vol. 191, No. 4, pp. 915-925, 2011.

81. Deshmukh, R., H. Sonah, G. Patil, W. Chen, S. Prince, R. Mutava, T. Vuong, B. Valliyodan, and H. T. Nguyen, "Integrating Omic Approaches for Abiotic Stress Tolerance in Soybean", *Frontiers in plant science*, Vol. 5, No. 244, pp. 1-12, 2014.

82. Libault, M., A. Farmer, T. Joshi, K. Takahashi, R. Langley, L. Franklin, J. He, D. Xu, G. May, and G. Stacey, "An Integrated Transcriptome Atlas of the Crop Model *Glycine Max*, and Its Use in Comparative Analyses in Plants", *The Plant Journal*, Vol. 63, No. 1, pp. 86-99, 2010.

83. Yun-Qing, C., L. Jian-Feng, Y. Xiangdong, M. Rui, L. Chunming, and L. Qiang, "Rna-Seq Analysis Reveals Ethylene-Mediated Reproductive Organ Development and Abscission in Soybean (*Glycine Max* L. Merr.)", *Plant Molecular Biology Reporter*, Vol. 31, No. 3, pp. 607-619, 2013.

84. Fu, X., N. Fu, S. Guo, Z. Yan, Y. Xu, H. Hu, C. Menzel, W. Chen, Y. Li, R. Zeng, and P. Khaitovich, "Estimating Accuracy of Rna-Seq and Microarrays with Proteomics", *BMC Genomics*, Vol. 10, No. 161, pp. 1-9, 2009.

85. Seki, M., M. Narusaka, J. Ishida, T. Nanjo, M. Fujita, Y. Oono, A. Kamiya, M. Nakajima, A. Enju, T. Sakurai, M. Satou, K. Akiyama, T. Taji, K. Yamaguchi-Shinozaki, P. Carninci, J. Kawai, Y. Hayashizaki, and K. Shinozaki, "Monitoring the Expression Profiles of 7000 Arabidopsis Genes under Drought, Cold and High-Salinity Stresses Using a Full-Length Cdna Microarray", *The Plant Journal*, Vol. 31, No. 3, pp. 279-92, 2002.

86. Wang, H., S. Miyazaki, K. Kawai, M. Deyholos, D. W. Galbraith, and H. J. Bohnert, "Temporal Progression of Gene Expression Responses to Salt Shock in Maize Roots", *Plant Molecular Biology*, Vol. 52, No. 4, pp. 873-91, 2003.

87. Rabbani, M. A., K. Maruyama, H. Abe, M. A. Khan, K. Katsura, Y. Ito, K. Yoshiwara, M. Seki, K. Shinozaki, and K. Yamaguchi-Shinozaki, "Monitoring Expression Profiles of Rice Genes under Cold, Drought, and High-Salinity Stresses and Abscisic Acid Application Using Cdna Microarray and Rna Gel-Blot Analyses", *Plant Physiology*, Vol. 133, No. 4, pp. 1755-67, 2003.

88. Jiang, Y. and M. K. Deyholos, "Comprehensive Transcriptional Profiling of Nacl-Stressed Arabidopsis Roots Reveals Novel Classes of Responsive Genes", *BMC Plant Biology*, Vol. 6, No. 25, pp. 1-20, 2006.

89. Walia, H., C. Wilson, L. Zeng, A. M. Ismail, P. Condamine, and T. J. Close, "Genome-Wide Transcriptional Analysis of Salinity Stressed Japonica and Indica Rice Genotypes During Panicle Initiation Stage", *Plant Molecular Biology*, Vol. 63, No. 5, pp. 609-23, 2007.

90. Qing, D. J., H. F. Lu, N. Li, H. T. Dong, D. F. Dong, and Y. Z. Li, "Comparative Profiles of Gene Expression in Leaves and Roots of Maize Seedlings under Conditions of Salt Stress and the Removal of Salt Stress", *Plant and Cell Physiology*, Vol. 50, No. 4, pp. 889-903, 2009.

91. Ge, Y., Y. Li, Y. M. Zhu, X. Bai, D. K. Lv, D. Guo, W. Ji, and H. Cai, "Global Transcriptome Profiling of Wild Soybean (*Glycine Soja*) Roots under Nahco3 Treatment", *BMC Plant Biology*, Vol. 10, No. 153, pp. 1-14, 2010.

