Molecular studies on abiotic stress and identification of stress tolerant genes in *Hevea brasiliensis*

Thesis Submitted to
MAHATMA GANDHI UNIVERSITY
KOTTAYAM
for the award of the degree of
DOCTOR OF PHILOSOPHY
in
BIOSCIENCES (BIOTECHNOLOGY)
(Faculty of Science)

By
LISHA P. LUKE

Under the supervision and guidance of
Dr. M.B. Mohamed Sathik

With compliments
Dr. Sugunan V.S.
30/8/2017

RUBBER RESEARCH INSTITUTE OF INDIA
KOTTAYAM, KERALA
INDIA- 686 009

March 2017

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M.B. MOHAMED SATHIK

M.B. Mohamed Sathik M.Phil., Ph.D.
Scientist
Crop Physiology Division
Rubber Research Institute of India.
Kottayam - 686 009.

Name & Signature of the HoD/HoI (Chairperson of the Doctoral Committee) : 10/03/17

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M.B. MOHAMED SATHIK

M.B. Mohamed Sathik M.Phil., Ph.D.
Scientist
Crop Physiology Division
Rubber Research Institute of India.
Kottayam - 686 009 Kerala

DECLARATION

I hereby declare that the thesis entitled **"Molecular studies on abiotic stress and identification of stress tolerant genes in *Hevea brasiliensis*"** is an authentic record of original research carried out by me under the supervision and guidance of Dr. M.B. Mohamed Sathik, Principal Scientist, Rubber Research Institute of India, Kottayam-9 in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY of the Mahatma Gandhi University, Kottayam and no part of this work has been presented for any degree, diploma or any other similar titles of any university.

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This is to certify that the thesis entitled "**Molecular studies on abiotic stress and identification of stress tolerant genes in *Hevea brasiliensis***" is an authentic record of original research work carried out by **Smt. Lisha P Luke**, at Rubber Research Institute of India, Kottayam-9, under my supervision and guidance for the award of the degree of **Doctor of Philosophy in Biotechnology**, under the faculty of Science, Mahatma Gandhi University, Kottayam. It is also certified that the work presented in this thesis have not been published or submitted earlier for any degree, diploma or any other similar titles of any university.

12.03.2017

Kottayam

Dr. M.B. Mohamed Sathik
Principal Scientist
Rubber Research Institute of India
Kottayam 686009

Tele Phone: 91- 481- 2353311 to 2353320 Fax: 91- 481- 2353327

To my son Rayan

Acknowledgement

Foremost, I bow before the **God Almighty** for strengthening me in all my infirmities and for bestowing upon me His blessings throughout these years for the successful completion of this endeavor.

I would like to express my immense sense of gratitude to my guide **Dr. M. B. Mohamed Sathik** for his excellent and splendid supervision over the research period which helped me a lot to complete the venture successfully. I am thankful to him for his warm personality, continual encouragement, support and perseverance in guiding me through the entire research and thesis writing process. I am especially indebted to him for his patience in my shortcomings and promoting self-confidence in me.

I sincerely thank **Dr. James Jacob**, Director of Research, Rubber Research Institute of India, for allowing me to conduct this doctoral research at Rubber Research Institute of India, Kottayam and his encouragement and support throughout the course of my work.

I wish to express my heartfelt gratitude to **Dr. Molly Thomas**, Principal Scientist, Crop Physiology Division, RRII, for the incredible help, moral support, motherly care and encouragement she rendered during my stay at RRII. I am very thankful to her as she had always been there for me at all stages of the work.

I convey my thanks to **Dr. K. Annamalainathan**, Joint Director, Crop Physiology Division, RRII, for his valuable and timely suggestions and providing all the facilities during the course of this work.

I would like to thank **Dr. R. Krishnakumar**, Joint Director (Retired), Climate Change and Ecosystem Studies, RRII, for the help he rendered to me.

I am deeply indebted to **Dr. K. V. Sumesh**, Scientist B, Crop Physiology Division, RRII for his timely help and support in this research.

I extend my heartfelt thanks to ***Mr.R.Ananthharamanan,*** *Scientist, Genome Analysis Division, RRII for all the timely help and the technical support he had rendered with the transcriptome data analysis.*

I wish to express my gratitude to ***Dr. T.R. Keerthi,*** *Director, School of Biosciences, M.G University,* ***Dr. J.G. Ray, Professor,*** *School of Biosciences, M.G University and all the other faculty members of School of Biosciences, M.G University, Kottayam.*

It is a great pleasure to express my deep sense of gratitude to ***Dr. S. Sreelatha, Dr. Jayasree Gopalakrishnan, Dr. D. Bhuvanendran Nair, Dr. Jayantha Sarkar, Dr. Sheela P. Simon, Dr. K.K .Jayasooryan and Mr. Harikumar*** *of Crop physiology Division, Rubber Research Institute of India, Kottayam*

I am grateful to ***Dr.M.A.Nazeer, Dr. Shaji Philip, Dr. Kavitha K. Mydin and Dr. A. Thulaseedharan*** *for their support and advices.*

I owe my thanks to ***Rajan Mathew, P.M. Sebastian, M.B. Rajan*** *and all other members of Crop Physiology Division for their timely help in different stages of this work.*

My heartfelt thanks for the support and companionship of ***Mrs. Ambily P.K, Mrs. Smitha M. Xavier, Dr. Mrudula P Musthapha, Mrs. Rejitha Rajan, Dr. Satheesh P. R., Dr. Amith Abraham*** *and* ***Mr. S. Pramod*** *during my amazing days in RRII.*

I am grateful to ***Mr. Aneesh,*** *Assistant Statistician, Rubber Research Institute of India, for the help in statistical data analysis. I convey my thanks to staff members of library, computer section and other staff members of RRII, Kottayam.*

·I am extremely grateful to my sister ***Mrs. Anu Mary Joseph,*** *Georgi and sweet Chakkara for the wonderful days we spent together as family and the happy and hard moments we shared will always remain in the heart forever.*

I extend my heartfelt thanks to my labmate and my dear friend ***Mrs. Linu Kuruvilla****, her husband Mr. Binu Philip and little Ivin for the help rendered and wholeheartedly allowing me to stay in their home during thesis writing days.*

Words fail to express my heartfelt gratitude to ***Viji aunty, Joychettan*** *and family who kept my son happy and comfortable during my long hours of absence through all these years which gave me a peaceful mind to work. This thesis will not be a reality without their help.*

I bow down to my ***parents and my in-laws*** *for their moral support, immense patience, and loving care that enabled me to complete this venture successfully.*

No words to express my gratitude to my beloved husband ***Mr. Danny Abraham*** *for his immense patience and support. Without his personal hardships and sacrifices all along these years, it wouldn't have been possible to complete this task and my lovable son* ***Rayan*** *who had always been my inspiration to pursue and accomplish this venture successfully.*

I sincerely thank M/s Minitek computers, Opp.M.G.University, Kottayam for the excellent and neat documentation of the thesis.

I wish to thank all my EV family members especially ***Mr. Johns K Lukose, Mrs. Jobina Johns and Mrs. Soly Cheriyan*** *whose prayers and kind words had always been a support to me.*

Last but not the least, I am indebted to my ever loving friend ***Mrs. Ann Mary Joseph*** *who always had positive feeling for me and fills my thoughts with her energy and enthusiasm.*

Lisha P Luke

ABSTRACT

Plants are often challenged by adverse stress factors from the environment they live against which they develop intricate mechanisms in order to overcome and adapt to the stressful environment. The adaptive changes that occur at physiological, biochemical and molecular levels depend upon the type, duration and severity of the stress factors. The molecular level responses include changes at the transcriptional level either by activating or repressing particular set of genes. Changes in the gene expression level ultimately adds on to the tolerance or susceptibility of a particular variety/genotype and investigations on such changes through gene expression profiling can help us understand the molecular mechanisms underlying the tolerance trait.

Hevea brasiliensis is the sole source of natural rubber which grows well in the traditional rubber growing regions of India which is conducive for its optimal growth and productivity. But non-availability of cultivable land in the traditional regions and its ever increasing demand resulted in expansion of its cultivation to non-traditional regions where *Hevea* is often challenged by extreme environmental conditions like drought and cold. So, it is imperative to evolve clones or genotypes which can withstand and perform well under such adverse agro-climatic conditions. This work is an attempt for the identification of stress tolerant genes associated with abiotic stress and identification of genes/factors contributing for the abiotic stress tolerance. Such genes or factors could be used as candidate genes in the molecular breeding programmes for drought and cold tolerance in *Hevea*.

In this study, attempts were made to identify drought and cold stress responsive genes from *Hevea brasiliensis* through quantitative PCR and next generation high throughput sequencing methods. Expression analysis of selected genes carried out in different clones with varying levels of stress tolerance revealed differential expression in drought and cold treated plants. Quantitative gene expression analysis of selected drought responsive transcripts in *Hevea* clones led to the discovery of drought tolerance associated transcripts like NAC tf, LEA, peroxidase and MAPK.

Using RNA-Seq technology, abiotic stress responsive transcripts of *Hevea* were identified. After sequence clustering, 58581 and 68482 transcripts were obtained from control and drought stressed samples respectively and 66019 transcripts from cold stressed samples. The differential gene expression analysis indicated up-regulation of 268 transcripts and down regulation of 566 transcripts under drought stress, while between control and cold stressed samples 961 and 109 transcripts were found up and down regulated, respectively. Further, when quantitative gene expression analysis of 17 prominent drought responsive transcripts was conducted, transcripts such as ferritin, DNA binding protein, NAC tf and aquaporin were found to exhibit stronger association with drought tolerance. Gene expression analysis conducted with regard to cold stress revealed the association of an ethylene responsive transcription factor with cold tolerance. These transcripts can be further utilized in the crop improvement programmes by the breeders to identify or develop drought/cold tolerant genotypes of *H. brasiliensis*.

Keywords: *Hevea brasiliensis*, drought tolerance, cold tolerance, expression analysis, RNA-Seq, qPCR

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ABBREVATIONS

A	CO_2 assimilation rate
ABA	Abscisic acid
CAT	Catalase
COR	Cold-responsive
Ct	Threshold cycle
DE	Differentially expressed
DEPC	Diethyl pyrocarbonate
dNTPs	Deoxyribonucleoside triphosphates
DTT	Dithiothreitol
EST	Expressed sequence tags
GAPDH	Glyceraldehyde 3 phosphate dehydrogenase
GO	Gene ontology
g_s	Stomatal conductance
H. brasiliensis	*Hevea brasiliensis*
HP	Hypothetical protein
kb	Kilobase
LEA	Late Embryogenesis abundant
MAPK	Mitogen Activated Protein Kinase
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
NR	Natural rubber
NRG	Non responding gene
PCR	Polymerase Chain Reaction
PSII	Photosystem II
qPCR	Quantitative PCR
QTL	Quantitative Trait Loci

RNA	:	Ribonucleic acid
RNA Seq	:	RNA sequencing
ROS	:	Reactive oxygen species
RQ	:	Relative quantification
RRII	:	Rubber Research Institute of India
RRIM	:	Rubber Research Institute of Malaysia
SSR	:	Simple-Sequence Repeats
Tf	:	Transcription factor
TPD	:	Tapping Panel Dryness

Units

°C	:	degree Celsius
g	:	gram(s)
hr	:	hour(s)
Kb	:	kilobase
l	:	litre(s)
M	:	molar
min	:	minutes
mol	:	mole(s)
nM	:	nanomole
rpm	:	revolutions per minute

Prefixes

k	:	kilo
m	:	milli
μ	:	micro

Chapter 1

Introduction

1.1 *Hevea brasiliensis*

Hevea brasiliensis Muell-Arg, a native species of the Amazonian rainforest is the primary source of natural rubber (NR). NR is known to be produced by about 2500 plant species among which only a few species *viz.* *Hevea brasiliensis* (rubber tree), *Parthenium argentatum* (guayule) and *Taraxacum koksaghyz* (Russian dandelion) produce high quality NR (Gronover *et al.*, 2011). Among these species, *H. brasiliensis* is the major source of NR which accounts for about 99% of world's NR production (Cornish, 2001). The genus *Hevea* under the family Euphorbiaceae comprises eleven species of which *H. brasiliensis* is the most economically important species (Clement *et al.*, 2007; Priyadarshan and Goncalves, 2002; Pires *et al.*, 2002). *Hevea* is a tropical perennial tree species with a proposed amphidiploid origin stabilized during the course of evolution (Ong *et al.*, 1975; Lespinasse *et al.*, 2000).

NR which is a *cis*-1, 4-polyisoprene of high molecular weight polymer (molecular weight > 1 million daltons) made of repeating isoprene units in the *cis*-configuration, is produced in the milky cytoplasm (latex) of specialized cells called laticifers. NR synthesis is a typical plant secondary metabolism process in which NR is synthesized on the surface of rubber particles through sequential condensation of several isopentanyl diphosphate units suspended in the cytoplasm of laticifer cells (Cornish, 2001). The latex is extracted from the tree by a non-destructive method of harvesting by apping the bark systematically in regular intervals. NR is an industrial ommodity used in the manufacturing of over 50,000 products including nedical devices, heavy-duty tires and high-performance engineering omponents. The superior properties in terms of elasticity, flexibility, esilience and efficient heat dispersion exhibited by NR make it irreplaceable y synthetic rubber (Mooibroek and Cornish, 2000). Rubber trees are also sed as a source of timber, once their latex productivity is no longer conomically viable.

Hevea brasiliensis is a deciduous tree that grows up to a height of 30–40 m. After an immaturity period of 5–7 years, the trees will have a productive period of 25–30 years. Rubber is now widely cultivated in South East Asia, especially in Malaysia, Indonesia, India, Sri Lanka, Vietnam, China and Thailand. Rubber was promoted as a plantation agriculture crop in South East Asia to feed raw material for the rubber based industries located in Europe during late 19th Century (Thomas and Panikkar, 2000). Commercial cultivation of rubber in India was started in 1902 by establishing rubber cultivation in Kerala and Kanyakumari district of Tamil Nadu. Later the cultivation was expanded to parts of Karnataka, North Konkan region, Madhya Pradesh, Orissa, West Bengal and North Eastern region of India (Fig 1.1).

Growth and yield of rubber are optimal in its native region, owing to the warm, hot and humid climate. Due to the constraints in available cultivable land in the optimal traditional regions, the cultivation has been expanded to dryer and colder regions across the globe (Raj et al., 2005) such as South East Asian countries which includes North-Eastern regions of India, Vietnam, Southern China, etc. The unfavourable climate in such regions affects the overall growth and yield (Rao et al., 1998; Jacob et al., 1999).

Fig1.1.Rubber plantation

In India, rubber cultivation has been traditionally confined to the Kanyakumari District of Tamil Nadu and Kerala. This traditional rubber growing belt lies between 8° and 12°N latitudes. The traditional rubber growing tract has almost been saturated for rubber cultivation and the possibility for further expansion of the crop is much limited. In order to increase the production, rubber cultivation was expanded to newer non-traditional regions with diverse agro-climatic conditions. Attempts were also made to develop or identify clones that can withstand unfavorable climatic conditions in such areas.

The perennial nature, long immature period and a narrow genetic base were the major challenges for the genetic improvement of *Hevea brasiliensis* (Saha and Priyadarshan, 2012). Molecular work was initiated in the 1990s with the cloning and characterization of latex biosynthesis genes (Goyvaerts *et al.*, 1991; Chye *et al.*, 1991; 1992). Later, gene expression studies were taken up on various biotic and abiotic stresses, a physiological disorder called 'Tapping Panel Dryness' (TPD) and on ethylene stimulation (Saha *et al.*, 2010; Thomas *et al.*, 2011; 2012; Sathik *et al.*, 2011; 2012; Luke *et al.*, 2015). Many attempts were made to identify markers for early selection of clones with high biomass, improved yield along with disease and stress tolerance. In rubber, genetic markers were also developed to study the inheritance and diversity of natural variation prevailing among different genotypes. Attempts were also made to develop transgenic Hevea plants to over-express genes of interest (Sobha *et al.*, 2003; Jayashree *et al.*, 2003; Leclercq et *al.*, 2012; Rekha *et al.*, 2013).

Rubber tree breeding is time consuming and expensive owing to the long time and larger areas required for field evaluations to release a new genotype. With the introduction of molecular biological techniques, field evaluations could be optimized which, shortened the time and area required for these experiments. Substantial developments were also made in the field of microsatellite markers, EST-simple sequence repeats (EST-SSRs), linkage maps and gene expression profiles which led to the accumulation of enormous data (Besse *et al.*,1994; Low *et al.*, 1996; Varghese *et al.*, 1997; Lespinasse *et al.*, 2000; Roy *et al.*, 2004; Saha *et al.*, 2005; Le Guen *et al.*, 2011 ; Mantello *et al.*, 2012., Souza *et al.*, 2013; Silva *et al.*, 2014; Mantello *et al.*, 2014). Discovery of such molecular markers has made the processes like characterizing of various genotypes, identification of different cultivars and analyzing their genetic diversity, identification of QTL's and establishing their association with agricultural traits and identification of specific genes much easier. Draft genome sequence of the rubber tree was published by Rahman *et al.* (2013). Later, report of high quality genome analysis of *Hevea* was also made available (Tang *et al.*, 2016). High-throughput genomic techniques in conjunction with bioinformatics tools has started playing important role in breeding for clones best suited to different agro-climatic conditions (Saha and Priyadarshan, 2012).

Abiotic stresses significantly affect plant survival, growth and development and thus decrease yield and biomass production (Wang *et al.*, 2003; Mittler, 2006). Plants have developed intricate mechanisms for responding to the environmental changes (Wani *et al.*, 2016) and the responses to these abiotic stresses reflect at biochemical, physiological, cellular and molecular levels within a plant (Fernandez, 2014; Mathur *et al.*, 2014; Suzuki *et al.*, 2014). Diverse sets of signal transduction pathways are triggered and stress-responsive genes are activated as a response to stress and as a part of developing tolerance to short or long term stresses. A number of functional and regulatory genes related to abiotic stress tolerance have been identified and validated (Shinozaki *et al.*, 2003). Several important genes like those encoding transcription factors have been implicated in response to abiotic stresses. Over expression of these genes in model plant species, such as *Arabidopsis*, and other agriculturally important crops have resulted in significant improvement in tolerance to individual stresses (Ganesan *et al.*, 2012; Diaz-Vivancos *et al.*, 2013; Gong *et al.*, 2014., Li *et al.*, 2014; Tamirisa *et al.*, 2014).

Plants have the ability to dramatically alter their gene expression patterns in response to environmental changes. Sometimes these transcriptional changes successfully lead to tolerance whereas in other cases plants ultimately fail to adapt to the new environment and succumb to the adverse environmental condition. Expression profiling can define both tolerant and sensitive responses and helps to understand the response of organism to such environmental changes (Hazen *et al.*, 2003) and can reveal various gene regulatory networks involved in its stress response (Seki *et al.*, 2007). The pace of data collection on gene expression has been increased with the introduction of next generation sequencing methods. High throughput sequencing helps to generate functional genomic data and to discover differentially expressed genes in different cultivars, organs or different treatment conditions (Kakamanu *et al.*, 2012; Xu *et al.*, 2013; Zhang *et al.*, 2014).

Traditional plant breeding approaches for improving abiotic stress tolerance of crops had limited success owing to the multigenic and quantitative nature of stress tolerance (Flowers and Yeo, 1995; Collins *et al.*, 2008). Molecular techniques are also helpful to understand the mechanisms by which plants perceive environmental signals and eventually the way by which it is transmitted to cellular machinery to activate adaptive responses. This knowledge is critical for the development of rational breeding and to design transgenic strategies to develop stress tolerant crops.

1.2 Drought and cold stress responses in *Hevea*

Constraints in cultivable land, competition from other crops and ever increasing demand for NR led rubber cultivation to marginal and subtropical environments. In India, cultivation of *Hevea* is being extended to non-traditional regions with adverse climatic conditions which limit the growth, yield and performance of the crop. These regions include North Konkan where the summer is very severe and northeastern states where the temperature during winter is too low. The drought prone non-traditional regions experience soil and atmospheric drought, warmer atmospheric temperatures concomitant with high light and low relative humidity during extreme summer season, which affect the performance of the crop (Chandrasekhar *et al.*, 1990; Jacob *et al.*, 1999; Devakumar *et al.*, 1998). Several reports in *Hevea* confirmed the adverse effect of drought stress on yield and general performance of the crop (Sethuraj *et al.,* 1984; Huang and Pan, 1992; Sreelatha *et al.,* 2007; 2011). Gas exchange parameters have been reported to be severely inhibited under drought stress. The reports also indicated that clone RRIM 600 had lesser inhibition when compared to clone RRII 414 (Sumesh *et al.*, 2011). In rubber, few studies have reported expression and quantification of drought stress responsive transcripts which also indicated the existence of altered expression in different *Hevea* clones with varying levels of tolerance (Thomas *et al.*, 2011; 2012; Luke *et al.*, 2015).

In South Central China and northeastern states of India, low temperature stress (between 0 to 10°C) strongly affects the cultivation of rubber trees and latex production (Priyadarshan *et al.*, 2005). In addition to growth reduction, low temperature is responsible for loss in yield due to tapping rest for a period of 1-3 months every year in NE regions of India (Jacob *et al.*, 1999). Young rubber plants are more susceptible to abiotic stress conditions (Jacob *et al.*, 1999). Low temperature stress ~~in~~ concomitant with high light is reported to damage PS II and photosynthetic apparatus from its normal functioning (Annamalainathan *et al.*, 2010). Clonal variation in cold tolerance has been reported (Polhamus, 1962; Das *et al.*, 2013). Cold responses of different *Hevea* clones under controlled environmental conditions were also reported (Sarkar *et al.*, 2013). During winter, high light during day time combined with cold stress in the previous nights led to severe inhibition in photosynthesis and chlorophyll bleaching (Powles, 1984; Jacob *et al.*, 1999; Devakumar *et al.*, 2002; Ray *et al.*, 2004) and PSII activity as evidenced by the reductions in the maximum and the effective quantum yield of PSII (Sathik *et al.*, 1998b; Jacob *et al.*, 1999; Devakumar *et al.*, 2002). Under cold stress, differential gene expression was noticed in *Hevea* clones with varying levels of tolerance to cold stress (Sathik *et al.*, 2012).