92. Xu, P., Z. Liu, X. Fan, J. Gao, X. Zhang, X. Zhang, and X. Shen, "*De Novo* Transcriptome Sequencing and Comparative Analysis of Differentially Expressed Genes in *Gossypium Aridum* under Salt Stress", *Gene*, Vol. 525, No. 1, pp. 26-34, 2013.

93. Postnikova, O. A., J. Shao, and L. G. Nemchinov, "Analysis of the Alfalfa Root Transcriptome in Response to Salinity Stress", *Plant and Cell Physiology*, Vol. 54, No. 7, pp. 1041-55, 2013.

94. Ramirez, M., M. A. Graham, L. Blanco-Lopez, S. Silvente, A. Medrano-Soto, M. W. Blair, G. Hernandez, C. P. Vance, and M. Lara, "Sequencing and Analysis of Common

Bean Ests. Building a Foundation for Functional Genomics", *Plant Physiology*, Vol. 137, No. 4, pp. 1211-1227, 2005.

95. Melotto, M., C. B. Monteiro-Vitorello, A. G. Bruschi, and L. E. Camargo, "Comparative Bioinformatic Analysis of Genes Expressed in Common Bean (*Phaseolus Vulgaris* L.) Seedlings", *Genome*, Vol. 48, No. 3, pp. 562-70, 2005.

96. Kalavacharla, V., Z. Liu, B. C. Meyers, J. Thimmapuram, and K. Melmaiee, "Identification and Analysis of Common Bean (*Phaseolus Vulgaris* L.) Transcriptomes by Massively Parallel Pyrosequencing", *BMC Plant Biology*, Vol. 11, No. 135, pp. 1-18, 2011.

97. Liu, Z., M. Crampton, A. Todd, and V. Kalavacharla, "Identification of Expressed Resistance Gene-Like Sequences by Data Mining in 454-Derived Transcriptomic Sequences of Common Bean (*Phaseolus Vulgaris* L.)", *BMC Plant Biology*, Vol. 12, No. 42, pp. 1-9, 2012.

98. Liao, D., D. Cram, A. Sharpe, and F. Marsolais, "Transcriptome Profiling Identifies Candidate Genes Associated with the Accumulation of Distinct Sulfur Γ-Glutamyl Dipeptides in *Phaseolus Vulgaris* and *Vigna Mungo* Seeds", *Frontiers in Plant Science*, Vol. 4, No. 1, pp. 60, 2013.

99. Wu, J., L. Wang, L. Li, and S. Wang, "*De Novo* Assembly of the Common Bean Transcriptome Using Short Reads for the Discovery of Drought-Responsive Genes", *PLoS One*, Vol. 9, No. 10, pp. e109262, 2014.

100. Garg, R., R. K. Patel, A. K. Tyagi, and M. Jain, "De Novo Assembly of Chickpea Transcriptome Using Short Reads for Gene Discovery and Marker Identification", *DNA Research*, Vol. 18, No. 1, pp. 53-63, 2011.

101. Hiremath, P., A. Farmer, S. Cannon, J. Woodward, H. Kudapa, R. Tuteja, A. Kumar, A. Bhanuprakash, B. Mulaosmanovic, N. Gujaria, L. Krishnamurthy, P. Gaur, P. Kavikishor, T. Shah, R. Srinivasan, M. Lohse, Y. Xiao, C. Town, D. Cook, G. May, and R. Varshney, "Large-Scale Transcriptome Analysis in Chickpea (*Cicer Arietinum* L.), an Orphan Legume Crop of the Semi-Arid Tropics of Asia and Africa", *Plant Biotechnology journal*, Vol. 9, No. 8, pp. 922-931, 2011.

102. Huang, J., X. Lu, H. Yan, S. Chen, W. Zhang, R. Huang, and Y. Zheng, "Transcriptome Characterization and Sequencing-Based Identification of Salt-Responsive Genes in *Millettia Pinnata*, a Semi-Mangrove Plant", *DNA Research*, Vol. 19, No. 2, pp. 195-207, 2012.

103. Chen, Y., F. Li, Y. Ma, K. Chong, and Y. Xu, "Overexpression of *Orbhlh001*, a Putative Helix-Loop-Helix Transcription Factor, Causes Increased Expression of *Akt1* and Maintains Ionic Balance under Salt Stress in Rice", *Journal of Plant Physiology*, Vol. 170, No. 1, pp. 93-100, 2013.