1.3 Rationale of the study

In order to obtain maximum growth and yield, it is necessary to identify clones with better tolerance to withstand these suboptimal conditions. However, lack of suitable methods for early evaluation of cold and drought tolerance and the long time required for breeding programmes in rubber are challenging. In *Hevea*, breeding for drought and cold tolerant clones is progressing. Though there are few reports available in *Hevea* on differential gene expression, no information is available on gene expression among clones with varying levels of drought/cold tolerance. Similarly, abiotic stress responsive transcriptome sequencing data was not available in *Hevea*. Hence, it is imperative to carry out clone wise expression studies in order to identify genes or transcripts that are associated with drought or cold tolerance in *Hevea*.

The rationale of this study was to find out drought and cold responsive genes from *Hevea brasiliensis* and to further identify genes having stronger association with stress tolerance/susceptibility through transcriptome sequencing and quantitative PCR. This study was conceived with an objective to identify potential abiotic stress responsive genes which could eventually be employed by the breeders to either develop crops with improved stress tolerance or to use them as markers to screen clones or germplasm accessions for identifying abiotic stress tolerant genotypes.

1.4 Objectives

- Identification of drought/cold stress responsive genes contributing for stress tolerance in *Hevea brasiliensis*
- Selection of candidate genes based on their functions/role
- To employ them in screening for tolerant varieties or to use them in crop improvement programmes

Chapter 2

Review of Literature

2.1 *Hevea brasiliensis*

Hevea brasiliensis (the Para rubber tree), indigenous to Amazonian rain forests is a cross pollinated tree species belonging to the family Euphorbiaceae (Spurge) (Archer and Audley, 1987) and the genus *Hevea* comprises of 11 inter-crossable species (Clement-Demange *et al.*, 2007). For the optimum growth of rubber tree, a temperature range of 28-35°C, a high humidity and an annual rainfall of about 2000 - 4000 mm is required (Webster and Baulkwill, 1989; Jacob *et al.*, 1999; Priyadarshan *et al.*, 2005). Within the genus *Hevea*, *H. brasiliensis* is the most abundant tree species that can be cultivated profitably and is capable of producing large quantity of latex that makes up to 99% of the world's natural rubber production. From the place of its origin, the Amazon basin of South America, Sir Henry Wickham collected rubber seeds in the year 1876 and transported to Kew Gardens from where subsequently seedlings were transported to Sri Lanka and other South East Asian Countries (Chan, 2000). Rubber was introduced in India during 1902 in Nilambur, Kerala and was later extended to other parts of Kerala and Kanyakumari District of Tamil Nadu.

Cultivation of rubber tree had contributed much to the world's economy by its wonder product, natural rubber, which has revolutionized the modern world by its wide usage in almost every field. Natural rubber (*cis*-1, 4-polyisoprene) is produced in the cytoplasm of laticifer cells and this polymer is considered to be most important one among the polymers produced by the plants. Rubber comprises 94% of *cis*-1, 4- polyisoprene plus 6% of proteins and fatty acids (Sakdapipanich, 2007). Natural rubber exhibits properties like better flexibility, liquid impermeability and abrasion resistance which are unique and superior. Due to these remarkable properties, natural rubber is preferred over synthetic rubber for use in various applications (Cornish, 2001; Priyadarshan and Goncalves, 2003). Production of natural rubber is sustainable and environment friendly when compared to synthetic rubber (Jones, 1994). An increased demand for natural rubber in the international market has contributed to the extension of rubber cultivation to larger areas.

The climatic conditions prevailing in the Amazon region are optimum for the crop based on which rubber plantations were introduced in suboptimal regions of South East Asian countries which includes North-Eastern regions of India, Southern Brazil, Vietnam, Southern China, etc. (Pushparajah, 2001). *Hevea* is cultivated mostly in the South and South East Asian countries where the climatic conditions are favouring its growth and productivity. In India, Kerala and the Kanyakumari District of Tamil Nadu together constitute the traditional rubber growing regions and it constitutes about 85 % of the rubber cultivation. In order to meet the ever growing demand for natural rubber, it has become necessary to produce more rubber by increasing the productivity and extending its cultivation to non-traditional regions like North Eastern States, Goa, Maharashtra, Odisha, West Bengal and Andhra Pradesh. In the non- traditional regions or marginal zones with suboptimal conditions, rubber cultivation is often challenged by abiotic stress factors such as low temperatures and dry periods which limit the overall growth and productivity (Pushparajah, 1983; Jacob *et al.*, 1999; Priyadarshan and Goncalves, 2003).

In India, drought and high temperature in the North Konkan and chilling stress during winter in the North East are the two factors that limit the growth and productivity of *Hevea.* North Konkan region is characterized by the fast depletion of soil moisture coupled with high intensity of solar radiation, high temperature and very low atmospheric relative humidity (Chandrasekhar *et al.*, 1990). These environmental conditions inhibit the growth and productivity of *Hevea* (Sethuraj, 1986; Chandrashekar *et al.,* 1990; Bhaskar *et al.,* 1991; Mohanakrishna *et al,* 1991; Rao *et al.,* 1998). In *Hevea,* drought had been reported to cause growth retardation in seedlings and mature tapping trees, shortening of tapping period, reduced latex flow, lesser dry rubber contents and higher incidence of TPD (tapping panel dryness) and in certain cases tree death (Huang and Pan, 1992). Drought stress also affects yield and general performance of the crop (Buttery and Boatman, 1976; Sethuraj *et al.*, 1984; Sreelatha *et al.*, 2007; 2011). Biochemical investigations on antioxidant system like super oxide dismutase (SOD) and peroxidase, C-serum invertase activity, C-serum thiols, sucrose and ATP levels also confirmed the drought susceptibility in *Hevea* clones (Sreelatha *et al.*, 2007). Clonal variation in the level of tolerance to drought stress was also reported in *Hevea* (Chandrasekhar, 1997). The physiological parameters like CO_2 assimilation rate and stomatal conductance varied among different clones under drought stress (Annamalainathan *et al.*, 2010; Sumesh *et al.*, 2011). Parameters like transpiration coefficient (Nair *et al.*, 1996), membrane integrity (Reddy, 2000), osmoregulation, laticifer turgor pressure (Ranasinghe and Milburn, 1995) and low solute potential (Ayutthaya *et al.*, 2011) were also found related to drought tolerance in rubber tree.

Low temperature is responsible for loss in yield due to tapping rest for a period of 1-3 months every year in NE regions of India along with growth reduction (Jacob *et al.*, 1999). Clonal difference in low temperature tolerance has been reported in *Hevea* based on membrane stability (Sathik *et al.*, 1998a). Cold damage to rubber trees is a complex phenomenon as it involves differential response of clones, age and vigour of the plant. Higher levels of antioxidant enzymes such as superoxide dismutase, peroxidase, ascorbate peroxidase, catalase and antioxidants like glutathione found in the cold exposed leaves indicated the occurrence of oxidative stress (Devakumar *et al.*, 2002). Repeated and prolonged exposure to low temperature, particularly in concomitant with high sun light intensity damages the photosynthetic machinery as a result of oxidative stress eventually triggering senescence in the leaves (McKersie and Leshem, 1994). Exposure to abiotic stress affects latex production and overall tree productivity (Chen *et al.*, 2003). Photosynthesis is one of the first physiological processes that are inhibited when plants are exposed to abiotic stresses such as drought and chilling (Baker, 1996). Drought and cold stress inhibited the quantum yield for CO_2 assimilation in *Hevea* (Jacob *et al.*, 1999).

2.2 Abiotic stress in plants

Abiotic stress refers to numerous stresses on living organisms caused by non living environmental factors such as strong light, temperatures extremes, freezing, drought, salinity and heavy metals, i.e. abiotic factors or stressors from the environment imposing stress on organisms. In 1982, Boyer indicated that environmental factors may limit crop production by as much as 70% (Boyer, 1982). Abiotic stress leads to a series of changes at the morphological, physiological, biochemical and molecular levels adversely affecting plant growth and productivity (Bartels and Sunkar, 2005). Plants have to respond to these changing environmental attributes in order to mitigate the effects and have evolved adaptive mechanisms to attain stress tolerance. The plants evolve adaptive strategies which vary among different species based on the nature, severity and duration of the stress as well as the parts being exposed to the stress. Plant's response to abiotic stresses is complex and their responses ranges from changes in patterns of gene expression and cellular metabolism, growth and yield (Bray *et al.*, 2000). Tolerance or susceptibility of a plant to the stresses is a complex event as stress always affects the plants in concomitant with other stresses which affect different stages of development (Chinnusamy *et al.*, 2004). Plant's capacity to sustain under adverse environmental conditions depends on its intrinsic ability to effect distinct or a combination of modifications in it (Farooq *et al.*, 2009).

2.2.1. Drought stress

Drought or water deficit is an important environmental constraint limiting plant growth, development and productivity (Deikman *et al.*, 2012) and is the interplay of various factors like reduced rainfall, decreasing ground water table and limiting water availability with rise in temperature (Singh and Laxmi, 2015; Singh *et al.*, 2015). It brings about drastic changes in the overall functioning of the plant system at the physiological, biochemical and molecular levels. In order to alleviate the effects of drought stress, plants adopt two major strategies; drought avoidance and drought tolerance (Levitt, 1980). Drought avoidance helps the plants to overcome the negative impact of drought stress by exhibiting certain adaptive mechanisms like improved root traits, reduced leaf area, reduced epidermal conductance, *etc.* Drought tolerance is the inherent ability of the plant to survive, grow and reproduce under water deficit conditions (Turner, 1979) which is a quantitative and complex trait (McWilliam, 1989).

The response of any crop to drought stress largely depends on its stage of growth and water use efficiency (WUE) (Pareek *et al.*, 2010). Plant's capacity to survive with water deficit also depends on its water status which fluctuates with the changing environmental conditions (Joshi *et al.,* 2016). Plants initially respond to the water deficit stress by closing stomata that result in reduced stomatal conductance, CO_2 uptake and transpiration, which further ends up with reduction in photosynthetic activity. Several studies indicate the inhibition of photosynthesis and energy dissipation under drought stress in many species (Zhou *et al.*, 2007; Zivcak *et al.*, 2014). Plants also respond to drought stress by altering the membrane structures, cell walls and whole organs, in addition to accumulation of compatible solutes such as prolines, soluble sugars, spermines and betaine which act as osmoprotectants. Apart from this, plants also synthesize detoxifying enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) as a response to drought stress. Upon prolonged dehydration, rolling of leaves occurs followed by wilting and bleaching (Sahoo *et al.*, 2013). Drought also leads to increase in cellular temperature which along with accumulation of ROS leads to increase in viscosity of cellular components, modifications in protein interactions, aggregation of proteins and ultimately disintegration of cells and tissues (Farooq *et al.*, 2008).

Drought tolerance is a complicated process that involves a series of molecular, cellular and physiological processes. Under water deficit conditions, the presence of abscisic acid (ABA) causes stomatal closure and induces expression of various stress-related genes. At the molecular level, there occur changes in the expression of numerous genes, such as those related to stress signal transduction and the transcription and regulation of thousands of functional proteins altogether contributing to the molecular control of drought resistance. Drought inducible gene expression occurs either by ABA-dependent or ABA independent regulatory system (Yang *et al.*, 2011; Aguado *et al.*, 2014). For engineering drought stress tolerance in plants, it is essential to sort out the molecular mechanisms underlying the stress responses (Ray *et al.*, 2010; Sanchez *et al.*, 2011). To a great extent, developing drought tolerant cultivars using conventional breeding approach is constrained by the low heritability of drought tolerance, complex and multigenic nature of the trait (Pardo, 2010; Liu *et al.*, 2014). Under changing climatic conditions, incorporating drought stress tolerance in plants is imperative and it is important to develop genotypes with better stress tolerance and adaptability through simple, cost-effective and eco-friendly approaches (Jha *et al.*, 2014).

2.2.2. Cold stress

Apart from drought stress, cold stress is also a major environmental constraint limiting plant growth, productivity and causes significant crop losses (Thomashow, 1999). Plants have evolved mechanisms to enhance tolerance to freezing during exposure to periods of low, but non-freezing temperatures. Plants in the temperate regions adopt 'cold acclimation', during which the plants adjust their metabolism to cold temperature and acquire better freezing tolerance after being exposed to nonfreezing cold temperatures. In contrast, plants of tropical and subtropical origins are intolerant to chilling and freezing temperatures. Plants have evolved various protective mechanisms to minimize potential damages caused by low temperature. Plants have various ways by which they respond to and tolerate cold stress. These include changes in the composition, structure, and function of the plasma membrane, transient changes in Ca^{2+} influx, accumulation of cryoprotectant molecules and an increase in the scavenging activity of reactive oxygen species (Steponkus, 1984; Apel and Hirt, 2004; Wang *et al.*, 2013).

Most cold stress responses occur through regulation of cold responsive (COR) gene expression through several transcription actors (Chinnusamy *et al.*, 2007). The transcriptional regulation of cold responsive gene expression is complex. ICE-CBF pathway (inducer of CBF expression

1/C-repeat/drought-responsive element binding factor) is a well documented pathway for gene regulation under cold conditions in many species and the genes that are regulated by these pathways are collectively identified as *cor* genes and is conserved in diverse plant species. This transcriptional cascade involve a class of DREB/CBF tfs (dehydration-responsive-element-binding/C-repeat binding factors) which specifically bind to the DRE/CRT (dehydration-responsive-element/C-repeat) *cis*-elements in the promoter regions of target genes like molecular chaperones and LEA proteins and activate them (Thomashow, 1999; Maruyama *et al.*, 2009). These genes itself are upstream regulated by several proteins such as ICE1, MYB15 and ZAT12 (Shinozaki *et al.*, 2003; Maruyama *et al.*, 2009), CAMTA and HOS1 (Miura *et al.*, 2007; Doherty *et al.*, 2007; Dong *et al.*, 2006). Cold response is a very complex trait involving many different metabolic pathways, gene regulations and cell compartments (Hannah *et al.*, 2005).

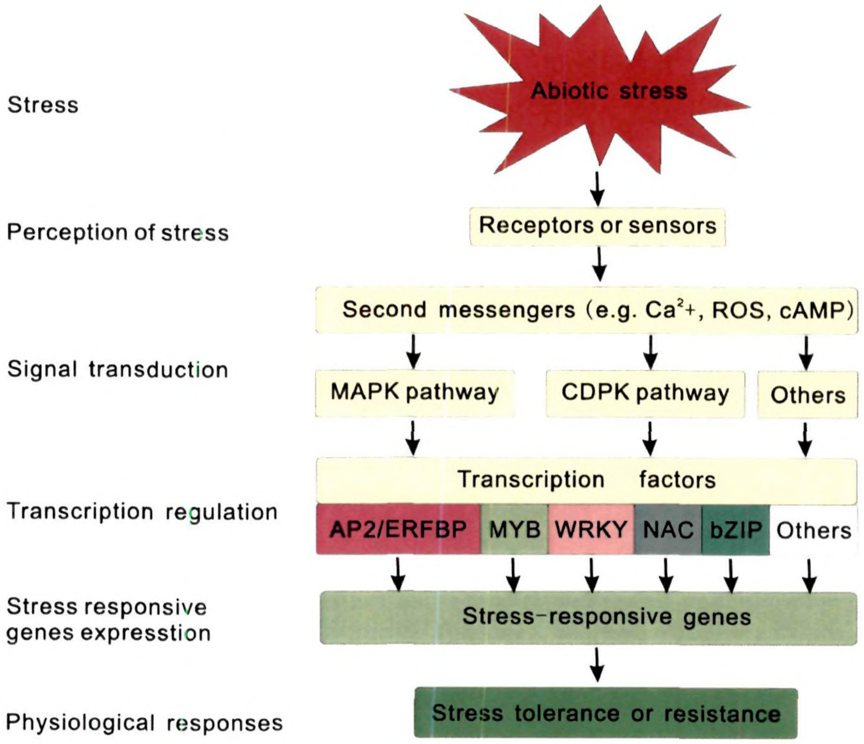
2.3 Molecular responses under drought and cold stresses

Plants have evolved complex and intricate mechanisms to withstand and survive under changing environmental conditions. These mechanisms include signal perception and transduction, transcriptional activation of stress responsive genes and synthesis of stress-related proteins and other molecules, which assist plants to cope with adverse environmental conditions through biochemical and physiological manifestations. Molecular responses to abiotic stress on the other hand include perception, signal transduction, gene expression and ultimately metabolic changes in the plant thus providing stress tolerance (Agarwal *et al.*, 2006). In order to identify the genes and pathways with regard to the development of stress-tolerant crop plants, it is essential to study the molecular mechanisms by which the plants perceive and transduce the stress signals to cellular components for adaptive responses (Heidarvand and Amiri, 2010; Sanchez *et al.*, 2011).

Although abiotic stresses are different in nature, they are known to activate certain common sets of genes. At the molecular level this involves induction of stress responsive and tolerance genes often mediated by the phytohormone abscisic acid (Matsui *et al.*, 2008). ABA, the plant stress hormone, in addition to its role in plant development, plays significant roles in responses to abiotic stress factors like regulation of stomatal closure and activation of many stress-related genes (Finkelstein *et al.*, 2002; Cutler *et al.*, 2010). Till now, a large number of abiotic stress tolerance genes have been identified and validated which have been grouped under two types of genes such as functional genes and regulatory genes (Shinozaki *et al.*, 2003). The functional group of genes encode detoxification enzymes and stress tolerant proteins such as water channel protein like aquaporin, heat shock proteins (HSPs), osmoprotectant proteins like LEA proteins, etc. These proteins are directly involved in protecting the cells from stress effects. The second group comprises of regulatory genes involves various transcription factors, protein kinases and protein phosphatases which are directly involved in regulating the signal transduction and trigger a set of specific genes as a response to particular stress (Shinozaki and Yamaguchi-Shinozaki, 1997; Seki *et al.*, 2003).

Over the course of evolution, plants have developed short and long term tolerance mechanisms to protect themselves through diverse sets of signal transduction pathways and through expression of several stress responsive genes. Signal transduction of abiotic stress starts with perception of abiotic signals primarily which are later transduced via a set of chemicals eventually triggering the stress responsive gene expression along with the activation of physiological and metabolic responses (Chaves *et al.*, 2003; Perez-Clemente *et al.*, 2013). Signals are perceived by sensors or receptors located in the cell wall or membrane which are later carried on through the secondary messengers such as calcium ions, inositol phosphate, reactive oxygen species, cyclic nucleotides nd nitric oxide into the intracellular signalling pathway that induces specific ene expression (Chaves *et al.*, 2009; Bhargava and Sawant, 2013).

The protein kinases and phosphatases mediated phosphorylation-ephosphorylation is a significant mechanism in signal transduction athways. Mainly two signal transduction pathways are involved in abiotic tress responses in plants, one is mitogen activated protein kinase (MAPK) ediated pathway and the other is calcium dependent protein kinase (CDPK) ediated pathway. These signal transduction pathways finally ends up in anscriptional activation or repression of stress responsive genes thereby ontributing for the stress tolerance (Danquah *et al.*, 2014).



eneric signaling pathway involved in plant abiotic stress responses (Wang *et al.*, 2016)

Protein kinases play an important role in responses to environmental hanges and signal transduction in plants. The expression of calcium-ependent and calmodulin dependent protein kinase (CDPK) genes, including *NtCDPK4, OsCPK6, OsCPK13, OsCPK17* and *OsCPK25* increases under drought, high salt or low temperature stress. *AtMPK4* and *AtMPK6* are activated by cold stress. *Arabidopsis MAPKK, AtMKK2*, also got up-regulated in response to cold stress. Mitogen-activated protein kinase (MAPK) mediated signal transduction cascades have provided another pivotal understanding of the integration of physiological and cellular responses to environmental adversity. Involvement of MAPKs in drought and salt adaptation has been reported in wide ranges of plant species (Ning *et al.*, 2010; Yu *et al.*, 2010). *Arabidopsis MKK4* plays a central role in the osmotic stress response by regulation of MPK3 activity, accumulation of ROS and targeting the ABA biosynthetic process (Kim *et al.*, 2011). Abiotic stress induced ROS accumulation under control of *MPK6, MKK1* and *MKKK20* was reported (Xing *et al.*, 2008; Kim *et al.*, 2012). It has been reported that MAPKs and CDPKs can be activated in drought and other abiotic stresses as a link between sensor and pathway targets. In *Arabidopsis thaliana*, *CPK10* participates in abscisic acid (ABA) and Ca^{2+} -mediated stomatal regulation in response to drought stress (Zhu *et al.*, 2007). In *Oryza sativa*, transgenic plants over expressing *OsCDPK7/OsCPK13* showed enhanced resistance to cold, drought and salt stress (Saijo *et al.*, 2000). *OsCDPK13/ OsCPK7* and *OsCPK21* are involved in responses to cold and salt stress, respectively (Asano *et al.*, 2011). In *Nicotiana tabacum*, two CDPK genes, *NtCDPK2* and *NtCDPK3* play important role in the defense response and *NtCDPK2* functions together with the stress-induced MAPKs (mitogen-activated protein kinases) to control response specificity to abiotic and biotic stresses (Ludwig *et al.*, 2005; Romeis *et al.*, 2005).

2.3.1. Transcription factors in stress responses

Transcriptional regulation is a major stress response mechanism in plants and plant genomes assign approximately 7% of their coding sequence to transcription factors(Tfs), which prove the complexity of transcriptional regulation (Udvardi *et al.*, 2007). Some of these Tfs are master regulators of signaling and regulatory pathways of stress adaptation, and genetic engineering of one or a few of these may be sufficient to enhance stress tolerance in plants, making these tfs attractive targets for engineering (Wang *et al* ., 2016). Transcription factors bind to specific *cis*-elements in the promoter region and regulate the expression of downstream target genes (Yamaguchi-Shinozaki and Shinozaki, 2006; Agarwal and Jha, 2010).

NAC tfs have diversified roles in plant development and growth (Tran *et al.*, 2010; Nakashima *et al.*, 2012; Nuruzzaman *et al.*, 2013). NAC family may contribute to ABA- dependent gene expression under various stresses, including cold (Nakashima *et al.*, 2007; 2012). In *Arabidopsis*, *ANAC019*, *ANAC055*, and *RD26 (ANAC072)* were induced by drought, high salinity, ABA and they bind to the promoter of *early response to dehydration stress 1* (*ERD1*) and over expression of these three NAC tfs conferred improved drought tolerance by up-regulation of several stress-inducible genes in transgenic plants (Tran *et al.*, 2004). Over expression of *ANAC019* displayed enhanced tolerance to cold (Jensen *et al.*, 2010) and *MLNAC5* enhanced tolerance to drought and cold (Yang *et al.*, 2015) in transgenic *Arabidopsis*.

MYB tfs are reported to be involved in various abiotic stresses. In *Arabidopsis AtMYB60* and *AtMYB61* enhanced drought tolerance by the regulation of stomatal opening (Cominelli *et al.*, 2005; Liang *et al.*, 2005). *AtMYB96* modulates ABA and auxin signals in response to drought stress and confer improved tolerance (Seo *et al.*, 2009). Transgenic plants over expressing *OsMYB 4* improved drought and cold tolerance in *Arabidopsis*, tomato and apple (Vannini *et al.*, 2004 ; 2007; Pasquali *et al.*, 2008).

WRKY tfs, another family of transcription factors play important roles in abiotic stress responses in plants (Chen *et al.*, 2012; Rushton *et al.*, 2012). Over expression of *OsWRKY11 gene* in rice displayed significant tolerance to heat and drought stress (Wu *et al.*, 2009). Over expression of *GmWRKY21* gene exhibited improved tolerance to cold stress and *GmWRKY54* gene enhanced tolerance to drought and salt stress in *Arabidopsis* (Zhou *et al.*, 2008). Over expression of *TaWRKY79* resulted in enhanced drought tolerance in *Arabidopsis* (Qin *et al.*, 2013). WRKY-type transcription factor *ThWRKY4* from *Tamarix hispida* controls cellular accumulation of ROS via regulating expression and activity of antioxidant genes such as superoxide dismutase and peroxidase (Zheng *et al.*, 2013).

AP2/ERF family of plant-specific tfs are characterized by a highly conserved DNA-binding domain which interact with DRE/CRT *cis* acting elements in the promoter region of downstream target genes (Riechmann and Meyerowitz,1998). Over-expression of *DREB1s/CBFs* displayed significantly improved tolerance to freezing, drought and high salinity in transgenic *Arabidopsis* (Gilmour *et al.*, 1998; Jaglo- Ottosen *et al.*, 1998), increased freezing tolerance in oil seed rape (Jaglo *et al.*, 2001) and chilling tolerance in tomato, tobacco and rice (Kasuga *et al.*, 2004; Ito *et al.*, 2006). Over expression of *OsERF4a* in rice displayed enhanced drought tolerance (Joo *et al.*, 2013) and *TaPIE1* in wheat showed enhanced cold tolerance (Zhu *et al.*, 2014).

Table 2.1. Reports on transgenic plants over-expressing transcription factor genes (adopted from Wang *et al.*, 2016)

Family	Gene	Transgenic Plants	Increased tolerance	References
AP2/ERF	*OsERF4a*	Rice	Drought	Joo *et al.*, 2013
	AtDREB1A	Rice	Drought	Ravikumar *et al.*, 2014
	TaPIE1	Wheat	Cold	Zhu *et al.*, 2014
MYB	*GmMYBJ1*	*Arabidopsis*	Drought and cold	Su *et al.*, 2014
	TaMYB3R1	*Arabidopsis*	Drought and salinity	Cai *et al.*, 2015
	TaPIMP1	Tobacco	Drought and salinity	Liu *et al.*, 2011
	LeAN2	Tobacco	Chilling and oxidative stresses	Meng *et al.*, 2014
	OsMYB2	Rice	Drought, cold and salinity	Yang *et al.*, 2012
	MdSIMYB1	Apple	Drought, cold and salinity	Wang *et al.*, 2014
WRKY	*TaWRKY79*	*Arabidopsis*	Drought	Qin *et al.*, 2013
	VvWRKY11	*Arabidopsis*	Drought	Liu *et al.*, 2011a
	GsWRKY20	*Arabidopsis*	Drought	Luo et al., 2013
	BdWRKY36	Tobacco	Drought	Sun *et al.*, 2015
	ZmWRKY58	Rice	Drought and salinity	Cai *et al.*, 2014
	MtWRKY76	*Medicago truncatula*	Drought and salinity	Liu *et al.*, 2016
NAC	*ANAC019*	*Arabidopsis*	Cold	Jensen *et al.*, 2010
	TaNAC67	*Arabidopsis*	Cold, salinity and drought	Mao *et al.*, 2014
	MLNAC5	*Arabidopsis*	Drought and cold	Yang *et al.*, 2015
	TaNAC2a	Tobacco	Drought	Tang *et al.*, 2012
	AhNAC3	Tobacco	Drought	Liu *et al.*, 2013
	SNAC1	Wheat	Drought and salinity	Saad *et al.*, 2013
	OsNAP	Rice	Cold, salinity and drought	Chen *et al.*, 2014

2.4 Recent advances in gene expression studies

Significant progress has been made on molecular mechanisms of response to abiotic stress in plants through high throughput sequencing approach and functional genomics tools. Next-generation sequencing (NGS) technologies, such as Illumina/Solexa (San Diego, CA, USA), the SOLiD platform from Applied Biosystems, and Roche 454 sequencing, have revolutionized genomics because they allow faster and less expensive sequencing. These methods are employed to characterize genes, detect gene expression pattern and level, recognize and quantify rare transcripts without prior information of the particular gene or reference genome, and to generate information on alternative splicing and sequence variations (Strickler *et al.*, 2012; Jia *et al.*, 2015). RNA-Seq has been successfully used to identify abiotic stress response mechanism in many plant species. This powerful high throughput sequencing technology has been widely applied to transcriptome sequencing by which functional genomic data can be generated and differentially expressed genes (DEGs) among different cultivars, organs and different treatment conditions can be discovered (Li *et al.*, 2012; Bharadwaj *et al.*, 2013; Wu *et al.*, 2014).

When RNA seq transcriptome analysis was carried out to compare drought stressed and well watered leaf meristem and pollinated ovaries of maize using Illumina deep sequencing, higher levels of expression of drought responsive genes were observed in ovary when compared to leaf meristem (Kakumanu *et al.*, 2012). Xu *et al.* (2013) performed transcriptome sequencing in *Chrysanthemum* under dehydration stress by Illumina sequencing technology and identified 8558 dehydration responsive unique transcripts including many transcription factors, protein kinases and stress responsive genes. Gene expression profiling by high throughput Illumina sequencing of soya bean leaves and roots under different stress conditions like salt, saline-alkali and drought stress identified a total of 1415 and 480 genes differentially expressed in roots and leaves respectively and among these genes 69 were co- regulated in roots and leaves (Fan *et al.*, 2013).

Transcriptome analysis in two contrasting genotypes (sensitive and tolerant) of red clover for drought response revealed that drought sensitive plants had approximately twice the number of differentially expressed transcripts than the tolerant plants (Yates *et al.*, 2014). Zhang *et al.* (2014) analysed differences in the transcriptomes of the transgenic (multiple resistance genes) and non-transgenic poplar lines using high throughput sequencing (Illumina Hiseq 2000) and reported the occurrence of transcriptome reprogramming in the transgenic poplar when compared with the non-transgenic line. *De novo* transcriptome sequencing (Illumina GA IIx) performed in *Brassica juncea* seedlings revealed differential regulation of 19110 transcripts by high temperature and/or drought stress compared to control sample and of these, 92 and 72 transcription factors were found altered in response to high temperature and drought stress respectively (Bhardwaj *et al.*, 2015). Ma *et al.* (2015) when analyzed transcriptome sequencing data of root tissues in the desert plant *Cynanchum komarovii* using Illumina sequencing technology found differential expression of genes encoding regulatory and functional proteins under drought stress. Transcriptome sequencing analysis made in *Abies alba* (Behringer *et al.* 2015) revealed 296 drought responsive genes (247 up and 49 down regulated). Transcriptome profiling of the potato under water deficit stress and re-watering treatment conditions carried out by high throughput Illumina sequencing (Gong *et al.*, 2015) revealed a total of 3189, 1797 and 4230 differentially expressed genes in control, drought treated and re-watered plants respectively.

Transcriptome sequencing in *Lilium lancifolium* facilitated the identification of cold regulated genes and transcriptional networks and signal transduction genes such as *LlICE* and *LlCDPK* and transcription factor genes such as *LlDREB1/CBF, LlAP2/EREBP, LlNAC1, LlR2R3-MYB* and *LlBZIP* (Wang *et al.*, 2014). *De novo* transcriptome sequencing of cold stressed *Phlox subulata* revealed the expression of twenty major cold-related genes, including transcription factors, antioxidant enzymes, osmoregulation proteins, and Ca^{2+} and ABA signaling components (Qu *et al.*, 2015). Tan *et al.* (2016) analyzed the global transcriptome of two asparagus bean cultivars; Dubai bean and Ningjiang-3 that vary in their cold tolerance under level and could identify 3510 and 2868 differentially expressed genes from two cultivars, respectively. *De novo* transcriptome assembly of *Ocimum americanum var. pilosum* grown under normal and low temperatures revealed cold temperature significantly affects genes related to protein translation and cellular metabolism (Zhan *et al.*, 2016). Calzadilla *et al.* (2016) identified differentially expressed genes in *Lotus japonicus* under cold treatment and they identified 41 cold-inducible transcription factors including members of the *AP2/ERF, NAC, MYB, and WRKY* families.

2.5 Development of molecular markers in *Hevea*

Conventional genetic analysis in *Hevea* is difficult because of its perennial nature, long breeding and selection cycles and difficulties in raising F_2 progenies. Various molecular markers were developed for *Hevea* to study the genetic diversity like isozymes, restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), microsatellites, *etc.* RAPD, RFLP and microsatellite markers have been reported in *Hevea* for the clonal identification and studies of genetic variability (Varghese *et al.*, 1997; Luo *et al.*, 1995; Venkatachalam *et al.*, 2002; Roy *et al.*, 2004; Besse *et al.*, 1994; Saha *et al.*, 2005). Identification of ESTs from *H. brasiliensis* had been going on for more than 10 years with regard to various stress and latex biosynthesis related cDNA libraries. Ko *et al.* (2003) analyzed more than 20,000 CDNA –AFLP based transcription fragments and 1176 ESTs from latex transcriptome. Chow *et al.* (2007) sequenced 10,040 ESTs and obtained 3441 unique transcripts. Large-scale EST studies have only been initiated in the last few years which led to the development of EST-derived SSR markers for rubber (Feng *et al.*, 2008; Xia *et al.,* 2011; Triwitayakorn *et al.*, 2011; Pootakham *et al.*, 2011; Li *et al.*, 2012). Development of genomic microsatellite markers (Mantello *et al.*, 2012) and linkage maps were also reported in *Hevea* (Lespinasse *et al.*, 2000; Souza *et al.*, 2013).

2.6 Transgenics of *Hevea*

In order to develop clones with greater adaptability to the stress conditions, rubber breeding programs were initiated (Priyadarshan and Goncalves, 2003). Though traditional breeding methods are productive, they are time-consuming, laborious and expensive (Goncalves and Fontes, 2012). These challenges can be circumvented through the interventions of molecular biological approaches. The first transformation by *Agrobacterium* mediated gene transfer was reported in *Hevea* by Arokiaraj and Wan (1991). Later transgenic plants were developed from anther derived calli (Arokiaraj *et al.*, 1996; 1998). Blanc *et al.* (2005) developed transgenic plants of *H. brasiliensis* PB 260 through *Agrobacterium* mediated transformation. Drought tolerant transgenic plants of *Hevea* also were developed with SOD gene placed under the control of CaMV 35S and FMV 34S promoters (Jayasree *et al.*, 2003; Sobha *et al.*, 2003). Physiological and molecular analysis made under drought condition revealed enhanced SOD expression and significant reduction in oxidative stress in the transgenic plants compared with control plants (Jayashree *et al.*, 2011). The recovery of transgenic plants upon re-watering following the exposure to drought was found better (Sumesh *et al.*, 2014). Transgenic plants of *Hevea* transformed with gene coding for 3-hydroxy-3-methylglutaryl-coenzymeA reductase 1 (*hmgr1*) were developed and established. Similarly, transgenic plants integrated with osmotin protein were also developed to impart abiotic as well as biotic stress tolerance in *Hevea brasiliensis* (Rekha *et al.*, 2013). Leclercq *et al.* (2012) developed *H. brasiliensis* plants integrated with a cytosolic isoform of the CuZnSOD gene from *H. brasiliensis* which could alter its response to water deficit. Transcript abundance and proline content were also found at higher levels in the transgenic plants upon induction of water stress. In their study, SOD transgenic plants were found surviving better under water deficit conditions. Attempts were also made to produce foreign proteins in the latex of *Hevea.* For e.g. Human serum albumin protein was expressed in transgenic *Hevea* plants (Arokiaraj *et al.*, 2002). Priya *et al.* (2006) characterized promoter sequence of rubber elongation factor gene by transgenic approaches. Attempts were also made to develop antibiotic marker-free *Hevea* transgenic plants (Leclercq *et al.*, 2010). Sunderasan *et al.* (2012) developed transgenic *Hevea* plants integrated with a gene encoding human atrial natriuretic factor (HANF), a peptide hormone regulating cardiac blood pressure. HANF transcripts were detected in the leaf samples and the expression of recombinant protein was detected by western immunoblot. Genetic transformation in *Hevea* has undergone great advancements and is progressing rapidly. However, large scale discovery and validation of functional genes are necessary.

2.7 Molecular studies in *Hevea*

Kush *et al.* (1990) reported laticifer-specific genes induced by ethylene in *H. brasiliensis.* Miao and Gaynor (1993) isolated *MnSOD* gene, which was found to express in all tissues, *i.e.* leaf, petiole, root, latex and callus and at higher levels in younger leaves. Thanseem *et al.* (2003; 2005) cloned and characterized b -1, 3-glucanase gene from various clones of *Hevea*. Priya *et al.* (2006) characterized promoter sequence of *REF* (rubber elongation factor) gene from *Hevea*. A full-length cDNA encoding cysteine protease, *HbCP1* was isolated from *Hevea brasiliensis* (Peng *et al.*, 2008). Cloning and characterization of full-length aquaporin cDNAs HbPIP2*; 1* and *HbTIP1; 1* (for plasma membrane intrinsic protein and tonoplast intrinsic protein respectively) from *Hevea* were also reported (Tungngoen *et al.*, 2009). cDNA encoding coronatine-insensitive 1 protein (*HbCOI1*) from *Hevea* was cloned by Peng *et al.*(2009). Genes that are differentially expressed during cold acclimation in *Hevea* were reported and genes like carbonic anhydrase, glutathione peroxidase, metallothionein, chloroplastic Cu/Zn SOD, serine/threonine protein kinase, transcription factor, DNA-binding protein, *etc.* showed significant increase in expression levels (Saha *et al.*, 2010). Tang *et al.* (2010) functionally characterized, *HbSUT3,* a sucrose transporter from *Hevea*. Expression analysis of various stress related transcripts under drought stress in different *Hevea* clones was carried out by Thomas *et al.* (2012). Liang *et al.* (2009) reported the constitutive expression of a translationally controlled tumour protein, *HbTCTP* in latex, leaves and barks upon ethylene (ET) treatment. Deng *et al.* (2012) further analyzed the gene structure and developed molecular markers of *HbTCTP*. Li *et al.* (2013) cloned *HbTCTP1*, another *TCTP* gene in *Hevea*. *HbTCTP1* was found expressed throughout different tissues and developmental stages of leaves and was found regulated by drought, low temperature, high salt, ethylene stimulation, wounding, H_2O_2, and methyl jasmonate. Further, Deng *et al.* (2016) elucidated functional characterization and expression profile of *HbTCTP*.

Decreased expression of *HbMyb1* in bark and latex of tapping panel dryness (TPD) affected rubber trees were reported (Chen et *al.*, 2003).

Venkatachalam *et al.* (2007) identified genes associated with TPD by suppression subtractive hybridization (SSH). Later, *HbTOM20* associated with TPD was identified by mRNA differential display (Venkatachalam *et al.*, 2009). Genes involved in TPD from *Hevea* latex were identified using suppression subtractive hybridization (Li *et al.*, 2010). In *Hevea*, genes related to ethylene biosynthesis and signalling also have been identified (Kuswanhadi *et al.*, 2010; Piyatrakul *et al.*, 2012; Duan *et al.*, 2013). Three MADS-box genes from *Hevea brasiliensis, HbMADS1, HbMADS2 and HbMADS3* were cloned and characterized by Li *et al.* (2011). Identification of DNA methylation patterns and their putative relationship with abiotic stress in *H. brasiliensis* was reported by Uthup *et al.* (2011). Gene expression analysis in *Hevea* clones revealed association of genes *viz LEA 5* protein, peroxidase, *ETR1, ETR2* and *NAC* transcription factor with cold tolerance (Sathik *et al.*, 2012). Function of sucrose synthase gene family in stress response and sucrose utilization in the laticifer cells was discussed by Xiao *et al.* (2013). Significant up-regulation of genes related to energy biosynthesis and ROS scavenging systems including *HbCuZnSOD, HbMnSOD, HbAPX, HbCAT, HbCOA, HbATP*, and *HbACAT* under drought stress was reported (Wang, 2014). Differential gene expression in *Hevea* clones with varying levels of drought tolerance was reported by Luke *et al.* (2015). Functional characterization of CRT/DRE Binding Factor 1 (*HbCBF1*) gene in *H. brasiliensis* was carried out by Cheng *et al.* (2015) while transgenic *Arabidopsis* over expressing *HbCBF1* was found having enhanced cold resistance. Identification of nine *ACS* (1-aminocyclopropane-1-carboxylic acid synthase) like genes and their differential expression in different tissues of *Hevea* was reported by Zhu *et al.* (2015). *ErbB-3 binding protein 1* (*EBP1*) which undergoes changes in its expression in response to cold, drought stress and ABA treatment was identified and characterized from *Hevea* and its over-expression in *Arabidopsis* enhanced resistance to freezing and drought stress (Cheng *et al.,* 2016). Identification and characterization of the glucose-6-phosphate dehydrogenase gene family in *Hevea* was carried out by Long *et al*., (2016) and identified four *HbG6PDH*s responding to temperature and drought stresses in root, bark, and leaves, thereby implicating their roles in maintaining redox balance and defense against oxidative stress.

2.8 Transcriptome sequencing in *Hevea*

Recent high-throughput sequencing technology and various genomic tools have facilitated the data acquisitions in several crops in general and rubber in particular over the years till date (Table 2.2). The recent development in the field of bioinformatics also added upon the capacity to extract informative and precision data from high-throughput genomic sequencing. These developments assume much importance in rubber tree breeding which can eventually facilitate developing superior clones that are best suited to different agro-climatic conditions. Recent technological developments made RNA sequencing a cost effective tool in gene expression profiling while providing qualitative and quantitative information at whole genome level compared to other conventional methods.

Rahman *et al.* (2013) reported the first draft genome of *Hevea* and it was a major boom in the transcriptomic studies related to natural rubber. cDNA libraries of cold-stressed clones and different tissues from *Hevea* were constructed for the development of EST-SSR and SNP markers (Silva *et al*., 2014). Salgado *et al.* (2014) reported *H. brasiliensis* transcriptome, covering a wide range of tissues and organs, leading to the production of the first developed SNP markers. Transcriptome sequencing and SNP marker development for rubber biosynthesis pathways were reported by Mantello *et al.,* (2014). ATP binding cassette proteins (ABC proteins) in the laticifers of *H. brasiliensis* was first identified by sequencing the latex transcriptome and its expression patterns were analyzed using real-time quantitative reverse transcript-polymerase chain reaction (RT-qPCR) (Zhiyi *et al.*, 2015). Comparative transcriptome analysis of healthy and TPD affected rubber trees were carried out and genes involved in rubber biosynthesis and jasmonate synthesis were found differentially expressed (Liu *et al.*, 2015). Comparative analysis of latex transcriptome between rubber tree clone PR107 (low yielder) and CATAS8-79 (high yielder) was performed to uncover the molecular mechanism for the regulation of latex regeneration and duration of latex flow and identified differential expression of several genes related to rubber biosynthesis, cellulose and lignin biosynthesis (Chao *et al.*, 2015).

Liu *et al.* (2016) carried out transcriptome analysis in ethylene induced cDNA library of *Hevea* and found expression of certain regulatory enzymes in the glycolytic pathway as well as genes in the carbon fixation pathway (Calvin cycle) up-regulated. Transcriptome analysis between healthy and TPD trees revealed expression of genes associated with ROS metabolism, jasmonate and ethylene biosyntheses, ubiquitin proteosomal pathway (UPP), programmed cell death (PCD) and rubber biosynthesis(RB) (Li *et al.*, 2016). Lau *et al.* (2016) reported a comprehensive genome-wide analysis of the widely planted *H. brasiliensis* clone, RRIM600 and attributed to *H. brasiliensis*'s capacity to produce high levels of latex to the expansion of rubber biosynthesis-related genes in its genome and its high expression in latex. A high quality genome assembly of *H. brasiliensis* was recently analyzed (Tang *et al.*, 2016) and reported REF/SRPP gene family to encompass the most highly expressed genes in latex.