104. Cheng, L., X. Li, X. Huang, T. Ma, Y. Liang, X. Ma, X. Peng, J. Jia, S. Chen, Y. Chen, B. Deng, and G. Liu, "Overexpression of Sheepgrass R1-Myb Transcription Factor *Lcmyb1* Confers Salt Tolerance in Transgenic Arabidopsis", *Plant Physiology and Biochemistry*, Vol. 70, No. 1, pp. 252-260, 2013.

105. Cho, H. Y., C. Lee, S. G. Hwang, Y. C. Park, H. L. Lim, and C. S. Jang, "Overexpression of the *Oschi1* Gene, Encoding a Putative Laccase Precursor, Increases Tolerance to Drought and Salinity Stress in Transgenic Arabidopsis", *Gene*, Vol. 552, No. 1, pp. 98-105, 2014.

106. Xu, N., K. Qian, Y. Dong, Y. Chen, Q. Yu, B. Zhang, L. Xing, and M. Li, "Novel Role of the Candida Albicans Ferric Reductase Gene *Cfl1* in Iron Acquisition, Oxidative Stress Tolerance, Morphogenesis and Virulence", *Research in Microbiology*, Vol. 165, No. 3, pp. 252-61, 2014.

107. Ma, X., X. Zhu, C. Li, Y. Song, W. Zhang, G. Xia, and M. Wang, "Overexpression of Wheat *Nf-Ya10* Gene Regulates the Salinity Stress Response in *Arabidopsis Thaliana*", *Plant Physiology and Biochemistry*, Vol. 86, No. 1, pp. 34-43, 2015.

108. Li, J.-B. B., Y.-S. S. Luan, and Z. Liu, "Overexpression of Spwrky1 Promotes Resistance to *Phytophthora Nicotianae* and Tolerance to Salt and Drought Stress in Transgenic Tobacco", *Physiologia Plantarum*, Vol. 12315, No. 1, pp. 1-19, 2014.

109. Xu, G.-Y. Y., P. S. Rocha, M.-L. L. Wang, M.-L. L. Xu, Y.-C. C. Cui, L.-Y. Y. Li, Y.-X. X. Zhu, and X. Xia, "A Novel Rice Calmodulin-Like Gene, *Osmsr2*, Enhances

Drought and Salt Tolerance and Increases Aba Sensitivity in Arabidopsis", *Planta*, Vol. 234, No. 1, pp. 47-59, 2011.

110. Marinova, K., L. Pourcel, B. Weder, and M. Schwarz, "The Arabidopsis Mate Transporter *Tt12* Acts as a Vacuolar Flavonoid/H^+-Antiporter Active in Proanthocyanidin-Accumulating Cells of the Seed Coat", *The Plant Cell*, Vol. 19, No. 6, pp. 2023-2038, 2007.

111. Debeaujon, I., "The Transparent Testa12 Gene of Arabidopsis Encodes a Multidrug Secondary Transporter-Like Protein Required for Flavonoid Sequestration in Vacuoles of the Seed Coat Endothelium", *The Plant Cell Online*, Vol. 13, No. 4, pp. 853-871, 2001.

112. Iuchi, S., K. Yamaguchi-Shinozaki, and T. Urao, "Novel Drought-Inducible Genes in the Highly Drought-Tolerant Cowpea: Cloning of Cdnas and Analysis of the Expression of the Corresponding Genes", *Plant and Cell Physiology*, Vol. 37, No. 8, pp. 1073-1082, 1996.

113. Song, Y., C. Zhang, W. Ge, Y. Zhang, A. L. Burlingame, and Y. Guo, "Identification of Nacl Stress-Responsive Apoplastic Proteins in Rice Shoot Stems by 2d-Dige", *Journal of Proteomics*, Vol. 74, No. 7, pp. 1045-1067, 2011.

114. Wallner, S., D. Corinna, D. Bastian, and P. Macheroux, *Berberine Bridge Enzyme and the Family of Bicovalent Flavoenzymes*, De Gruyter, Berlin, Boston, 2012.

115. Winter, D., B. Vinegar, H. Nahal, R. Ammar, G. V. Wilson, and N. J. Provart, "An "Electronic Fluorescent Pictograph" Browser for Exploring and Analyzing Large-Scale Biological Data Sets", *PloS One*, Vol. 2, No. 8, pp. e718, 2007.

116. Gonzalez-Candelas, L., S. Alamar, P. Sanchez-Torres, L. Zacarias, and J. F. Marcos, "A Transcriptomic Approach Highlights Induction of Secondary Metabolism in Citrus Fruit in Response to Penicillium Digitatum Infection", *BMC Plant Biology*, Vol. 10, No. 194, pp. 194, 2010.

117. Attila, C., A. Ueda, S. L. Cirillo, J. D. Cirillo, W. Chen, and T. K. Wood, "*Pseudomonas Aeruginosa* Pao1 Virulence Factors and Poplar Tree Response in the Rhizosphere", *Microbial Biotechnology*, Vol. 1, No. 1, pp. 17-29, 2008.

118. Daniel, B., T. Pavkov-Keller, B. Steiner, and A. Dordic, "Oxidation of Monolignols by Members of the Berberine Bridge Enzyme Family Suggests a Role in Plant Cell Wall Metabolism", *The Journal of Biological Chemistry*, Vol. 290, No. 5, pp. 18770-18781, 2015.

119. Hong, T. Y., C. W. Cheng, J. W. Huang, and M. Meng, "Isolation and Biochemical Characterization of an Endo-1,3-Beta-Glucanase from Streptomyces Sioyaensis Containing a C-Terminal Family 6 Carbohydrate-Binding Module That Binds to 1,3-Beta-Glucan", *Microbiology*, Vol. 148, No. 4, pp. 1151-1159, 2002.

120. Bosch, M., C. D. Mayer, and A. Cookson, "Identification of Genes Involved in Cell Wall Biogenesis in Grasses by Differential Gene Expression Profiling of Elongating and Non-Elongating Maize Internodes", *Journal of Experimental Botany*, Vol. 62, No. 10, pp. 3545-3561, 2011.

121. Zhu, Y. N., D. Q. Shi, M. B. Ruan, L. L. Zhang, Z. H. Meng, J. Liu, and W. C. Yang, "Transcriptome Analysis Reveals Crosstalk of Responsive Genes to Multiple Abiotic Stresses in Cotton (*Gossypium Hirsutum* L.)", *PLoS One*, Vol. 8, No. 11, pp. e80218, 2013.

122. Bohnert, H. J., P. Ayoubi, C. Borchert, and R. A. Bressan, "A Genomics Approach Towards Salt Stress Tolerance", *Plant Physiology and Biochemistry*, Vol. 39, No. 3-4, pp. 295-311, 2001.

123. Sadder, M., A. Alsadon, and M. Wahb-Allah, "Transcriptomic Analysis of Tomato Lines Reveals Putative Stress-Specific Biomarkers", *Turkish Journal of Agriculture and Forestry*, Vol. 38, No. 5, pp. 700-715, 2014.

124. Castresana, C., F. de Carvalho, and G. Gheysen, "Tissue-Specific and Pathogen-Induced Regulation of a Nicotiana Plumbaginifolia Beta-1, 3-Glucanase Gene", *The Plant Cell*, Vol. 2, No. 12, pp. 1131-1143, 1990.

125. Grenier, J., C. Potvin, and A. Asselin, "Barley Pathogenesis-Related Proteins with Fungal Cell Wall Lytic Activity Inhibit the Growth of Yeasts", *Plant Physiology*, Vol. 103, No. 4, pp. 1277-1283, 1993.

126. Conn, S. J., B. Hocking, M. Dayod, B. Xu, A. Athman, S. Henderson, L. Aukett, V. Conn, M. K. Shearer, and S. Fuentes, "Protocol: Optimising Hydroponic Growth Systems for Nutritional and Physiological Analysis of *Arabidopsis Thaliana* and Other Plants", *Plant Methods*, Vol. 9, No. 1, pp. 1-11, 2013.

127. Nakagawa, T., S. Ishiguro, and T. Kimura, "Gateway Vectors for Plant Transformation", *Plant Biotechnology*, Vol. 7, No. 5, pp. 193-195, 2009.

128. Shavrukov, Y., "Salt Stress or Salt Shock: Which Genes Are We Studying?", *Journal of Experimental Botany*, Vol. 64, No. 1, pp. 119-127, 2013.