Table 2. 2. Next Generation sequencing reports in *Hevea*

Publications	**Sequencing method**	**Transcriptome type**
Xia *et al* .(2011)	PE-RNA-Seq (Illumina)	Latex and leaf combined; clone RY7-33-97
Pootakham *et al.* (2011)	454 Pyrosequencing(Roche)	Leaf tissue; clone RRIM 600 RRII 105
Triwitayakorn *et al.* (2011)	454 Pyrosequencing(Roche)	Shoot apical meristem; clone RRIM 600
Chow *et al.* (2012)	RNA-Seq (Illumina)	Latex; clone RRIM 600
Li *et al.*(2012)	PE-RNA-Seq (Illumina)	Bark; clone RY7-33-97
Duan *et al.* (2013)	454 Pyrosequencing (Roche)	Leaf, bark, latex, root, embryogenic tissues; clone PB 260
Rahman *et al.*(2013)	PE-RNAseq (Illumina) 454 Pyrosequencing (Roche)	Leaf; clone RRIM 600 Leaf; clone RRIM 600
Salgado *et al.* (2014)	454 Pyrosequencing (Roche)	Pooled RNA extracts of different tissues and open pollinated seedlings; clone RRIM 600
Mantello *et al.* (2014)	RNA-Seq (Illumina)	Bark samples; clones GT1 and PR255
Zhiyi *et al.* (2015)	RNA-Seq (Illumina)	Latex; clone Reyan 7–33–97
Liu *et al.* (2015)	RNA-Seq (Illumina)	Bark; clone PR107
Chao *et al.* (2015)	Illumina paired-end sequencing	Latex; clone CATAS8-79 and PR107
Liu *et al.* (2016)	Illumina deep-sequencing	Bark; clone PR107
Li *et al.* (2016)	RNA-Seq (Illumina)	Latex; clones CATAS7-33-97 and HAIKEN 2
Li *et al.* (2016)	Illumina HiSeq 2000	Bark ; Clone RY 7–33–97
Lau *et al.* (2016)	Illumina Hiseq 2500	Leaf, bark, latex ; clone RRIM 600
Tang *et al.* (2016)	IlluminaGA2 and Hiseq2000	Different tissues and organs; clone Reyan7-33-97

Even though, thousands of stress associated ESTs of *Hevea* are available, quantitative gene expression analysis of these genes in *Hevea* clones with varying levels of tolerance using qPCR is only recently being attempted for the identification of candidate genes/factors associated with tolerance. While some reports are there on transcriptome sequencing in *Hevea,* no reports are available on transcriptome profiling of drought or cold stress responsive transcripts. In the present study, expression analyses of certain stress specific transcripts were carried out in *Hevea* clones with varying levels of drought tolerance. Transcriptome sequencing was performed on leaf tissues of *Hevea* exposed to drought/cold stress. Prominent drought/cold responsive transcripts were further quantified in order to identify markers associated with stress tolerance.

Chapter 3

Expression studies on selected drought responsive genes of *Hevea brasiliensis*

3.1 Introduction

In India, cultivation of natural rubber (*Hevea brasiliensis*) is being extended to non-traditional regions which are drought/ low temperature prone areas. The drought prone areas experience soil and atmospheric drought, higher atmospheric temperatures combined with high solar light and low relative humidity (RH) during summer which affect the performance of the crop severely (Chandrasekhar *et al.*, 1990; Devakumar *et al.*, 1998). Drought stress ~~is~~ concomitant with high sunlight aggravates the damage inflicted upon green leaves resulting in severe inhibition of photosynthesis (Sathik *et al.*, 1998a; Devakumar *et al.*, 2002). Drought tolerance capacity of *Hevea* has been reported to vary among the clones (Chandrasekhar, 1997; Alam *et al.*, 2006) and among the clones evaluated in the field conditions, clone RRIM 600 exhibited better growth and yield (Dey *et al.*, 1998). In order to obtain maximum growth and yield performance in such non-traditional regions, clones with better drought tolerance have to be identified /developed for which it is essential to understand the molecular mechanisms involved in drought tolerance.

Plants undergo various alterations at the physiological, biochemical and molecular levels under adverse environmental conditions like drought. To mitigate the effect of drought stress, plants respond inherently to produce stress responsive protein by altering the expression of an assortment of genes with diverse functions (Shinozaki *et al.*, 2003; Bartels and Sunkar, 2005). Drought tolerance is a complex trait that involves multiple genes, interactions among genes and environmental cues (Tardieu *et al.*, 2010). Such gene expression involved in acclimatization of plants to extreme weather conditions is influenced by the activation of complex signaling pathway as a result of perception of signals (Kasuga *et al.*, 1999; Tong *et al.*, 2007). It is well established that almost all biotic and abiotic stresses induce oxidative stress by producing higher levels of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and nitric oxide (NO). Higher levels of ROS which are extremely deleterious damages cellular membranes by oxidizing cellular components eventually leading to cell death (Mittler, 2002 ; Dat *et al.*, 2000; Kar, 2011).

Studies on the molecular basis of plant responses to drought are critical in order to understand the regulatory mechanisms involved in environmental stress tolerance which can be subsequently utilized to improve stress tolerance through genetic manipulation (Xiong *et al.*, 2002; Umezawa *et al.*, 2006) or through molecular breeding. Through molecular and genomic analyses of *Arabidopsis,* rice and other species, many drought-inducible genes with varying roles have been identified. Signal transduction and gene networks involved in the drought response and tolerance have been extensively reviewed (Golldack *et al.*, 2014; Nakashima *et al.*, 2014) and reports are available on signaling mechanism, transcriptional regulatory network and their cross-talk. Both the reviews highlight the central role of transcription factors (Tfs) in abiotic stress response and tolerance mechanisms.

In *Hevea*, drought stress has been reported to afflict yield and its general performance (Buttery and Boatman, 1976; Sethuraj *et al.*, 1984; Sreelatha *et al.*, 2007; 2011). The metabolic activity of clone RRII 105 has been found to be severely affected during drought stress. The gas exchange parameters (CO_2 assimilation rate and stomatal conductance) measured under drought stress indicated that clone RRIM 600 had least inhibition during summer proving that it is a drought tolerant clone while the clone RRII 414 was found susceptible (Sumesh *et al.*, 2011). The clones RRIM 600 and RRII 208 were reported to be surviving well under dry conditions (Singh *et al.*, 2012) and also better yielding (Mydin, 2014). All these reports indicate that clones RRIM 600 and RRII 208 as drought tolerant and RRII 105 as a moderately tolerant clone while RRII 414 as drought susceptible clone.

In this study, attempts were made to identify drought responsive transcripts by quantitative gene expression analysis with an objective to identify candidate genes/factors associated with drought tolerance. Despite the availability of thousands of stress associated ESTs of *Hevea*, quantitative gene expression analysis of these genes is only recently being attempted for the identification of candidate genes/factors. With the advent of qPCR technique, it is easier to quantify each gene and establish their relevance under the given stress situations. Even though few genes associated with tolerance/susceptibility have been reported in few clones, a comprehensive study on genes/factors directly involved in drought tolerance has not been reported in *Hevea*. Hence in the present study, attempts were made to quantify expression of a set of stress responsive genes using quantitative real time PCR in *Hevea* clones with varying levels of drought tolerance and to identify the candidate genes for drought tolerance.

3.2 Materials and methods

3.2.1. Plant material and stress induction

In one experiment, two year old field planted *Hevea* plants belonging to moderately drought tolerant group (RRII 105) and drought tolerant group (RRIM 600, RRII 208 and RRII 430) grown in Regional Research Station, Dapchari, Maharashtra, India were selected. The control plants were irrigated on every third day, while the drought imposed plants were left unirrigated for 15 days. In another experiment, four *Hevea* clones, RRII 105 (moderately drought tolerant), RRIM 600 and RRII 208 (drought tolerant) and RRII 414 (drought susceptible) were employed. The clones were generated by budding of seedlings raised from *Hevea* seeds at Rubber Research Institute of India (RRII) farm with clonal buds collected from *Hevea* budwood nursery maintained at RRII. The budded stumps were grown in polythene bags (size, 65x 35 cm) in open field conditions of RRII nursery. After growing for six months (two to three whorl stage), the plants were transferred to glass house conditions before imposing drought treatment. One set of plants from each clone was subjected to water stress by withholding irrigation for 10 days and the other set of plants was watered on alternate days. After confirming the impact of stress by gas exchange parameters, leaf samples were collected and frozen in liquid nitrogen before transporting in dry ice to the laboratory.

3.2.2. Physiological parameters

The degree of impact of drought stress on young plants was assessed by measuring the net CO_2 assimilation rate (A) and stomatal conductance (g_s) using a portable photosynthesis system (LI-6400 XT), LI-COR, U.S.A. All the gas exchange measurements were made at a constant CO_2 concentration of 400 ppm using a CO_2 injector (LI-6400-01, LI-COR, USA) and at 500 μmol m^{-2} s^{-1} of light intensity using red LED source (with 10 % blue light) attached with the leaf chamber of LI-6400. Leaf samples from these plants were collected for the gene expression studies.

3.2.3. Total RNA isolation

Total RNA was extracted from the leaf samples using the Spectrum Plant Total RNA Kit (Sigma-Aldrich). Leaf tissues were ground to fine powder in liquid nitrogen using mortar and pestle and 100 mg tissue was transferred to 2ml collection tubes containing 500 µl lysis solution and β mercaptoethanol (0.01%) followed by vortexing vigorously for 30 seconds.

The mixture was further incubated at 56°C for 5 minutes and then centrifuged. The supernatant was transferred to a filtration column followed by centrifugation. After discarding the filtration column, 500 µl of binding solution was mixed with the clarified lysate. The mixture was then transferred to a binding column placed on a 2ml collection tube and centrifuged at maximum speed for a minute to facilitate binding of RNA. The column was then washed with wash solution I by centrifugation followed by on column DNase digestion. For this purpose, 80 µl of DNase-buffer mixture was added to each column and the column was incubated at room temperature for 15 minutes. 500 µl of wash solution I was again added and centrifuged for a minute to remove digested DNA. The flow through was discarded and the column was washed again with 500 µl of wash solution II and by centrifuging at maximum speed for 30 seconds. The column was dried, after which RNA was eluted with 70 µl of sterilized DEPC water by transferring to fresh collection tubes. The concentration and quality of total RNA prepared was determined by spectrophotometer (Nanodrop ND 1000, USA) and denaturing (1.4%) gel electrophoresis.

3.2.4. cDNA synthesis

cDNA synthesis was carried out using Superscript III first strand synthesis system (Invitrogen). 3µg of total RNA was combined with 1µl of oligo dT primer (50 µM) and 1µl of 10 mM dNTP mix and made up to 10 µl by adding sterilized DEPC treated water. The mixture was kept at 65°C for 5 minutes and immediately chilled on ice for a minute. The cDNA synthesis system mix (10 µl) was prepared by combining 2 µl of 10X RT buffer, 4 µl of MgCl2 (25mM), 2 µl 0.1M DTT, 1 µl of RNase OUT (40 U/µl) and 1 µl of Superscript III reverse transcriptase (200 U/µl). This reaction mix was added to 10 µl of prepared RNA-primer mixture and incubated at 50 °C for 50 minutes for cDNA synthesis. After that, reaction was terminated by incubating at 85°C for 5 minutes followed by chilling on ice. RNA was removed by adding 1 µl of RNase H and kept the tube at 37°C for 20 minutes. The tubes containing the cDNA were stored at -20°C for further use.

3.2.5. Quantitative real time PCR

Suitable primers were designed for 23 gene transcripts selected from *Hevea* ESTs using Primer Express (Applied Biosystems, USA) as given in (Table 3.1). Quantitative gene expression analysis was eventually carried out using Light Cycler 480 II, Roche Real Time PCR System. qPCR was performed in a 20 µl reaction mixture containing 1 µl from 1/10 dilution of first-strand cDNA reaction, 125 nM of each primer and 10 µl of Lightcycler 480 SYBR Green I Master (Roche Diagnostics Gmbh, Germany). qPCR was performed by incubating the mixture at 95 °C for 7 min, followed by 40 cycles of 95 °C for 20 s and 60 °C for 30 s. This was followed by a melt curve analysis (95 °C for 20 s, 60 °C for 1 min and 95 °C for about 5 min). Each experiment was repeated twice and each PCR reaction was performed in triplicate with no template controls (NTC). Reaction efficiency of both the target genes and the endogenous control was calculated based on the formula, Efficiency $=10^{(-1/\text{slope})}-1$. Primers with slope values between primers between −3.2 and −3.5 only were employed for these reactions. GAPDH was used as endogenous control for the qPCR analysis. The relative quantification (RQ) values were analyzed using Light Cycler 480 Software (release 1.5.0).

3.2.6. Data analysis

The $2^{-\Delta\Delta Ct}$ method was adopted to analyze the relative changes in gene expression from qPCR data (Livak and Schmittgen, 2001) and the data are presented as the fold change. Three biological replications were included in the qPCR analysis for each treatment. Statistical analysis was performed with the relative quantification data using ANOVA. The ratio with P-value <0.05 was adopted as significant for either down or up-regulation.

Table 3.1. List of genes and the corresponding primers used for qPCR analysis

Sl.No	Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
1	HbDRT 5b	TCAAACACTGTCATGTCCAAGAAA	GAATCAGGGCAACCTTTTAAACC
2	HbTPD 24	TCAGGGCAACCTTTTAAACCA	ACCAGGCAAATCATTGAAATTCA
3	HbTPD27	CCAGAACCAGAAAGGAAATGAATT	ACCAAACATCATTAACTAGAACAGAAACA
4	HbDRT50	TCGGAAGCTTCGACTGTGATG	TACTGCTAGGATTTCGACCTTAAACA
5	HbNRG18	GAGGATCGACGCATCAAATGA	AAAGTTTGATTCAGCGGGAGAA
6	HbNRG 21	ATAAAAACACCCACAATGATAACCAA	CTAGTTATATATTATGGGTTTGTGCAAAGC
7	HbDRT 82b	GCTTAGTAGGCATAGCTAGTGGGATT	AGCAAGTATTCTGTTTTTCCATTCTCT
8	LEA5	CTCGCTTTCCCTCCAATG	TTCCTCACCATACCACTCC
9	Peroxidase	AATTGGCACGAATTTCCC	CACGGATAAGAGAACAAGG
10	HbHP 33	GGCCGTGCAATACGTGAGA	GCCATTTTCTTCGCGTAAGG
11	ABCT	ACCGGCGTCCTATGTTCTCA	TTTAATCCCAGCAATCCATATCG
12	WRKY tf	AGGGAATGGAGATGAGGGAAA	GGGACATAACCAGGTGGCTAGA
13	TF MBF1	GTTGGTAGGCACTCTCACTTGAC	AGAAGCGTGGACATAAGAAGAAGG
14	GPX	GCCTGCGTTGTGTTCTTTGA	TCAACATCGTTTCCCCTAGCA
15	Hb HP 20	CTCGACATCCCTTCGTTCCA	TTGGTGGCCTTGTAGGTGTTC
16	Hb HP 22	CACCCCAACGAGTGACAACA	TGCTCAGAAGGTGGACTTTGC
17	NFYA1	TTGGTGGAAGGATGTGTTTGC	ACTCCTCTGGTGGTCAGCTAGAA
18	MAPK	CTGTTGTGTGCAAGCAGGTTTT	CCCTATGTATGACATGTCGCTCAT
19	CRT/DRE	AGTCCCGGCATTGCAAAA	GAGTCAGCGCCGGAGGAT
20	HSP 70	ATGCCTGAGAGCTCAAATTTGC	GGAAACTGCTGGTGGTGTCA
21	MYB	TGTGACCACTAGAACACCAACTCA	TCCTGTGCTCTGCCTGATAAAA
22	APX	GTAGAGGATGGTGCCGACAAC	CCAAACTTCCCCAGTTACAAGAAA
23	CATALASE	TCTCCTTTTCAATGACGCACTTC	GCTGAGAGAACCCCACTTCCT
24	GAPDH	GCCTGTGATAGTCTTCGGTGTTAG	GCAGCCTTATCCTTGTCAGTGAAC

3.3 Results

a. Gene expression studies in field grown plants

In the first experiment comprising four *Hevea* clones *viz*, RRII 105, RRIM 600, RRII 208 and RRII 430 exposed to drought stress at RRS, Dapchari were utilized for studying physiological parameters as well as for the quantitative gene expression. Stomatal conductance was least in clone RRIM 600 under drought while clone RRII 430 had the highest value. Percentage of reduction in stomatal conductance was lesser in RRII 105 followed by RRII 430, RRII 208 and RRIM 600 (Fig 3.1a). The gas exchange parameters indicated that all the three relatively tolerant clones (RRIM 600, RRII 208 and RRII 430) maintained significantly higher rate of CO_2 assimilation rate than RRII 105 under drought stress condition. The reduction in the CO_2 assimilation rate in clone RRII 430 was meager under drought condition (Fig 3.1b). Clones RRIM 600 and RRII 208 exhibited nearly 30 per cent reduction in photosynthetic rate under drought condition while the clone RRII 105 had more than 40 percent reduction. Both CO_2 assimilation rate as well as stomatal conductance got reduced under drought stress in all the clones compared to their irrigated plants thus confirming impact of the drought effect on these plants.

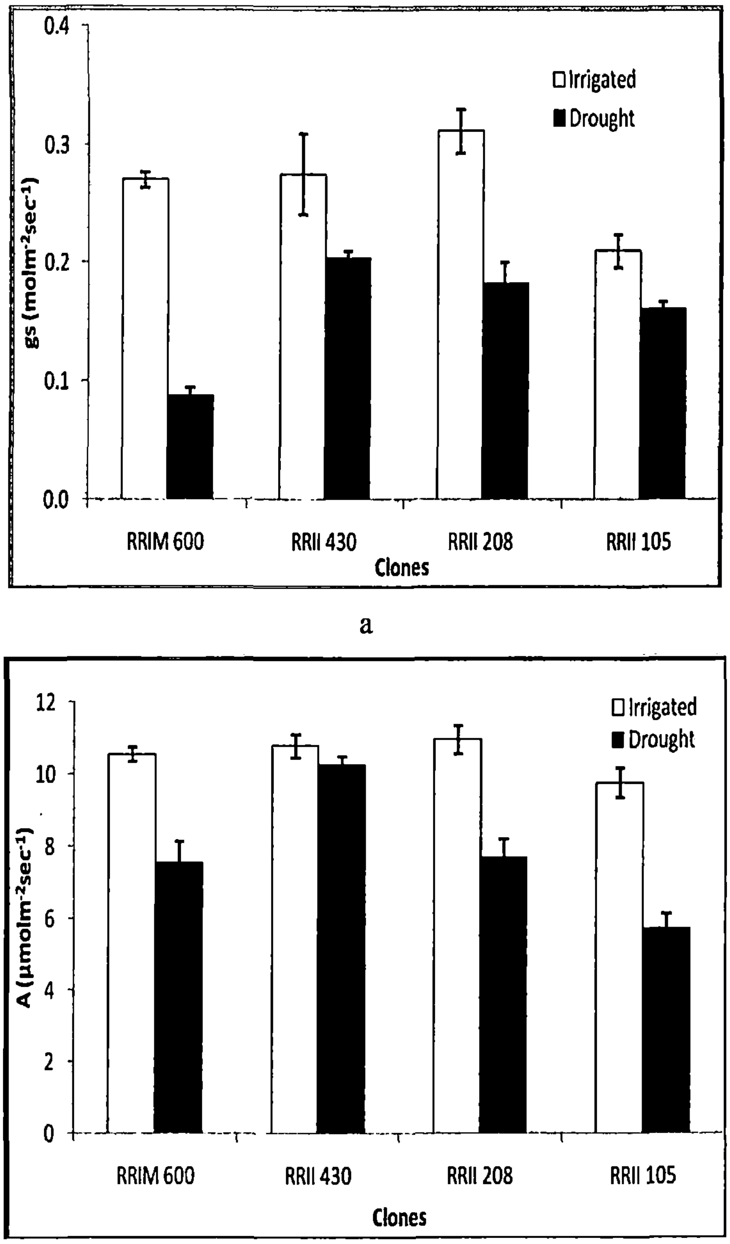


Fig 3.1. (a) Stomatal conductance (gs) and (b) CO_2 assimilation rate (A) in different *Hevea* clones under irrigated and drought conditions.

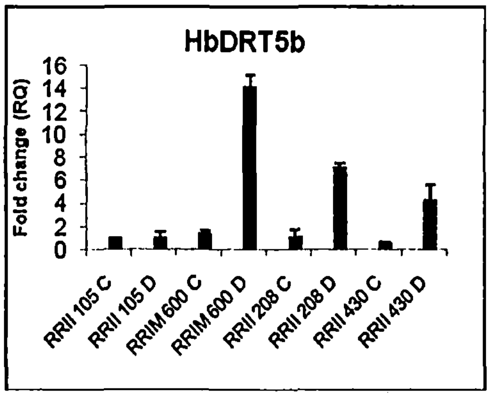
To assess the molecular effect of drought stress on *Hevea* plants, expression analysis of 16 selected gene transcripts were carried out using qPCR and the data of relative quantification in comparison with RRII 105 control plants (as calibrator) was analysed. (Table 3.2; Fig 3.2.). Two gene transcripts, HbDRT 5b and HbTPD 24, both homologous to NAC transcription factor were found significantly up-regulated in all the relatively tolerant clones RRIM 600, RRII 208 and RRII 430. HbDRT 5b got about 14 fold increase in RRIM 600 followed by RRII 208 (7.1) and RRII 430 (4.3) whereas Hb TPD 24 got 14.4, 3.8 and 3.4 fold increase in RRIM 600, RRII 208 and RRII 430 respectively. Transcripts of LEA 5 protein and peroxidase also got up regulated in all the tolerant clones. LEA 5 showed 5 fold increase in RRIM 600 followed by RRII 208 (3.2 fold) and RRII 430 (2.9 fold). Peroxidase showed significant up-regulation in RRIM 600 (95 fold), RRII 208 (12.8 fold) and RRII 430 (26.6 fold). ABC transporter protein and a hypothetical protein (HbHP20) showed significant up regulation in control and treated plants of RRIM 600 and RRII 208 and drought stressed plants of RRII 430. Levels of glutathione peroxidase (GPX) were significantly higher in both the treatments of the tolerant clones when compared to irrigated plants of RRII 105. But only in clone RRII 208, it was significantly up-regulated when compared to its own control. WRKY tf showed significant up-regulation in drought stressed plants of RRII 208. The other genes investigated neither exhibited any trend with the drought treatment when compared with the RRII 105 control nor with their own respective control.