129. Henson, I. E., V. Mahalakshmi, F. R. Bidinger, and G. Alagars–Wamy, "Genotypic Variation in Pearl Miller (*Pennisetum Americanum* L.) Leeke in the Ability to Accumulate Abscisic Acid in Response on Water Stress.", *Journal of Experimental Botany*, Vol. 32, No. 1, pp. 899-910, 1981.

130. Dlugokecka, E. and A. Kacperskapalacz, "Re-Examination of Electrical-Conductivity Method for Estimation of Drought Injuries", *Biologia Plantarum*, Vol. 20, No. 4, pp. 262-267, 1978.

131. Fan, S. H. and T. J. Blake, "Abscisic-Acid Induced Electrolyte Leakage in Woody Species with Contrasting Ecological Requirements", *Physiologia Plantarum*, Vol. 90, No. 2, pp. 414-419, 1994.

132. Mohr, K. F., "Lehrbuch Der Chemisch-Analytischen Titrirmethode", *Zeitschrift Für Analytische Chemie*, Vol. 26, No. 1, pp. 61-61, 1887.

133. Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev, "Full-Length Transcriptome Assembly from Rna-Seq Data

without a Reference Genome", *Nature Biotechnology*, Vol. 29, No. 7, pp. 644-652, 2011.

134. Iseli, C., C. V. Jongeneel, and P. Bucher, "Estscan: A Program for Detecting, Evaluating, and Reconstructing Potential Coding Regions in Est Sequences", *Proc Int Conf Intell Syst Mol Biol*, Vol., No., pp. 138-148, 1999.

135. Ye, J., L. Fang, H. Zheng, Y. Zhang, J. Chen, Z. Zhang, J. Wang, S. Li, R. Li, L. Bolund, and J. Wang, "Wego: A Web Tool for Plotting Go Annotations", *Nucleic Acids Research*, Vol. 34, No. 1, pp. 293-297, 2006.

136. Ashburner, M., C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, M. A. Harris, D. P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G. Sherlock, "Gene Ontology: Tool for the Unification of Biology. The Gene Ontology Consortium", *Nature Genetics*, Vol. 25, No. 1, pp. 25-29, 2000.

137. Li, R., C. Yu, Y. Li, T. W. Lam, S. M. Yiu, K. Kristiansen, and J. Wang, "Soap2: An Improved Ultrafast Tool for Short Read Alignment", *Bioinformatics*, Vol. 25, No. 15, pp. 1966-7, 2009.

138. Benjamini, Y. and D. Yekutieli, "The Control of the False Discovery Rate in Multiple Testing under Dependency", *Annals of Statistics*, Vol. 29, No. 4, pp. 1165-1188, 2001.

139. Du, Z., X. Zhou, Y. Ling, Z. Zhang, and Z. Su, "Agrigo: A Go Analysis Toolkit for the Agricultural Community", *Nucleic Acids Research*, Vol. 38, No. Web Server issue, pp. 70, 2010.

140. Al-Shahrour, F., R. Díaz-Uriarte, and J. Dopazo, "Fatigo: A Web Tool for Finding Significant Associations of Gene Ontology Terms with Groups of Genes", *Bioinformatics*, Vol. 20, No. 4, pp. 578-580, 2004.

141. Borges, A., S. M. Tsai, and D. G. Caldas, "Validation of Reference Genes for Rt-Qpcr Normalization in Common Bean During Biotic and Abiotic Stresses", *Plant Cell Reports*, Vol. 31, No. 5, pp. 827-38, 2012.

142. van Die, I. M., H. E. Bergmans, and W. P. Hoekstra, "Transformation in *Escherichia Coli*: Studies on the Role of the Heat Shock in Induction of Competence", *The Journal of General and Applied Microbiology*, Vol. 129, No. 3, pp. 663-70, 1983.

143. Holsters, M., D. Dewaele, A. Depicker, E. Messens, M. Vanmontagu, and J. Schell, "Transfection and Transformation of Agrobacterium-Tumefaciens", *Molecular & General Genetics*, Vol. 163, No. 2, pp. 181-187, 1978.

144. Bent, A., "Arabidopsis Thaliana Floral Dip Transformation Method", *Methods in Molecular Biology*, Vol. 343, No. 1, pp. 87-103, 2006.

145. Harrison, S. J., E. K. Mott, K. Parsley, S. Aspinall, J. C. Gray, and A. Cottage, "A Rapid and Robust Method of Identifying Transformed Arabidopsis Thaliana Seedlings Following Floral Dip Transformation", *Plant Methods*, Vol. 2, No. 19, pp. 1-7, 2006.

146. Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, "Nih Image to Imagej: 25 Years of Image Analysis", *Nature Methods*, Vol. 9, No. 7, pp. 671-675, 2012.

147. Ábrahám, E., C. Hourton-Cabassa, and L. Erdei, "Methods for Determination of Proline in Plants", *Methods in Molecular Biology*, Vol. 639, No. 1, pp. 317-331, 2010.

148. Rewald, B., O. Shelef, J. E. Ephrath, and S. Rachmilevitch, *Adaptive Plasticity of Salt-Stressed Root Systems*, Springer New York, 2013.

149. Barrs, H. D. and P. E. Weatherley, "A Re-Examination of Relative Turgidity Technique for Estimating Water Deficits in Leaves", *Australian Journal of Biological Sciences*, Vol. 15, No. 3, pp. 413-428, 1962.

150. Trovato, M., R. Mattioli, and P. Costantino, "Multiple Roles of Proline in Plant Stress Tolerance and Development", *Rendiconti Lincei*, Vol. 19, No. 1, pp. 325-346, 2008.

151. Mao, X., S. Chen, A. Li, C. Zhai, and R. Jing, "Novel Nac Transcription Factor *Tanac67* Confers Enhanced Multi-Abiotic Stress Tolerances in Arabidopsis", *PLoS One*, Vol. 9, No. 1, pp. e84359, 2014.

152. Jaradat, M. R., J. A. Feurtado, D. Huang, and Y. Lu, "Multiple Roles of the Transcription Factor *Atmybr1*/*Atmyb44* in Aba Signaling, Stress Responses, and Leaf Senescence", *BMC Plant Biology*, Vol. 13, No. 192, pp. 19, 2013.

153. Yang, A., X. Dai, and W. H. Zhang, "A R2r3-Type Myb Gene, *Osmyb2*, Is Involved in Salt, Cold, and Dehydration Tolerance in Rice", *Journal of experimental Botany*, Vol. 63, No. 7, pp. 2541-2556, 2012.

154. Hiz, M. C., B. Canher, H. Niron, and M. Turet, "Transcriptome Analysis of Salt Tolerant Common Bean (*Phaseolus Vulgaris* L.) under Saline Conditions", *PLoS One*, Vol. 9, No. 3, pp. e92598, 2014.

155. James, R. A., C. Blake, C. S. Byrt, and R. Munns, "Major Genes for Na^+ Exclusion, *Nax1* and *Nax2* (Wheat Hkt1;4 and Hkt1;5), Decrease Na^+ Accumulation in Bread Wheat Leaves under Saline and Waterlogged Conditions", *Journal of Experimental Botany*, Vol. 62, No. 8, pp. 2939-2947, 2011.

156. Zahaf, O., S. Blanchet, A. de Zelicourt, B. Alunni, J. Plet, C. Laffont, L. de Lorenzo, S. Imbeaud, J. L. Ichante, A. Diet, M. Badri, A. Zabalza, E. M. Gonzalez, H. Delacroix, V. Gruber, F. Frugier, and M. Crespi, "Comparative Transcriptomic Analysis of Salt Adaptation in Roots of Contrasting *Medicago Truncatula* Genotypes", *Molecular Plant*, Vol. 5, No. 5, pp. 1068-1081, 2012.

157. Fan, X.-D., J.-Q. Wang, N. Yang, Y.-Y. Dong, L. Liu, F.-W. Wang, N. Wang, H. Chen, W.-C. Liu, Y.-P. Sun, J.-Y. Wu, and H.-Y. Li, "Gene Expression Profiling of Soybean Leaves and Roots under Salt, Saline-Alkali and Drought Stress by High-Throughput Illumina Sequencing", *Gene*, Vol. 512, No. 2, pp. 392-402, 2013.