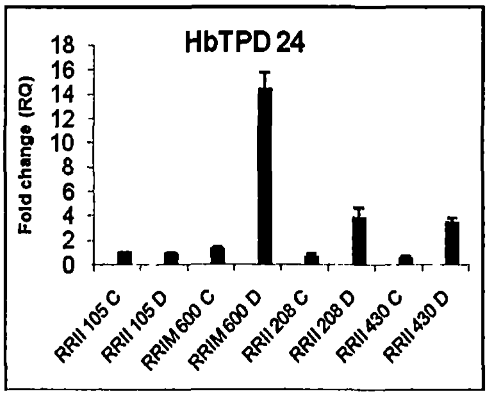
Table 3.2. Relative quantification (RQ) of sixteen genes in four clones of *Hevea* under drought condition using RRII 105 irrigated control as calibrator

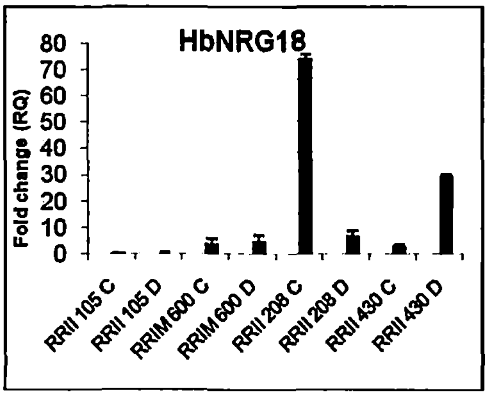
with? Tukey's

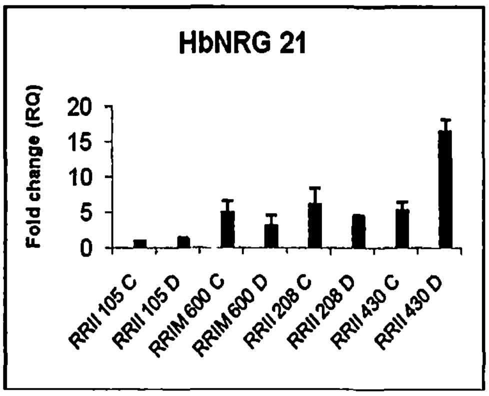
Genes	RRII 105 C	RRII 105 D	RRIM 600 C	RRIM 600 D	RRII 208 C	RRII 208 D	RRII 430 C	RRII 430 D	CD ?
HbDRT 5b	1	1.04	1.44	14.26	1.11	7.16	0.61	4.30	2.10
HbTPD 24	1	0.89	1.34	14.41	0.69	3.82	0.60	3.47	1.90
HbTPD27	1	1.83	2.99	1.99	4.58	4.97	3.04	9.83	3.50
HbDRT50	1	1.65	4.58	3.75	3.90	3.15	2.20	4.72	2.41
HbNRG18	1	0.47	4.34	5.23	74.39	7.22	3.25	29.65	3.63
HbNRG 21	1	1.28	4.93	3.23	6.24	4.27	5.39	16.45	4.13
HbDRT 82b	1	0.99	1.40	1.37	2.50	1.10	0.95	1.66	-
Peroxidase	1	2.195	42.31	95.74	1.441	12.84	5.140	26.65	18.63
LEA 5	1	0.856	1.150	5.001	2.583	3.219	0.902	2.973	1.029
WRKY tf	1	0.897	2.063	3.778	4.524	3.729	2.258	7.508	4.345
Tf MBF	1	1.075	2.448	1.582	0.957	1.435	1.3421	1.624	0.524
GPX	1	1.204	2.237	2.756	3.198	4.418	2.480	2.852	1.012
ABCT	1	1.672	2.813	4.865	3.034	3.175	1.068	2.059	0.9718
Hb 33 HP	1	0.631	0.835	1.149	0.694	1.897	1.358	1.386	0.545
Hb 22HP	1	1.54	2.28	4.54	3.39	3.16	2.06	2.56	1.878
Hb 20 HP	1	1.27	1.65	3.86	3.34	3.060	1.308	2.853	1.478

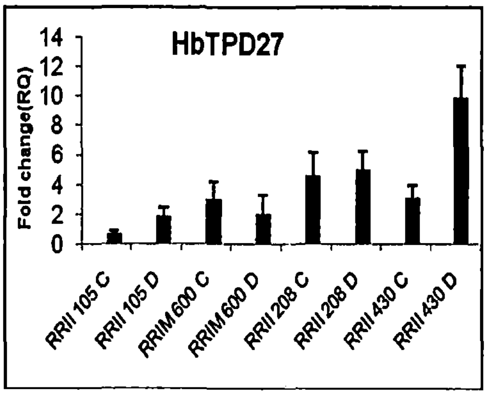
C-Control D- Drought

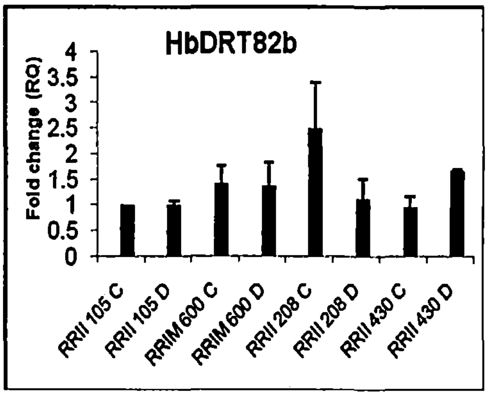

HbDRT5b
Fold change (RQ)
16
14
12
10
8
6
4
2
0
RRII 105 C
RRII 105 D
RRIM600 C
RRIM600 D
RRII 208 C
RRII 208 D
RRII 430 C
RRII 430 D

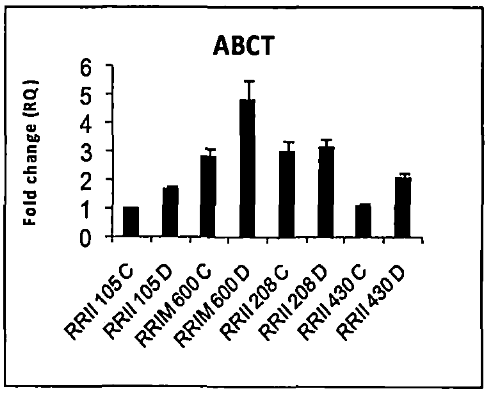

HbTPD 24
Fold change (RQ)
18
16
14
12
10
8
6
4
2
0
RRII 105 C
RRII 105 D
RRIM600 C
RRIM600 D
RRII 208 C
RRII 208 D
RRII 430 C
RRII 430 D

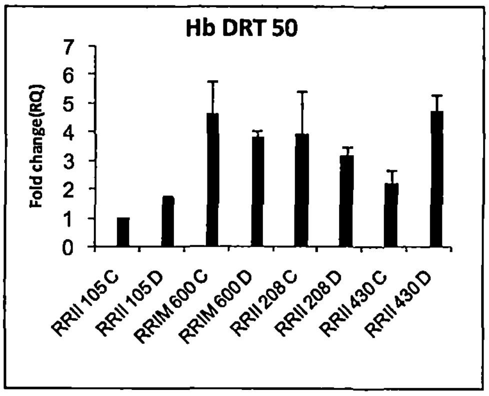

HbNRG18
Fold change (RQ)
80
70
60
50
40
30
20
10
0
RRII 105 C
RRII 105 D
RRIM600 C
RRIM600 D
RRII 208 C
RRII 208 D
RRII 430 C
RRII 430 D


HbNRG 21
Fold change (RQ)
20
15
10
5
0
RRII 105 C
RRII 105 D
RRIM600 C
RRIM600 D
RRII 208 C
RRII 208 D
RRII 430 C
RRII 430 D


HbTPD27
Fold change(RQ)
14
12
10
8
6
4
2
0
RRII 105 C
RRII 105 D
RRIM600 C
RRIM600 D
RRII 208 C
RRII 208 D
RRII 430 C
RRII 430 D


HbDRT82b
Fold change (RQ)
4
3.5
3
2.5
2
1.5
1
0.5
0
RRII 105 C
RRII 105 D
RRIM600 C
RRIM600 D
RRII 208 C
RRII 208 D
RRII 430 C
RRII 430 D


ABCT
Fold change (RQ)
6
5
4
3
2
1
0
RRII 105C
RRII 105D
RRIM600C
RRIM600D
RRII 208C
RRII 208D
RRII 430C
RRII 430D


Hb DRT 50
Fold change(RQ)
7
6
5
4
3
2
1
0
RRII 105C
RRII 105D
RRIM600C
RRIM600D
RRII 208C
RRII 208D
RRII 430C
RRII 430D

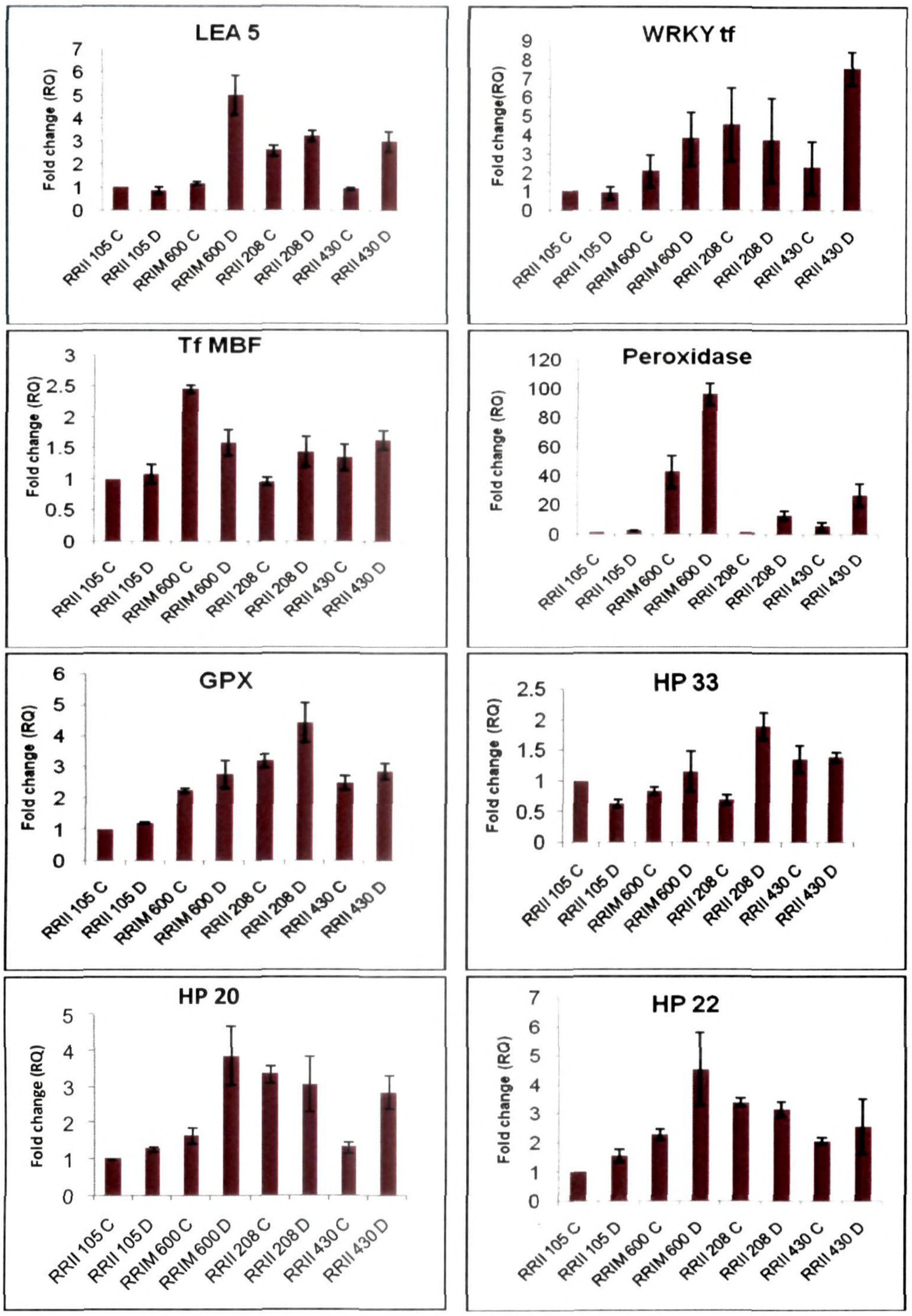
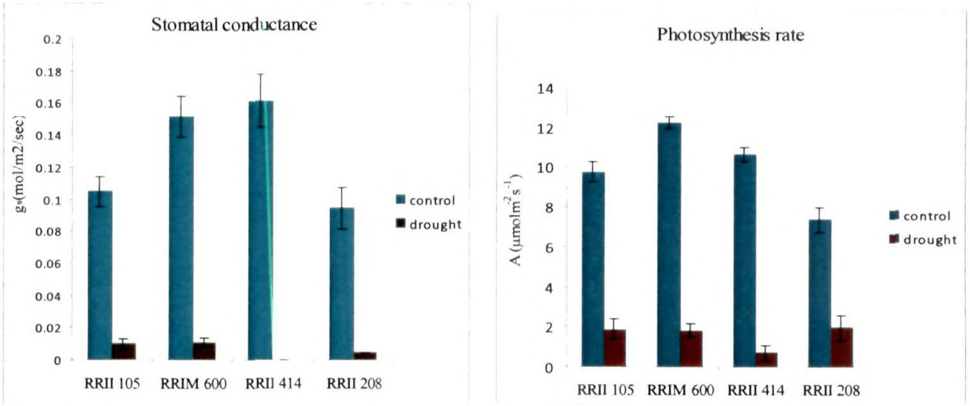


Fig 3.2. Expression analysis of sixteen genes in four clones of *Hevea* under drought condition in Dapchari (Fold change). ± Error bars indicate standard error of three biological replicates.

b. Gene expression studies on polybag grown plants

This study was conducted in RRII by exposing the poly bag grown plants to drought in summer season with an objective to study the gene expression pattern of seven drought responsive genes from *Hevea.* Polybag plants of four *Hevea* clones *viz,* RRII 105, RRII 414, RRIM 600 and RRII 208 were exposed to drought stress and the impact of the stress was assessed by measuring gas exchange parameters. Stomatal conductance was found declining significantly in all the clones under drought stress while it was almost nil in RRII 414 (Fig 3.3a). Among the clones studied, RRIM 600 had better stomatal conductance under drought stress indicating its inherent drought tolerance nature. Similarly, 'A' was also found significantly inhibited in plants of all the drought imposed clones (Fig 3.3b). While 'A' was severely affected in relatively susceptible clone RRII 414, it was stable in both the tolerant clones (RRIM 600 and RRII 208). Thus the effect of drought on these plants was confirmed.



a b

Fig 3.3. (a) Stomatal conductance (g_s) and (b) CO_2 assimilation rate (A) in irrigated and drought imposed polybag plants of *Hevea*

Gene expression analysis of seven drought related transcripts was carried out by qPCR and the results are given in Table 3.3; Fig 3.4. Among them, four transcripts namely NFYA, MYB tf, CRT/DRE bf and MAPK showed a stronger association with drought tolerance. Even though all these four transcripts were up-regulated in the relatively tolerant clones, only MAPK exhibited a trend in expression pattern. The level of expression of MAPK was 13 fold, 7 and 3.6 fold in RRIM 600, RRII 208 and RRII 105 respectively whereas no change could be observed in RRII 414. APX and HSP 70 did not show any trend in their expression pattern. Catalase was found down regulated in all the clones studied.

Table 3.3. Relative quantification (fold change) of seven genes in four clones of *Hevea* under drought condition using its own irrigated control as calibrator

Genes	RRII 105	RRIM 600	RRII 414	RRII 208
APX	1.5	5.73	0.213	0.246
catalase	0.377	0.362	0.138	0.212
CRT/DRE bf	7.33	7.49	0.208	3.269
HSP	3.97	2.955	0.099	0.39
Myb tf	1.69	2.02	0.084	12.28
NFYA	3.97	6.68	0.864	1.869
MAPK	3.61	13.08	1.18	7.06

Significant up-regulation (gene expression > 2 fold)

Significant down regulation (gene expression < 0.5 fold)

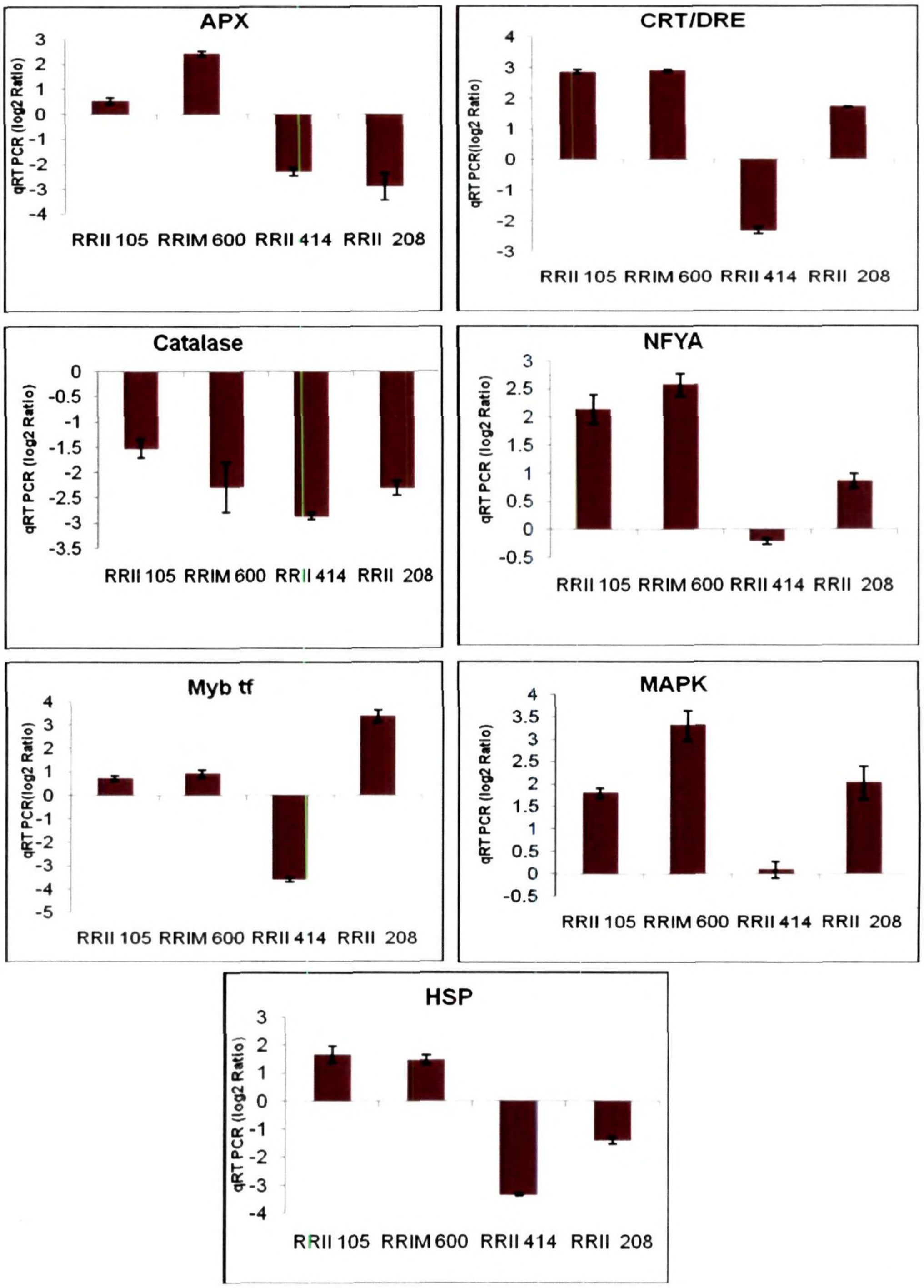


Fig 3.4. Expression analysis of seven genes in four clones of *Hevea* under drought condition (Fold change in log 2 ratio) with its own irrigated control as calibrator. Log 2 ratio>0 up regulated; < 0 down regulated. ± Error bars indicate standard error of three biological replicates

3.4 Discussion

Photosynthesis is very much sensitive to environmental adversities and is negatively influenced by water deficit and high solar light.The plants respond to water stress by closing the stomata which ultimately ends up with reduction in CO_2 assimilation as a consequence of limitation in CO_2 diffusion (Lawlor and Tezara, 2009). In field grown *Hevea* plants drought induced reduction in photosynthetic rate has earlier been reported by Devakumar *et al.* (2002) and Alam *et al.* (2006). Clonal variation in photosynthesis has also been reported in young plants of *Hevea* (Dey *et al.*, 1995; Nataraja and Jacob, 1998). The instantaneous water use efficiency values indicate RRIM 600 as the most efficient among the clones tested under drought conditions. The high water use efficiency of this clone at low g_s reflects its photosynthetic capability under severe drought conditions. Many studies in *Hevea* also indicate the negative impact of high light intensity and warmer temperature on overall performance of the clone in drought prone areas (Nair *et al.*, 1998; Jacob *et al.*, 1999) in terms of declining gas exchange parameters rather than shortage of water during the peak summer. Popular clone RRII 105 is relatively more susceptible to soil water deficit (Annamalainathan *et al.*, 2010) compared to the other clones selected for this study. Recent studies indicated that RRII 430 is on par with RRIM 600 displaying better photosynthetic rate and effective quantum yield when compared to other clones under drought condition (Annamalainathan *et al.*, 2010).