158. Molina, C., M. Zaman-Allah, F. Khan, N. Fatnassi, R. Horres, B. Rotter, D. Steinhauer, L. Amenc, J. J. Drevon, P. Winter, and G. Kahl, "The Salt-Responsive Transcriptome of Chickpea Roots and Nodules Via Deepsupersage", *BMC Plant Biology*, Vol. 11, No. 31, pp. 1-26, 2011.

159. O'Rourke, J. A., L. P. Iniguez, F. Fu, B. Bucciarelli, S. S. Miller, S. A. Jackson, P. E. McClean, J. Li, X. Dai, and P. X. Zhao, "An Rna-Seq Based Gene Expression Atlas of the Common Bean", *BMC Genomics*, Vol. 15, No. 1, pp. 866, 2014.

160. Eticha, D., M. Zahn, M. Bremer, Z. B. Yang, A. F. Rangel, I. M. Rao, and W. J. Horst, "Transcriptomic Analysis Reveals Differential Gene Expression in Response to Aluminium in Common Bean (*Phaseolus Vulgaris*) Genotypes", *Annals of Botany*, Vol. 105, No. 7, pp. 1119-1128, 2010.

161. Wang, C., H. Chen, Q. Hao, A. Sha, Z. Shan, L. Chen, R. Zhou, H. Zhi, and X. Zhou, "Transcript Profile of the Response of Two Soybean Genotypes to Potassium Deficiency", *PLoS One*, Vol. 7, No. 7, pp. e39856, 2012.

162. Pang, T., C.-Y. Ye, X. Xia, and W. Yin, "*De Novo* Sequencing and Transcriptome Analysis of the Desert Shrub, *Ammopiptanthus Mongolicus*, During Cold Acclimation Using Illumina/Solexa", *BMC Genomics*, Vol. 14, No. 1, pp. 488, 2013.

163. Parida, A. and A. Das, "Salt Tolerance and Salinity Effects on Plants", *Ecotoxicology and Environmental Safety*, Vol. 60, No. 3, pp. 324-349, 2005.

164. Tomoaki, H., K. Ichirou, and K. Maki, "Salinity Tolerance Mechanisms in Glycophytes: An Overview with the Central Focus on Rice Plants", *Rice*, Vol. 5, No. 11, pp. 1-18, 2012.

165. Mariana Lins de Oliveira, C., H. Bety Shiue de, G. João Antônio de Almeida, C. Rafaela Moura, A.-C. Jarcilene Silva de, and P. Marcelo Francisco, "Photosynthesis and Antioxidant Activity in *Jatropha Curcas* L. Under Salt Stress", *Brazilian Journal of Plant Physiology*, Vol. 24, No. 1, pp. 55*67, 2012.

166. Carillo, P., G. M. Annunziata, G. Pontecorvo, A. Fuggi, and P. Woodrow, *Salinity Stress and Salt Tolerance*, InTech, Crotia, 2011.

167. Ghosh, D. and J. Xu, "Abiotic Stress Responses in Plant Roots: A Proteomics Perspective", *Frontiers in Plant Science*, Vol. 5, No. 6, pp. 1-13, 2014.

168. Flexas, J., J. Bota, F. Loreto, G. Cornic, and T. D. Sharkey, "Diffusive and Metabolic Limitations to Photosynthesis under Drought and Salinity in C(3) Plants", *Plant Biology*, Vol. 6, No. 3, pp. 269-279, 2004.

169. Flexas, J., A. Diaz-Espejo, J. Galmes, R. Kaldenhoff, H. Medrano, and M. Ribas-Carbo, "Rapid Variations of Mesophyll Conductance in Response to Changes in Co2

Concentration around Leaves", *Plant Cell and Environment*, Vol. 30, No. 10, pp. 1284-1298, 2007.

170. Lawlor, D. W. and G. Cornic, "Photosynthetic Carbon Assimilation and Associated Metabolism in Relation to Water Deficits in Higher Plants", *Plant Cell and Environment*, Vol. 25, No. 2, pp. 275-294, 2002.

171. Matsuda, K. and A. Riazi, "Stress-Induced Osmotic Adjustment in Growing Regions of Barley Leaves", *Plant Physiology*, Vol. 68, No. 3, pp. 571-576, 1981.

172. Akula, R., M. J. Galligan, and H. Ibrahim, "Hydrogen Peroxide or Peracetic Acid Mediated Self-Titrating Alpha-Halogenation of 1,3-Dicarbonyl Compounds", *Synthesis*, Vol. 1, No. 2, pp. 347-351, 2011.