Among the genes studied in the first experiment, only two genes such as HbDRT 5b and HbTPD 24 which are homologous to NAC transcription factor (NAC tf) were found expressed significantly under drought conditions in all the three tolerant clones investigated. There were another set of genes LEA 5 protein, peroxidase, ABCT protein and a hypothetical protein (Hb HP 20) which got up-regulated significantly in only two of the three tolerant clones studied. Looking at the overall relative quantification results, intrinsically higher level of expression was found for peroxidase (about 96 fold in RRIM 600) when compared with RRII 105 irrigated control. ATP binding cassette protein (ABCT) was also found up-regulated significantly in control and treated plants of RRIM 600 and RRII 208 and drought stressed plants of RRII 430 when compared to irrigated control plants of RRII 105. Expression of HbHP20 was found significantly up-regulated in drought treated plants of both RRII 430 and RRIM 600. But in the case of RRII 208, it got up-regulated in the control plants. In the case of HbHP22, it was significantly up-regulated only in drought stressed plants of RRIM 600 and in both drought stressed and control plants of RRII 208. Level of glutathione peroxidase (GPX) was found significantly higher in all the tolerant clones when compared to irrigated plants of RRII 105. But only in clone RRII 208 it was found significantly up-regulated when compared to its own control.

There are other genes which got up-regulated only in one or two clones and not in all stress tolerant clones. For *e.g.* WRKY tf got significantly up-regulated only in the drought exposed plants of clone RRII 430. The transcript of HbHP33 got significantly up-regulated only in drought exposed plants of RRII 208. Tf MBF1 got significantly up-regulated in control and treated plants of RRIM 600 and treated plants of RRII 430. Tf MBF1 is a transcriptional co-activator that mediates transcriptional activation by bridging a sequence-specific activator and TATA-box–binding protein (TBP). In *Arabidopsis*, MBF1c has been reported to be produced more in response to pathogen infection, salinity, drought, heat, hydrogen peroxide and plant hormones such as abscisic acid or salicylic acid (Rizhsky *et al.*, 2004). Tf MBF1 expression had also been shown to enhance tolerance to heat and osmotic stress by partially activating, or perturbing ethylene-response signal transduction pathway in transgenic plants (Suzuki *et al.*, 2005). The other genes investigated neither exhibited any trend with the drought treatment when compared with the RRII 105 control nor with their own respective control.

The overall results of the quantitative expression analysis indicate the significant up-regulation of two transcripts *viz.* HbDRT 5b and HbTPD 24 homologous to NAC tf in all the three tolerant clones investigated. This significant level of up-regulation in drought tolerant clones indicates its close association with drought stress. NAC tfs are a family of genes specific to plants and are found to play significant role in a diverse set of developmental processes (Kikuchi *et al.*, 2000; Xie *et al.*, 2000). Over-expression of certain NAC transcription factors in plants has been shown to significantly improve drought tolerance (Tran *et al.*, 2004; Zheng *et al.*, 2009). Xu *et al.* (2013) reported that *NAC096* is associated with drought stress response by binding to ABA responsive element in the promoters of many drought stress responsive genes. In rice, *SNAC3* has been found to play a positive role in heat and drought tolerance by controlling genes involved in ROS pathway (Fang *et al.*, 2015).

The other two genes which appeared promising were LEA 5 protein and peroxidase. LEA 5 protein is significantly up-regulated in both RRIM 600 and RRII 430. The level of peroxidase was remarkable by the magnitude of its expression (about 96 fold) when compared with the expression of other genes. LEA proteins are generally hydrophilic, whereas LEA 5 proteins are hydrophobic proteins that contain a significantly higher proportion of hydrophobic residues and are assumed to be sequestering ions during water loss (Bhatnagar-Mathur *et al.*, 2008). LEA 5 protein transcripts accumulate during the late stage of seed development and in response to stress conditions, such as drought, UV light, salinity, cold, and wounding. Higher levels of LEA5 protein found under drought stress conditions in tolerant clones RRIM 600 and RRII 430 indicate their possible association with drought tolerance.

Similarly, higher level of peroxidase expressed in RRIM 600 and RRII 430 indicate its relevance in the context of stress amelioration through detoxification of reactive oxygen species which are likely be produced in large scale under drought stress condition. ROS which are highly reactive can disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids in the absence of any protective scavenging mechanism (Allen, 1995). Antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase are the most important components in the scavenging system of ROS. So up-regulation of peroxidase in tolerant clones indicate the existence of better ROS scavenging system that could possibly be imparting enhanced drought tolerance. The rest of the genes which did not change much under drought could be presumed as not drought responsive.

Tolerant clones develop several adaptive mechanisms to ameliorate the impact of adverse environmental conditions. One of the major molecular responses that plants exhibit when exposed to drought stress is altered expression of genes related to different pathways associated with stress perception, signal transduction, regulators and synthesis of a number of compounds (Ramanjulu and Bartels, 2002; Montalvo-Hernandez *et al.*, 2008). Complete understanding of the mechanisms involved in plant's response to such abiotic stresses (drought) is required for the development of plants with better tolerance to such stresses. Several hundred genes that respond to drought stress at the transcriptional level have been identified in plants (Shinozaki and Yamaguchi- Shinozaki, 2007; Govind *et al.*, 2009; Wilkins *et al.*, 2009). Studies on transcriptome level changes in response to drought stress in two hybrid *Populus* clones indicated that although there are a number of conserved transcriptome-level changes between the genotypes, some changes are only particular to one or the other genotypes (Wilkins *et al.*, 2009). To understand the molecular basis of drought tolerance in *Hevea*, genes/factors associated with drought tolerance have to be identified. Although some mechanism of stress tolerance is common to all *Hevea* clones, the degree of tolerance varies among different clones. An understanding of gene regulation is particularly important in the case of multigenic trait like drought tolerance as different regulatory pathways determine the expression of a set of drought responsive genes.

With the quantitative gene expression analysis, it is easy to quantify each gene and to establish their relevance in drought tolerance. The present study reveals for the first time the existence of strong association of NAC tf (both Hb DRT 5b and Hb TPD 27) with drought tolerance in *Hevea*. LEA 5 protein and peroxidase also exhibited a correlation with drought stress tolerance. This opens up the possibility of using these genes as markers for drought tolerance in *Hevea* after validating them in more drought tolerant clones.

In the second set of experiment, four clones of *Hevea viz*. RRII 414 (drought susceptible), RRII 105 (moderately tolerant clone), RRIM 600 and RRII 208 (drought tolerant) were exposed to water deficit stress by withholding irrigation for ten days. Among the seven genes investigated in this study, APX was found significantly up-regulated in the relatively tolerant clone RRIM 600 and slightly higher in RRII 105 whereas it was found down regulated in RRII 414 and RRII 208. Under drought stress conditions, ROS such as, singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) are produced in higher quantities and only the cultivars/genotypes having an improved ROS detoxification process can protect the cells and their organelles from the toxic effect of these species (Apel and Hirt, 2004; Mittler, 2002; Caverzan *et al.*, 2012). APX is the key enzyme that is involved in detoxification of hydrogen peroxide in plant chloroplasts, cytosol, mitochondria and peroxisomes (Asada, 1992). APX activity has been reported to shoot up with other ROS scavenging enzymes such as catalase, SOD and glutathione reductase (GR) (Shigeoka *et al.*, 2002) under stress conditions. The cytosolic *APX1* (Koussevitzky *et al.*, 2008), *Populus* peroxisomal ascorbate peroxidase (*PpAPX*) (Li *et al.*, 2009) and tomato (*Solanum lycopersicum*) thylakoid-bound *APX* (*StAPX*) genes (Sun *et al.*, 2010) have all been shown to improve abiotic stress tolerance. Reduction of its expression in drought tolerant clone like RRII 208 needs to be revisited and involvement of other isoforms of this gene need to be investigated further.

The results of gene expression analysis for CAT indicated down regulation in all the four clones analyzed. Catalases have high specificity for H_2O_2 and are unique that they do not require cellular reducing equivalent. CATs have a very fast turnover rate, but a much lower affinity for H_2O_2 than APX. They are known to remove H_2O_2 produced in the peroxisomes by photorespiration (Noctor *et al.*, 2000), β-oxidation of fatty acids and other enzyme systems such as xanthine oxidase (XOD) coupled to SOD (Sharma *et al.*, 2012). CAT activity has also been found to decrease under conditions that suppress photorespiration, such as elevated CO_2 (Azevedo *et al.*, 1998) and high light intensities (Shang and Feierabend, 1999). The reduction in catalase expression observed in all the clones need to be further investigated.

Expression of MYB transcription factor (Myb tf) was found significantly higher in both the tolerant clones (RRII 208 and in RRIM 600), whereas it was found moderately up- regulated in RRII 105 which is a moderately tolerant clone. But in drought susceptible clone RRII 414, it got significantly down regulated. This trend coincides with the *in vivo* performance of clones studied and surely indicates the existence of strong correlation between the Myb tf and stress tolerance. The Myb tfs are induced by various abiotic stress factors such as dehydration, osmotic or salinity stress, ABA, wound signalling pathways, cold stress, salt stress, *etc.* Myb tfs interact with *cis*-elements in the promoter regions of several stress-related genes through ABA-dependent pathway of stress signalling for the up-regulation of abiotic stress responsive genes. Over-expression of Myb tf results in improved drought and salt tolerance in *Arabidopsis* (Ding *et al.*, 2009), osmotic tolerance (Abe *et al.*, 2003), drought stress in tomato (Vannini *et al.*, 2007) and drought and cold tolerance in the apple transgenic (Pasquali *et al.*, 2008). Over-expression of a *StMYB1R-1* transgene in potato plants improved tolerance to drought stress while not influencing significant effects on other agronomical traits (Shin *et al.*, 2011). Higher levels of Myb tf observed in tolerant clones might be attributed to enhanced tolerance.

MAP Kinase was found significantly up-regulated in RRIM 600 followed by RRII 208 and RRII 105 where it got moderately up-regulated when compared to RRII 414 in which no much change was noticed. MAP kinases play pivotal role in signal transduction pathways. Protein phosphorylation is the most important mechanism for controlling many fundamental cellular processes in all living organisms including plants. Specific classes of serine/threonine protein kinases, MAP kinases play a central role in the transduction of various extra and intracellular signals (Rodriguez *et al.*, 2010). In a cascade of signalling event, MAPK is phosphorylated and activated by MAPK kinase (MAPKK), which itself is activated by MAPKK kinase (MAPKKK). MAPK signaling triggers expression of a cascade of genes that are involved in cellular protection, desiccation tolerance, ROS scavenging enzymes and DNA damage repair by activating their corresponding transcription factors (Neil *et al.*, 2002; Desikan *et al.*, 2000; Hung *et al.*, 2005). Involvement of MAPKs in abiotic stress adaptation has been widely reported in many plant species (Yu *et al.*, 2010; Ning *et al.*, 2010). The distinctly higher level of expression observed in RRIM 600 and RRII 208, moderate up-regulation in RRII 105 and no change in RRII 414 clearly indicate its strong association with drought tolerance. Probably, this particular transcript can be employed for further use after proper validation in large number of clones with wide range of tolerance/susceptibility.

The transcript abundance of Nuclear Factor Y subunit A (NFYA) was observed in RRIM 600 and RRII 105 while it was moderate in RRII 208. But it got slightly down regulated in RRII 414. Nuclear Factor Ys which are sequence-specific transcription factors with histone-like subunits activate transcription by recognizing the CCAAT- box *cis*-acting element present in about 30% of eukaryotic promoters in a highly specific manner via the DNA binding domain present in the NF-YA subunit. In the case of plants, the CCAAT-box is present mainly in the promoters of genes involved in photosynthesis. In *Arabidopsis thaliana, NFYA5* transcript is strongly induced by drought stress in an abscisic acid (ABA)-dependent manner (Li *et al.*, 2008). They bind DNA at CCAAT sites as heterotrimeric complexes composed of single subunits and are reported to be regulating drought responses in many plant species (Stephenson *et al.*, 2007; Hackenberg *et al.*, 2012). NFYA's expression is regulated at transcriptional and post-transcriptional levels by miR169 (Petroni *et al.*, 2012) and has positive and negative effects on gene expression (Gonzalez *et al.*, 2012). Up-regulation of NFYA in tolerant clones of *Hevea* in this study indicates its strong association with drought tolerance.

Heat shock protein (HSP70) was found significantly up-regulated in both RRII 105 and in RRIM 600 while it got significantly down regulated in RRII 414 and RRII 208. HSPs perform fundamental role in protecting plants against abiotic stresses (Ahuja *et al.,* 2010; Timperio *et al.*, 2008). They act as molecular chaperones to prevent denaturation or aggregation of target proteins as well as facilitate protein refolding. Many studies have established the influence of Hsp70 genes in enhancing the plant's tolerance to environmental stresses (Ono *et al.*, 2001; Rouch *et al.*, 2004; Zhang *et al.*, 2008; Al-Whaibi, 2011). Expression of HSP70 in this study did not exhibit any trend with tolerance or susceptibility of clones. HSP being a strong contributing gene for drought tolerance, its down regulation seen in RRII 208 does not support its relevance as candidate gene for drought tolerance.

Expression of CRT/DRE bf got up-regulated in RRII 105, RRIM 600 and RRII 208 whereas it got down regulated in RRII 414. CRT/DRE bf has been reported to be upstream transcription factors that play vital regulatory roles in abiotic stress responses in plants. They bind to promoter *cis*-element CRT/DRE and activate the expression of downstream genes. Among the DRE binding proteins, the DREB2 subfamily has been reported to be induced by drought and high salinity stress. Transgenic *Arabidopsis* plants expressing DREB1 B/CBF1 or DREB1 A/CBF3 under the control of CaMV 35 S promoter showed strong tolerance to freezing, drought and high salinity stresses suggesting that DREBs/CBFs target multiple genes (Jaglo-Ottosen *et al.*, 1998; Liu *et al.*, 1998; Kasuga *et al.*, 1999). Microarray analyses have indicated that these factors target stress inducible genes containing the conserved DRE or DRE-related core motifs in their promoter regions (Maruyama *et al.*, 2004). Significant up- regulation of this particular transcript in tolerant clones as well as moderate clone and significant down regulation in the susceptible clone indicate its strong association with drought tolerance in young plants of *Hevea*.

In this study, seven stress responsive transcripts selected specifically from the data of *Hevea brasiliensis* were validated for their association with drought stress tolerance by quantifying its expression in four different clones among which two are drought tolerant, one moderate clone and one drought susceptible clone. The data of photosynthetic gas exchange parameters also corroborated with the tolerance level exhibited by these plants in field conditions. Previous reports on *Hevea* also indicated RRIM 600 and RRII 208 as tolerant clones that are suitable for drought and cold prone regions (Priyadarshan *et al.*, 2000; Sumesh *et al.*, 2011; Mydin, 2014). From the gene expression analysis data, four transcripts have been identified to have stronger association with drought tolerance in clones of *Hevea* with different levels of drought tolerance.

The clone suitable for drought regions should have a better ROS scavenging system. As it is known that elevated levels of ROS under drought conditions can induce oxidation of cellular components that could damage cellular membranes thus eventually leading to cell death, only clones with better ROS scavenging system can withstand the severity of summer in drought prone regions. Among the various free radicals, H_2O_2 has been known as a potential ROS which is highly damaging especially in the presence of transition metal like Fe^{2+}. H_2O_2 triggers MAPK signaling cascade that modulates expression of corresponding genes via activation of transcription factors (Neil *et al.*, 2002; Hung *et al.*, 2005). These genes are basically involved in cell wall protection, desiccation tolerance, production of ROS scavenging enzymes and DNA damage repair (Desikan *et al.*, 2000). Among the four transcripts having stronger association with drought tolerance, transcript of MAPK exhibited a perfect correlation with drought tolerance according to the level of drought tolerance they exhibit in the field condition. The level of MAPK expression was found 13, 7, 3.6 fold up-regulated in RRIM 600, RRII 208 and RRII 105 respectively and no change was noticed in clone RRII 414 conforming with previous findings that RRIM 600 and RRII 208 are drought tolerant (Priyadarshan, 2003).

In the case of MYB tf, 12, 2 and 1.6 fold up-regulation was found in RRII 208, RRIM 600 and RRII 105 respectively while there was down regulation in RRII 414. The up- regulation of Myb tf in clone RRIM 600 does not seem to be in proportion with its level in tolerant clone RRII 208, necessitating further investigation in more number of clones with varying levels of drought tolerance. In the case of CRT/DRE bf, higher magnitude of upregulation was found in RRIM 600 (7.4 fold) and RRII 105 (7.3 fold) while it exhibited only 3.2 fold increase in RRII 208. But its expression got down regulated in the drought susceptible clone RRII 414 (0.2 fold times). In the case of transcript NFYA also, there was 6.6, 1.8 and 3.9 fold upregulation in clones RRIM 600, RRII 208 and in RRII 105 respectively while there was 0.8 fold reduction in clone RRII 414. Among the four transcripts, though all of them got up-regulated in tolerant clones and moderate clone, only MAPK exhibited a stronger correlation with the drought tolerance potential of the clones studied. In the case of APX and HSP70, no definite trend could be observed while catalase got fully down regulated in all the clones. Catalase activity has been reported to be suppressed under elevated CO_2 and high light intensity conditions. The overall down regulation of catalase in all the clones also necessitate further investigation.

3.5 Conclusion

This study mainly focused on quantification and validation of selected drought responsive transcripts in young *Hevea* plants experiencing drought stress. In the first set of experiment, three transcripts *viz* NAC tf, LEA 5 and peroxidase exhibited association with drought tolerance among the clones selected for study whereas in the second experiment, MAPK exhibited a strong correlation with drought tolerance. But to identify more drought candidate genes, it is imperative to use a tool by which more number of genes could be screened at one go. The recent technological innovations in new generation sequencing methods such as Illumina column sequencing and larger screening method like microarray can be employed to identify overall drought responsive transcripts. Moreover, more number of clones with varying levels of drought response/tolerance has to be incorporated in future experiments to narrow down on genes contributing for drought tolerance. It would also be possible to identify various isoforms of genes through new generation sequencing technology and subsequent validation of each isoform by microarray and quantitative PCR in order to establish their association with drought tolerance.

Chapter 4

De novo transcriptome analysis of drought and cold responsive genes of *Hevea brasiliensis*

4.1. Introduction

RNA-Sequencing, an emerging tool for profiling of expressed genes is being employed to identify key genes/factors associated with specific situations like abiotic/biotic stresses or diseases *etc.* in plants and other organisms (Schuster, 2008; Wang *et al.*, 2009; Van *et al.*, 2013). The method RNA Seq, has obvious advantages such as enabling large scale functional assignment of genes, thorough qualitative and quantitative analysis of gene expression, improved sensitivity and accurate profiling of eukaryotic transcriptomes including non-model organisms (Ekblom *et al.*, 2011; Tang *et al.*, 2011). It also provides information about alternative splicing, single nucleotide polymorphisms (SNPs) for SNP marker development which has direct applications in marker assisted selection (MAS) (Martin *et al.*, 2013). RNA-Seq approach serves as an efficient, reliable and cost effective means for sequencing of RNAs and has revolutionized plant transcriptomics research. In recent years, transcriptomes of several model and non-model species have been analyzed and reported.

Approximately, 53,000 EST sequences of *H. brasiliensis* have been deposited in the National Center of Biotechnology Information (NCBI) (as on March 2017) and it is amazing to see the progress made over the past two decades on data acquisition with regard to transcriptome of rubber. In *Hevea*, transcriptional profile of different tissues and organs such as latex, leaves, bark and shoot apical meristem were generated using NGS technology (Triwitayakom *et al.*, 2011; Xia *et al.*, 2011; Li *et al.*, 2012; Salgado *et al.*, 2014; Mantello *et al.*, 2014). Transcriptome analysis of healthy and TPD - affected rubber trees was carried out to identify genes and pathways related to TPD (Liu *et al.*, 2015). In order to understand the molecular mechanisms involved in regulation of latex regeneration and duration of latex flow, Chao *et al.* (2015) analyzed latex from clone CATAS8-79 and PR107 at the transcriptome level.

In the present study, transcriptome sequencing was performed in leaf samples of *Hevea* exposed to drought stress as well as cold stress using Illumina sequencing technology (RNA-Seq) in order to generate functional genomic resource data which might eventually provide details on molecular mechanisms underlying drought/cold responses in *Hevea.* These data can further be employed in the molecular breeding based crop improvement programmes. Annotated transcriptome data of both drought and cold stress responsive transcripts was analyzed and the transcripts that exhibited interesting trend were further validated using quantitative gene expression analysis. This is the first systematic investigation undertaken to characterize transcriptomes responsive to drought and cold stress in *Hevea.* The results will enrich the available transcriptome data on *Hevea* and would sure contribute to discover genes or markers related to drought or cold tolerance that can be employed in molecular breeding of *Hevea* for abiotic stress tolerance.

4.2 Materials and methods

4.2.1. Plant material and stress induction

With regard to drought stress, one set of six month old poly bag plants of clone RRIM 600, acclimatized in the growth chamber was subjected to water stress by withholding irrigation for 10 days while the other set of plants was watered on alternate days. The degree of impact of drought stress on young plants was assessed by measuring the net CO_2 assimilation rate (A) and stomatal conductance (gs) with the aid of a portable photosynthesis system

(LI-6400 XT), LI-COR, U.S.A. All the gas exchange measurements were made as described in 3.2.2. and leaf samples from these plants were collected in liquid N_2 and stored at -80°C.