173. Rejeb, I. B., V. Pastor, and B. Mauch-Mani, "Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms", *Plants*, Vol. 3, No. 4, pp. 458-475, 2014.

174. Fujita, M., Y. Fujita, Y. Noutoshi, and F. Takahashi, "Crosstalk between Abiotic and Biotic Stress Responses: A Current View from the Points of Convergence in the Stress Signaling Networks", *Current Opinion in Plant Biology*, Vol. 9, No. 4, pp. 436-442, 2006.

175. Achuo, E. A., E. Prinsen, and M. Höfte, "Influence of Drought, Salt Stress and Abscisic Acid on the Resistance of Tomato to *Botrytis Cinerea* and *Oidium Neolycopersici*", *Plant Pathology*, Vol. 55, No. 2, pp. 178-186, 2006.

176. Atkinson, N. J. and P. E. Urwin, "The Interaction of Plant Biotic and Abiotic Stresses: From Genes to the Field", *Journal of Experimental Botany*, Vol. 63, No. 10, pp. 3523-3543, 2012.

177. Golldack, D., C. Li, H. Mohan, and N. Probst, "Tolerance to Drought and Salt Stress in Plants: Unraveling the Signaling Networks", *Frontiers in Plant Science*, Vol. 5, No. 151, pp. 1-10, 2014.

178. Cutler, S. R. and P. L. Rodriguez, "Abscisic Acid: Emergence of a Core Signaling Network", *Annual Review of Plant Biology*, Vol. 61, No. 1, pp. 651-679, 2010.

179. Kim, T. H. and B. Maik, "Guard Cell Signal Transduction Network: Advances in Understanding Abscisic Acid, Co$_2$, and Ca^{2+} Signaling", *Annual Review of Plant Biology*, Vol. 61, No. 1, pp. 561-591, 2010.

180. Zhu, J.-K. K., "Salt and Drought Stress Signal Transduction in Plants", *Annual Review of Plant Biology*, Vol. 53, No. 1, pp. 247-273, 2002.

181. He, Y., W. Li, J. Lv, Y. Jia, and M. Wang, "Ectopic Expression of a Wheat Myb Transcription Factor Gene, *Tamyb73*, Improves Salinity Stress Tolerance in Arabidopsis Thaliana", *Journal of Experimental Botany*, Vol. 63, No. 3, pp. 1511-1522, 2011.

182. Puranik, S., P. P. Sahu, P. S. Srivastava, and M. Prasad, "Nac Proteins: Regulation and Role in Stress Tolerance", *Trends in Plant Science*, Vol. 17, No. 6, pp. 369-381, 2012.

183. Msanne, J., J. Lin, J. M. Stone, and T. Awada, "Characterization of Abiotic Stress-Responsive *Arabidopsis Thaliana Rd29a* and *Rd29b* Genes and Evaluation of Transgenes", *Planta*, Vol. 234, No. 1, pp. 97-107, 2011.

184. Hayat, S., Q. Hayat, M. N. Alyemeni, and A. S. Wani, "Role of Proline under Changing Environments: A Review", *Plant Signaling & Behavior*, Vol. 7, No. 11, pp. 1456-1466, 2012.

185. Strizhov, N., E. Abraham, L. Ökrész, and S. Blickling, "Differential Expression of Two *P5cs* Genes Controlling Proline Accumulation During Salt-Stress Requires Aba and Is Regulated by *Aba1*, *Abi1* and *Axr2* in Arabidopsis", *The Plant Cell*, Vol. 12, No. 3, pp. 557-569, 1997.

186. Zhang, C., Q. Lu, and D. P. S. Verma, "Characterization of Δ 1-Pyrroline-5-Carboxylate Synthetase Gene Promoter in Transgenic *Arabidopsis Thaliana* Subjected to Water Stress", *Plant Science*, Vol. 129, No. 1, pp. 81-89, 1997.

187. Fabro, G., I. Kovács, V. Pavet, L. Szabados, and M. E. E. Alvarez, "Proline Accumulation and *Atp5cs2* Gene Activation Are Induced by Plant-Pathogen Incompatible Interactions in Arabidopsis", *Molecular Plant-Microbe Interactions*, Vol. 17, No. 4, pp. 343-350, 2004.