With regard to cold stress, six-months-old poly bag grown plants of clone RRIM 600 were acclimatized in a growth chamber for three days with a minimum temperature of 15 °C during night (for 3 h) and a gradual rise in maximum temperature (T max) up to 25 °C in the day time. Fourth day onwards, cold treatment was imposed by reducing the minimum temperature to 8 °C during night followed by a gradual increase in T max up to 16 °C in the day time for five consecutive days. Light intensity regime was provided between 400 and 800 μ mol m^{-2} s^{-1} and the relative humidity (RH) was maintained between 60 and 70%. Control plants were maintained at stress free ambient weather conditions.

4.2.2. Total RNA isolation and cDNA synthesis

Total RNA was extracted from the leaf samples using the Spectrum Plant Total RNA Kit (Sigma-Aldrich). Quality and quantity of the isolated RNA were checked by nanodrop and gel electrophoresis. RNA integrity was evaluated using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Poly RNA was isolated with oligoDT beads from TruSeq RNA sample preparation kit, Illumina. The purified mRNA were then fragmented with divalent cations at an elevated temperature (94 °C, 4 min) and reverse transcribed with superscript II reverse transcriptase by priming with random hexamers. DNA polymerase I was used for second strand synthesis in the presence of RNaseH.

4.2.3. Library construction and sequencing

Transcriptome library was constructed by employing Illumina TruSeq RNA library protocol. Illumina adapters were ligated to the cDNA followed by PCR amplification (11 cycles) for enrichment. Sequencing was carried out using Illumina Hi-Seq 2000 system (outsourced from M/s. Genotypic, Bangalore).

4.2.4. *De novo* assembly

Raw sequencing reads were subjected to quality filtering using SeqQC V2.1. Reads with adapter contamination were trimmed and low-quality bases were discarded. Further reads with $\geq$ 50 bases were filtered for assembly. The filtered sequencing reads were assembled using Oases 0.1.21 (Schultz *et al.*, 2012) assembler tool. Oases assembler is a robust transcriptome assembly program which generates contigs using Velvet 1.2.07 (Zerbino and Birney, 2008) algorithm. Further, the transcripts are constructed using contigs with the help of paired-end sequencing information.

4.2.5. Open reading frames (ORFs) and Pfam domain detection

The likely coding regions from transcripts sequence were identified using Trans Decoder program (http://transdecoder.sf.net). To support the ORF prediction, Pfam domain database search methods was setup using Pfam-A database (Finn, 2014). Hmmscan3 program was used for Pfam detection. Further, the transcripts sequences were subjected to multi-level filtration to obtain complete ORF sequences using in-house perl scripts.

4.2.6. Differential gene expression and gene ontology (GO) enrichment analysis

In order to construct the reference sequence for read mapping, the transcripts sequences from control and treated samples were clustered using CD-HIT tool with 90% sequence identity. Read mapping was carried out using Bowtie2 tool. DESeq (Anders and Huber, 2010) program was used for finding differentially expressed (DE) transcripts from mapping data. The DE transcripts were annotated using blast program against *Ricinus communis* protein sequence collections from UniProt. The aligned transcripts were further annotated using Gene Ontology (GO) terms (Ashburner *et al.*, 2000). Gene ontology enrichment analysis was carried out for differentially expressed transcripts. The enrichment analysis was performed using agriGO (Du, 2010) tool. AgriGO uses hypergeometric test with Benjamini-Hochberg FDR correction and a 'P' value < 0.05 was employed for finding significantly enriched GO terms. Due to the unavailability of well annotated whole genome sequences of *Hevea brasiliensis*, the closely related species *Ricinus communis* was used as a background for finding GO enrichment. After successful completion of enrichment analysis, the transcripts which were enriched under various biological processes were extracted for further analyses.

4.2.7. Validation by quantitative PCR

The clones *viz.,* RRIM 600, RRII 430, RRII 208, RRII 105 and RRII 414 were selected for validation of expression pattern of selected transcripts by quantitative PCR (qPCR) analyses in order to identify marker gene/s for drought and cold tolerance. Imposition of stress to the plants and leaf sample collection was carried out as described earlier. Total RNA was extracted from the leaf samples using Spectrum Plant Total RNA Kit (Sigma-Aldrich) and cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen) following manufacturer's instructions. Levels of expression of selected genes in plants under stress and control conditions were determined by qPCR. Attempts were made to validate about two dozen genes from the DGE libraries of both drought and cold treated plants by qPCR. Suitable primers were designed with Primer 3 Express (Applied Biosystems, USA) and were synthesized by M/s. Sigma-Aldrich India (Table 4.1). Further, quantitative gene expression analyses were carried out using Light Cycler 480 II, Roche Real Time PCR System as described in 3.2.5. The relative quantification (RQ) values were analyzed using Light Cycler 480 Software (release 1.5.0).

Table 4.1. List of Primers used in qPCR analysis

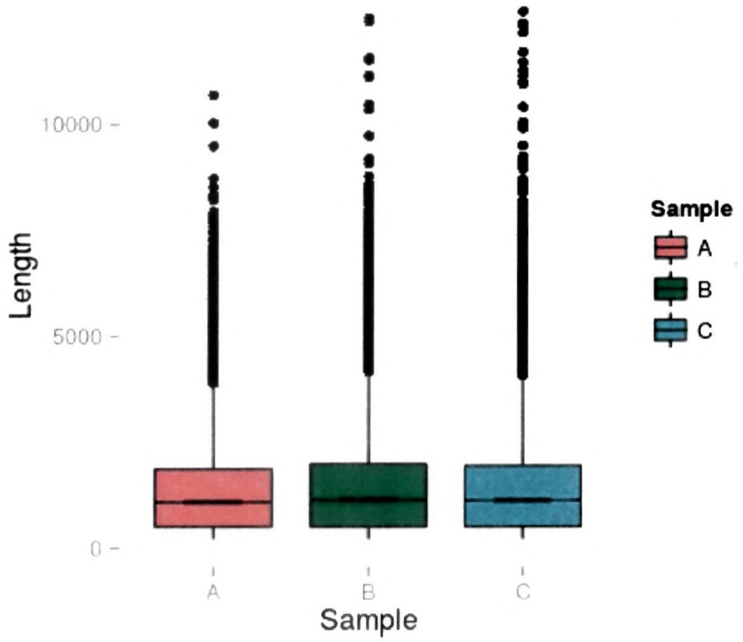
	ID No		Forward primer	Reverse primer
1	B_23441	pp2c	AAACGAAACAGAAGGAGAGATTAC	AGAGACGATGAAGGAGAG
2	B_5266	ferretin	CTAAACGAGAATAGAAAGCCCAAA	CAGAGCCACCATCCTTCAT
3	A_29734	MDCP	ACAAATTACAGAAGGAGAGATTAC	CAGCAACAATGGAAGCAAT
4	B_30709	GRX	GGTGAGTTGGTGCCTATT	GAAAGAAAACCCATCCATCAAA
5	B_19881	21 KDa	CTTGGCTCTTATCAATAACTATGC	CAACGAGAAAGGAGACATACAA
6	A_25234	ABCTP	AGGACTGTTATTGCTTCA	AATAGACTGTTCTGCCATA
7	B_11741	DNAbp	CTACGAGAAGAGAACAGA	AATGGATATGGAGTCACTA
8	A_7332	HSPbp	CAAGAGGCTTATTCAGTGTTATCA	CTTCATCATCATCATCTCCAA
9	A_5921	NACtf	TTCAATGGTGGCTTACTCT	CAAGACTGACTGGATTATGC
10	B_31589	MAPKKK	TAACAAGTCCTCCAATGATT	AGAGAAGACCGTGATACA
11	B_324	ERF	AGGATTATAGAGTCTTTGAGATTGA	GATAGTTCTTGTGGCTTGTAG
12	A_15463	MDH	ATAAGCACACCACCTCTC	CACTCAACCACTCAGGAT
13	A_28725	SCP	AAGGTTGACAGATGATGAAC	GGACTTCTACAGGCATTG
14	A_9060	PIP 2.1	GCGGGCTTTCCGTTTAGG	TGAGATCATCGCCACTTTCG
15	A_22946	Caspase	GTATGGAAAGGAACAAATGGT	GAAGTGTCAGCAGAGGTT
16	B_24758	TIP	CCCACTGGTCTCATGCCATTA	TGCCGCTATTGTGGCTTCTC
17	B_32804	DnaJ 11	CAAGTTGCCATGAGATAA	CAGACAAGGTAGAATAAGC
18	A_18065	HSP	TGAGAACATCGCGTTTGAGAA	TGGTACTGGCTTTAGGGATGGT
19	A_27682	OEC	GAAGAAGGCGAGGAAAGTGAAA	TGGTGGGTTGCATGAGTGTT
20	A_20212	ERF d	TTAGGCAACATTGATTGATTG	CATTCTTCAGTGGGTTACAA
21	A_16967	DRE bp	CCTGTATTGATGTGGATGTT	AACCAAGACGAGAATATGC
22	C_591	ERF U	TGGTGTTGAAGAGAAAAGGCATTAC	GATCTCCGCTGCGTATTTGC
23	C_1073	AUX/H Sym	CCCATCTGTGCTACATTTTCTGA	GTCCTGATTGCAGCTCTATGCA
24	C_9761	TF	CGTCGGTGTCATTAACTTC	CTATCAGTATGCGGTGGAA

4.3 RESULTS

4.3.1. *De novo* assembly and annotation

The filtered sequencing reads were assembled using Velvet/Oases transcriptome assembly program. Finding optimum kmer for RNA-Seq data is crucial for *de novo* assembly. This was achieved by performing multiple assemblies using different kmer sizes. The expected coverage and coverage cut off parameters were set as "auto" for the assembly runs. Kmer of 49, 51 and 49 was considered as optimum for control (A), drought (B) and cold (C) samples respectively. Around 88 % reads participated in the assembly of control sample and more than 90 % reads participated in drought/cold treated samples. In total, 90735 transcripts were generated in control, whereas as 104071 and 100120 transcripts were generated in drought and cold treated samples respectively. The N50 value is above 2 kb in both control and

Fig 4.1. Boxplot representation of transcript length



treated samples. The complete assembly statistics is given in Table 4.2. The boxplot of transcript length comparison is shown in Fig.4.1. Sequence clustering was performed to obtain non-redundant transcript sequences. The sequence clustering reduced the total count of transcripts to 58581, 67482 and 66019 in control, drought and cold treated samples respectively. The N50 statistics was still around 2 kb in these samples. The main aim of the sequence clustering was to avoid multiple hits in Open Reading Frame (ORF) and Pfam domain detection analyses. The maximum transcript lengths remained unchanged in all samples as expected of good clustering.

Table 4.2. Statistics of *de novo* transcriptome assembly

Sample	A	B	C
Kmer size	49	51	49
Total transcripts	90735	104071	100120
Maximum Transcript Length	10703	12514	12673
Minimum Transcript Length	200	200	200
Average Transcript Length	1454	1520	1505
Total Transcripts Length	133349657	160587432	152465368
N50 value	2046	2174	2128
Number of reads used	56312064	69710392	64217435
Total number of reads	63385498	77294164	70357568
Percentage of reads used	88.84%	90.19%	91.27%

4.3.2. Open Reading Frames (ORFs) and Pfam domain detection

Multi-level filtering such as (i) extract complete ORFs and filter sequences with partial ORFs (ii) discard sequences with ambiguities (N) and (iii) filter sequences which containing more than one ORF were applied on the predicted likely coding regions from transcripts sequences. After multi-level ORF filtration, 18789 final ORFs were obtained in control sample. Similar filtration was applied on drought and cold samples which yielded 21308 and 21077 ORFs respectively. The protein sequences obtained from

ORF pipeline were searched against pfam-A database for domain detection. ORFs with pfam domain hits were retained in the results. In total 32050, 35691 and 36313 transcript sequences were found to have at least one pfam domain hit in control, drought and cold samples respectively. The top 30 pfam domain hits from all three samples are given in (Fig 4.2). High abundance of pentatricopeptide repeat (PPR) motif was found in all three samples which had been found commonly present across the plant kingdom (Mingler, 2006).

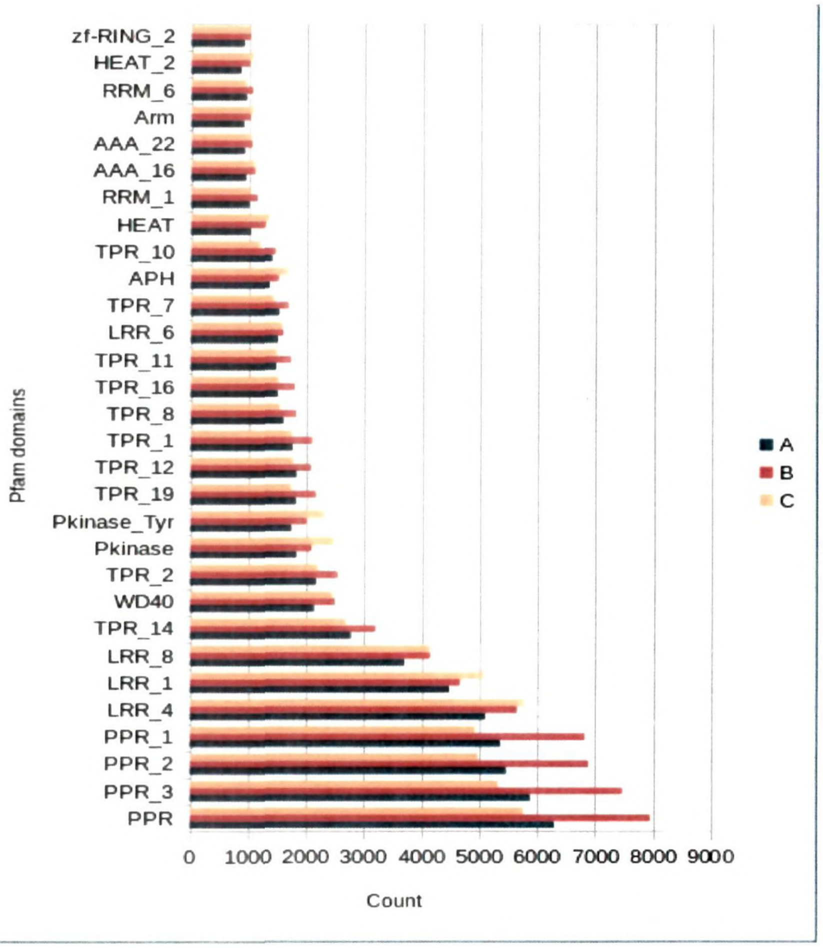


Fig 4.2. Top 30 Pfam domain detected in control, drought and cold treated samples (A – control, B – drought, C – cold)

4.3.3. Differential gene expression and gene ontology (GO) enrichment analysis

In total, 834 transcripts were identified as differentially expressed (DE) between control and drought stressed samples. Among the differentially expressed transcripts, 268 and 566 transcripts were found up and down regulated respectively. The differentially expressed transcripts were annotated against *Ricinus communis* protein database using homology search. Gene Ontology (GO) terms mapping was carried out for DE transcripts in order to classify them into biological process, molecular function and cellular component categories. A total of 292 GO terms were assigned to DE transcripts. Further, GO term enrichment analysis was performed using *Ricinus communis* as background reference. Significantly enriched DE drought responsive transcripts were extracted and their expression values were tabulated for further analyses and the GO term enrichment analysis result is given in Table 4.3 and 4.4. The majority of the DE transcripts were enriched under metabolic process (GO: 0008152) and cellular process (GO: 0009987) categories. In total, 130 down-regulated transcripts were grouped under metabolic process, whereas 59 up-regulated transcripts were assigned to the same process. A total of 180 DE transcripts were enriched under cellular process. Other biological processes such as carbohydrate metabolic process (GO: 0005975), primary metabolic process (GO: 0044238) and cellular amino acid and derivative metabolic process (GO: 0006519) were also found significantly enriched.

Similarly, 961 up-regulated and 109 down-regulated transcripts from control and cold stressed samples were mapped to a total of 353 Gene Ontology (GO) terms. These transcripts were further subjected to GO enrichment analysis. Biological processes such as response to stress (GO:0006950), response to stimulus (GO:0050896), protein folding (GO:0006457), photosynthesis (GO:0015979), response to oxidative stress

Table 4.3: GO term enrichment result for down regulated transcripts under drought stress condition

GO_acc	term_type	Term	Query item	Query total	Bg item	Bg total	P value	FDR
GO:0008152	P	metabolic process	130	207	7068	19659	5.7E-15	3.1E-13
GO:0005975	P	carbohydrate metabolic process	37	207	735	19659	6.2E-15	3.1E-13
GO:0009987	P	cellular process	112	207	5664	19659	3.4E-14	1.1E-12
GO:0051179	P	localization	42	207	1313	19659	1.4E-10	3.5E-09
GO:0044238	P	primary metabolic process	99	207	5309	19659	1.8E-10	3.5E-09
GO:0006810	P	transport	41	207	1287	19659	2.8E-10	3.9E-09
GO:0051234	P	establishment of localization	41	207	1287	19659	2.8E-10	3.9E-09
GO:0006519	P	cellular amino acid and metabolic process	17	207	232	19659	1.1E-09	1.4E-08
GO:0009058	P	biosynthetic process	52	207	2478	19659	0.00000087	0.0000097
GO:0050896	P	response to stimulus	17	207	494	19659	0.000027	0.00026
GO:0044237	P	cellular metabolic process	75	207	4616	19659	0.000028	0.00026
GO:0006950	P	response to stress	14	207	370	19659	0.000053	0.00045
GO:0006629	P	lipid metabolic process	14	207	410	19659	0.00015	0.0012
GO:0044249	P	cellular biosynthetic process	39	207	2279	19659	0.0017	0.012

Table 4.4: GO term enrichment result for up-regulated transcripts under drought stress condition

GO_acc	Term type	Term	query item	query total	bg item	bg total	p value	FDR
GO:0009987	P	cellular process	68	84	5664	19659	6.9E-23	1.9E-21
GO:0044237	P	cellular metabolic process	55	84	4616	19659	4.4E-16	6.1E-15
GO:0044238	P	primary metabolic process	55	84	5309	19659	2.4E-13	2.3E-12
GO:0044249	P	cellular biosynthetic process	35	84	2279	19659	3.2E-12	2.2E-11
GO:0009058	P	biosynthetic process	36	84	2478	19659	6.6E-12	3.7E-11
GO:0006807	P	nitrogen compound metabolic process	33	84	2240	19659	5.5E-11	2.5E-10
GO:0008152	P	metabolic process	59	84	7068	19659	2E-10	7.9E-10
GO:0044260	P	cellular macromolecule metabolic process	40	84	3605	19659	1E-09	3.6E-09
GO:0043170	P	macromolecule metabolic process	42	84	4081	19659	3E-09	9.2E-09
GO:0006519	P	cellular amino acid and metabolic process	11	84	232	19659	6.6E-09	1.8E-08
GO:0006350	P	transcription	18	84	1145	19659	0.0000015	0.0000038
GO:0065007	P	biological regulation	20	84	1559	19659	0.0000076	0.000018
GO:0034645	P	cellular macromolecule biosynthetic process	20	84	1766	19659	0.000045	0.000095
GO:0009059	P	macromolecule biosynthetic process	20	84	1772	19659	0.000048	0.000095
GO:0010467	P	gene expression	19	84	1722	19659	0.0001	0.00019
GO:0006139	P	nucleic acid metabolic process	20	84	1934	19659	0.00016	0.00028
GO:0050794	P	regulation of cellular process	15	84	1403	19659	0.00087	0.0014
GO:0050789	P	regulation of biological process	15	84	1486	19659	0.0015	0.0024
GO:0006464	P	protein modification process	13	84	1276	19659	0.003	0.0044
GO:0043412	P	macromolecule modification	13	84	1333	19659	0.0044	0.0061
GO:0044267	P	cellular protein metabolic process	16	84	1887	19659	0.006	0.008
GO:0010468	P	regulation of gene expression	11	84	1086	19659	0.0067	0.0085
GO:0019538	P	protein metabolic process	18	84	2319	19659	0.0086	0.01
GO:0060255	P	regulation of macromolecule metabolic process	11	84	1135	19659	0.0091	0.011
GO:0019222	P	regulation of metabolic process	11	84	1175	19659	0.012	0.013

Table 4.5: GO term enrichment result for down regulated transcripts under cold stress

GO_acc	term type	Term	query item	query total	bg item	bg total	p value	FDR
GO:0006950	P	response to stress	14	50	370	19659	4.5E-13	6.7E-12
GO:0050896	P	response to stimulus	14	50	494	19659	1.9E-11	1.4E-10
GO:0006457	P	protein folding	7	50	69	19659	8.4E-10	4.2E-09
GO:0015979	P	photosynthesis	7	50	88	19659	4.1E-09	1.5E-08
GO:0006979	P	response to oxidative stress	5	50	101	19659	0.0000072	0.000021
GO:0042221	P	response to chemical stimulus	5	50	204	19659	0.00018	0.00046
GO:0019538	P	protein metabolic process	14	50	2319	19659	0.0015	0.0033
GO:0006508	P	proteolysis	6	50	521	19659	0.0021	0.0039
GO:0009987	P	cellular process	24	50	5664	19659	0.0032	0.0048
GO:0008152	P	metabolic process	28	50	7068	19659	0.003	0.0048
GO:0044237	P	cellular metabolic process	19	50	4616	19659	0.015	0.021
GO:0044238	P	primary metabolic process	20	50	5309	19659	0.032	0.04
GO:0043170	P	macromolecule metabolic process	16	50	4081	19659	0.042	0.049

Table 4.6: GO term enrichment result for up-regulated transcripts under cold stress.

GO_acc	term type	Term	query item	query total	bg item	bg total	p value	FDR
GO:0009987	P	cellular process	184	301	5664	19659	8.8E-31	8.4E-29
GO:0007242	P	intracellular signaling cascade	28	301	58	19659	4.3E-30	2.1E-28
GO:0044238	P	primary metabolic process	162	301	5309	19659	2E-22	6.6E-21
GO:0008152	P	metabolic process	181	301	7068	19659	2.5E-17	6E-16
GO:0016311	P	dephosphorylation	14	301	25	19659	2.5E-16	4.1E-15
GO:0046700	P	heterocycle catabolic process	12	301	12	19659	2.6E-16	4.1E-15
GO:0006470	P	protein amino acid dephosphorylation	13	301	23	19659	2.7E-15	3.7E-14
GO:0008643	P	carbohydrate transport	11	301	14	19659	2.8E-14	3.4E-13
GO:0046034	P	ATP metabolic process	19	301	113	19659	1.2E-13	1.3E-12
GO:0009199	P	ribonucleoside triphosphate metabolic process	19	301	119	19659	2.8E-13	2.1E-12
GO:0046483	P	heterocycle metabolic process	27	301	284	19659	2.6E-13	2.1E-12
GO:0009205	P	purine ribonucleoside triphosphate metabolic process	19	301	119	19659	2.8E-13	2.1E-12
GO:0009144	P	purine nucleoside triphosphate metabolic process	19	301	119	19659	2.8E-13	2.1E-12
GO:0009141	P	nucleoside triphosphate metabolic process	19	301	120	19659	3.2E-13	2.2E-12
GO:0005975	P	carbohydrate metabolic process	42	301	735	19659	6.7E-13	4.3E-12
GO:0009150	P	purine ribonucleotide metabolic process	19	301	128	19659	9E-13	5.4E-12
GO:0009259	P	ribonucleotide metabolic process	19	301	131	19659	1.3E-12	7.4E-12
GO:0032501	P	multicellular organismal process	10	301	16	19659	2.2E-12	1.1E-11
GO:0044237	P	cellular metabolic process	126	301	4616	19659	2.2E-12	1.1E-11
GO:0006163	P	purine nucleotide metabolic process	19	301	138	19659	3E-12	1.4E-11
GO:0006073	P	cellular glucan metabolic process	15	301	73	19659	4E-12	1.8E-11
GO:0007165	P	signal transduction	28	301	349	19659	4.2E-12	1.8E-11
GO:0044042	P	glucan metabolic process	15	301	74	19659	4.7E-12	2E-11
GO:0055086	P	nucleotide metabolic process	23	301	232	19659	7.4E-12	3E-11

GO:0000003	P	reproduction	13	301	52	19659	1.3E-11	5E-11
GO:0044264	P	cellular polysaccharide metabolic process	15	301	92	19659	7.3E-11	2.7E-10
GO:0006807	P	*nitrogen compound metabolic process*	74	301	2240	19659	1.9E-10	6.9E-10
GO:0016998	P	cell wall macromolecule catabolic process	11	301	41	19659	2.7E-10	9.1E-10
GO:0009117	P	nucleotide metabolic process	19	301	191	19659	4.9E-10	1.6E-09
GO:0006753	P	nucleoside phosphate metabolic process	19	301	191	19659	4.9E-10	1.6E-09
GO:0044036	P	cell wall macromolecule metabolic process	11	301	44	19659	5.1E-10	1.6E-09
GO:0044262	P	cellular carbohydrate metabolic process	21	301	243	19659	6.4E-10	1.9E-09
GO:0005976	P	polysaccharide metabolic process	15	301	119	19659	1.8E-09	5.3E-09
GO:0006629	P	lipid metabolic process	26	301	410	19659	2.9E-09	8.1E-09
GO:0006351	P	transcription, DNA-dependent	35	301	737	19659	6.9E-09	1.9E-08
GO:0032774	P	RNA biosynthetic process	35	301	741	19659	7.8E-09	2.1E-08
GO:0051704	P	multi-organism process	11	301	61	19659	0.00000001	2.6E-08
GO:0009875	P	pollen-pistil interaction	10	301	49	19659	1.7E-08	3.8E-08
GO:0006810	P	transport	48	301	1287	19659	1.7E-08	3.8E-08
GO:0051234	P	establishment of localization	48	301	1287	19659	1.7E-08	3.8E-08
GO:0008037	P	cell recognition	10	301	49	19659	1.7E-08	3.8E-08
GO:0048544	P	recognition of pollen	10	301	49	19659	1.7E-08	3.8E-08
GO:0009856	P	pollination	10	301	49	19659	1.7E-08	3.8E-08
GO:0022414	P	reproductive process	10	301	51	19659	2.4E-08	5.2E-08
GO:0051179	P	localization	48	301	1313	19659	3.1E-08	6.5E-08
GO:0006950	P	response to stress	23	301	370	19659	3.4E-08	7.2E-08
GO:0006139	P	nucleotide metabolic process	60	301	1934	19659	0.00000014	0.00000028
GO:0006979	P	response to oxidative stress	12	301	101	19659	0.00000014	0.00000028
GO:0050896	P	response to stimulus	25	301	494	19659	0.00000037	0.00000072
GO:0050794	P	regulation of cellular process	46	301	1403	19659	0.0000012	0.0000024
GO:0044249	P	cellular biosynthetic process	64	301	2279	19659	0.0000015	0.0000029
GO:0034641	P	cellular nitrogen compound metabolic process	15	301	226	19659	0.0000042	0.0000077

GO:0030001	P	metal ion transport	12	301	146	19659	0.0000052	0.0000093
GO:0016070	P	RNA metabolic process	35	301	987	19659	0.0000053	0.0000094
GO:0050789	P	regulation of biological process	46	301	1486	19659	0.0000055	0.0000096
GO:0042221	P	response to chemical stimulus	14	301	204	19659	0.0000061	0.00001
GO:0044248	P	cellular catabolic process	14	301	221	19659	0.000014	0.000024
GO:0065007	P	biological regulation	46	301	1559	19659	0.000018	0.00003
GO:0009056	P	catabolic process	27	301	705	19659	0.000019	0.000031
GO:0009058	P	biosynthetic process	64	301	2478	19659	0.000022	0.000035
GO:0055085	P	transmembrane transport	10	301	124	19659	0.000037	0.000058
GO:0006350	P	transcription	35	301	1145	19659	0.0001	0.00016
GO:0043170	P	macromolecule metabolic process	88	301	4081	19659	0.00035	0.00053
GO:0016043	P	cellular component organization	12	301	248	19659	0.00062	0.00093
GO:0006812	P	cation transport	12	301	300	19659	0.0029	0.0043
GO:0006519	P	cellular amino acid and derivative metabolic process	10	301	232	19659	0.0038	0.0056
GO:0044260	P	cellular macromolecule metabolic process	74	301	3605	19659	0.0044	0.0064
GO:0006811	P	ion transport	12	301	358	19659	0.011	0.015
GO:0043436	P	oxoacid metabolic process	10	301	326	19659	0.032	0.044
GO:0019752	P	carboxylic acid metabolic process	10	301	326	19659	0.032	0.044
GO:0006082	P	organic acid metabolic process	10	301	327	19659	0.032	0.044
GO:0042180	P	cellular ketone metabolic process	10	301	329	19659	0.034	0.045
GO:0010467	P	gene expression	36	301	1722	19659	0.037	0.049

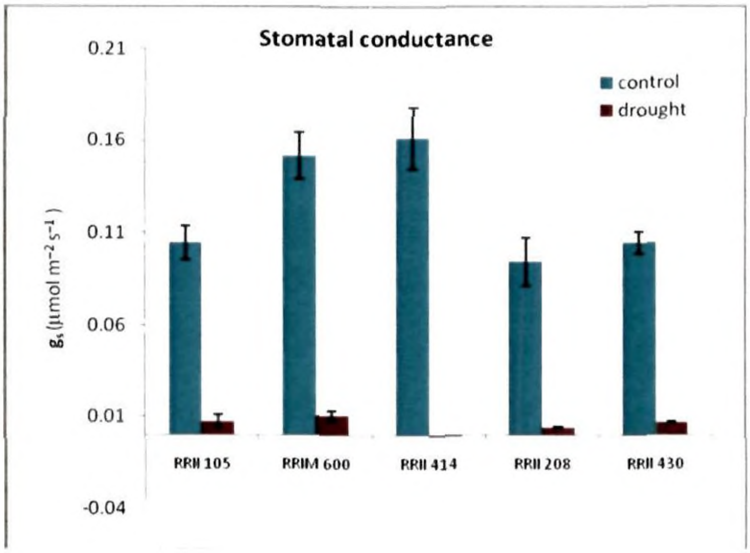
(GO:0006979), response to chemical stimulus (GO:0042221), protein metabolic process (GO:0019538), proteolysis (GO:0006508), cellular process (GO:0009987), metabolic process (GO:0008152), cellular metabolic process (GO:0044237), primary metabolic process (GO:0044238) and macromolecule metabolic process (GO:0043170) were found enriched in down-regulated transcripts. A high percentage of up-regulated transcripts were enriched under metabolic process (GO: 0008152), cellular process (GO: 0009987), primary metabolic process (GO: 0044238) and cellular metabolic process (GO: 0044237) categories. The GO term enrichment analysis result for control and cold stressed samples is given in Table 4. 5 and 4.6.

4.3.4. Validation of RNA-Seq results by qPCR

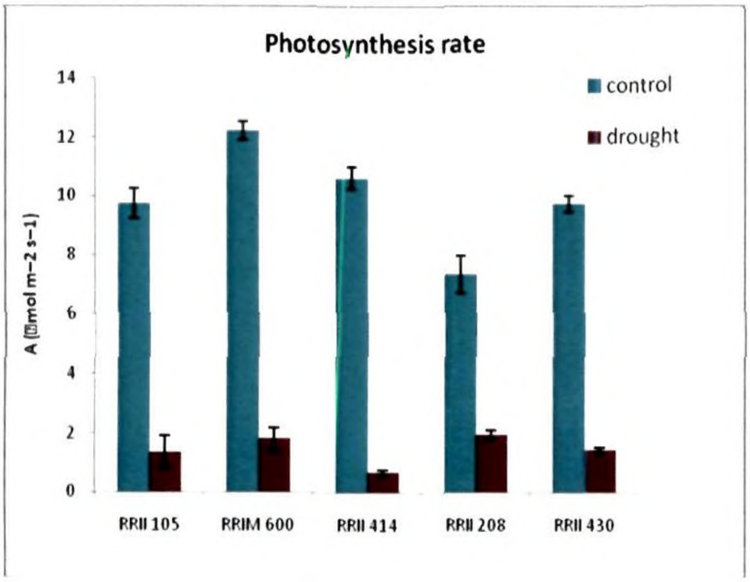
4.3.4.1. Expression analysis of drought responsive transcripts in different clones of *Hevea*

In order to determine the expression levels of drought specific transcripts from transcriptome sequencing, five clones of *Hevea viz.* RRII 105, RRIM 600, RRII 414, RRII 208 and RRII 430 with varying levels of drought tolerance were exposed to drought and further were subjected to gene expression analysis by qPCR. Prior to qPCR, physiological parameters were measured in drought exposed leaves of clones mentioned above. Reduction in stomatal conductance was noticed in all the clones under drought stress, while it was maximum in RRII 414 (Fig 4.3a). Similarly, reduction in CO_2 assimilation rate was found in all the clones, with clone RRII 414 being completely inhibited (Fig 4.3b).

Expression analysis was carried out in the above *Hevea* clones with qPCR being confined to 17 transcripts short-listed based on primer standardization (Table 4.7; Fig 4.4). Among the transcripts investigated, ferritin, DNA binding protein RAV1, NAC tf were found significantly up-regulated in tolerant clones *viz.* RRIM 600, RRII 208 and RRII 430 when compared to RRII 105 and RRII 414 which is moderately tolerant and susceptible clone respectively.



a



b

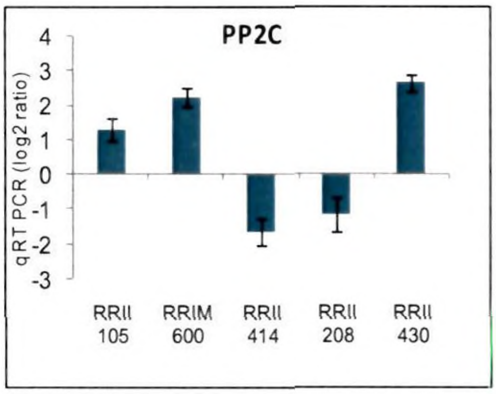
Fig.4.3 (a & b). Stomatal conductance and CO_2 assimilation rate in irrigated and drought imposed polybag plants of *Hevea*

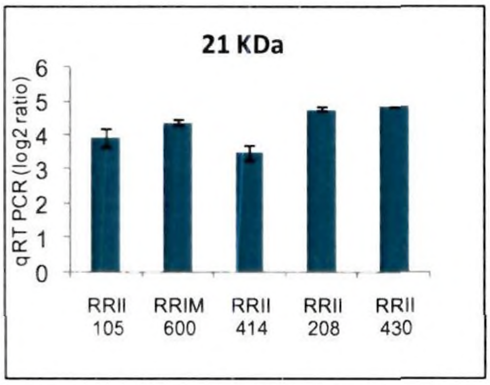
Table 4.7. Relative quantification of seventeen genes in five clones of *Hevea* under drought stress with its own control as calibrator

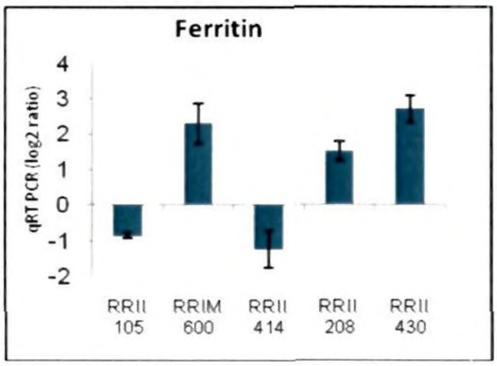
Transcripts	RRII 105	RRIM 600	RRII 414	RRII 208	RRII 430
PP2C	2.522	4.794	0.333	0.483	6.287
Ferritin	0.557	**5.68**	0.486	2.96	7.042
MDCP	0.748	**4.93**	0.153	0.699	12.417
GRX	7.741	**7.11**	**7.797**	**9.846**	9.848
21KDa	3.927	4.398	3.496	4.776	4.845
ABCT	0.716	0.499	0.003	1.305	1.089
DNAbp	2.35	3.913	0.378	3.338	25.434
HSPbp	3.704	0.588	0.01	0.024	0.059
NAC tf	1.072	6.676	1.76	16.217	20.166
MAP3K	7.108	8.743	3.31	11.614	8.702
ERF	0.542	2.686	0.583	2.523	1.31
MDH	0.464	0.581	0.239	0.59	0.255
SCP	0.454	1.194	0.162	1.109	0.562
Aquaporin	2.762	0.709	6.426	0.313	0.32
Caspase	3.033	0.871	0.384	0.687	5.621
TIP	7.409	14.532	2.757	4.222	12.056
Dnaj 11	2.014	2.268	13.22	34.79	3.234

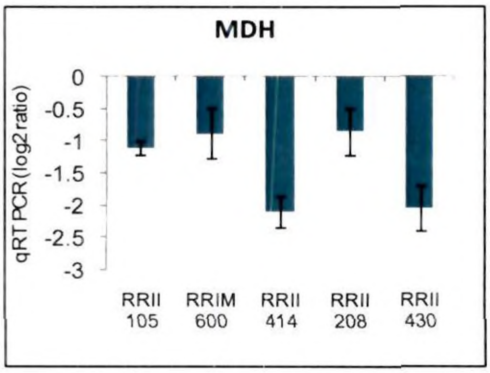
Significant up-regulation (gene expression > 2 fold)

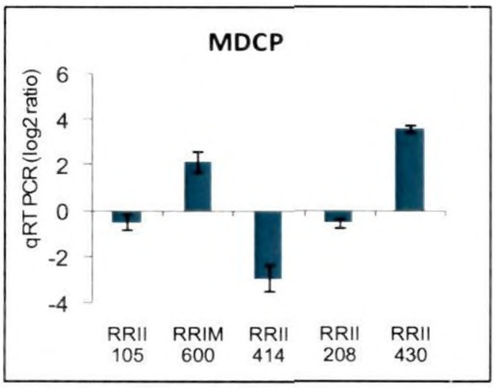
Significant down regulation (gene expression < 0.5 fold)

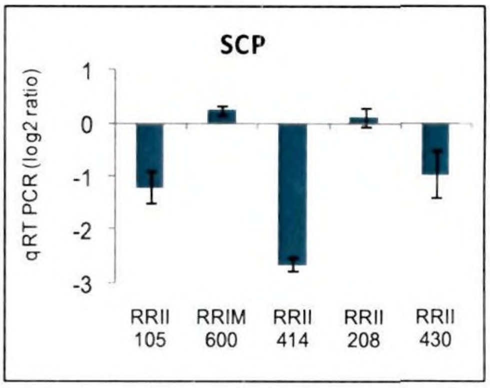

PP2C
qRT PCR (log2 ratio)
4
3
2
1
0
-1
-2
-3
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

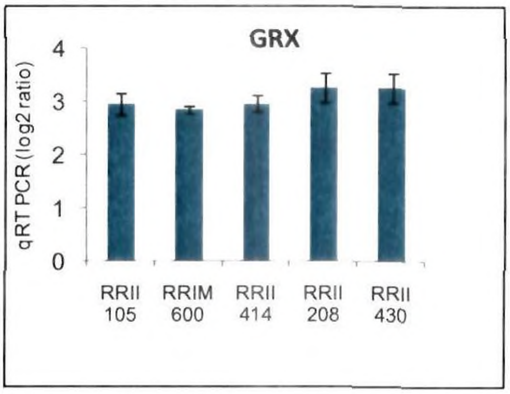

21 KDa
qRT PCR (log2 ratio)
6
5
4
3
2
1
0
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

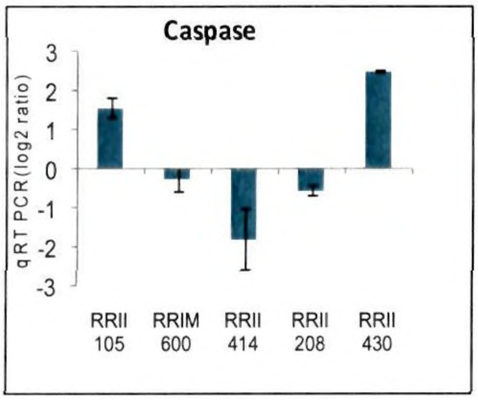

Ferritin
qRT PCR (log2 ratio)
4
3
2
1
0
-1
-2
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

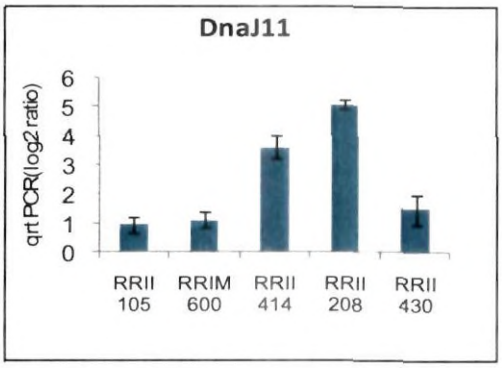

MDH
qRT PCR (log2 ratio)
0
-0.5
-1
-1.5
-2
-2.5
-3
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

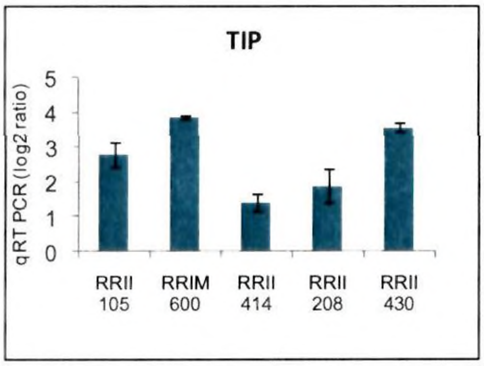

MDCP
qRT PCR (log2 ratio)
6
4
2
0
-2
-4
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

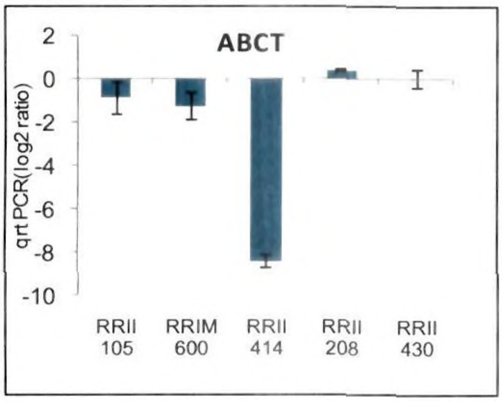

SCP
qRT PCR (log2 ratio)
1
0
-1
-2
-3
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

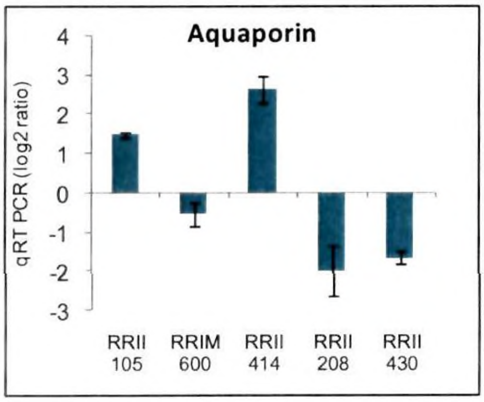

GRX
qRT PCR (log2 ratio)
4
3
2
1
0
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430


Caspase
qRT PCR (log2 ratio)
3
2
1
0
-1
-2
-3
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430


DnaJ11
qrt PCR(log2 ratio)
6
5
4
3
2
1
0
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430


TIP
qRT PCR (log2 ratio)
5
4
3
2
1
0
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430


ABCT
qrt PCR(log2 ratio)
2
0
-2
-4
-6
-8
-10
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430


Aquaporin
qRT PCR (log2 ratio)
4
3
2
1
0
-1
-2
-3
RRII 105
RRIM 600
RRII 414
RRII 208
RRII 430

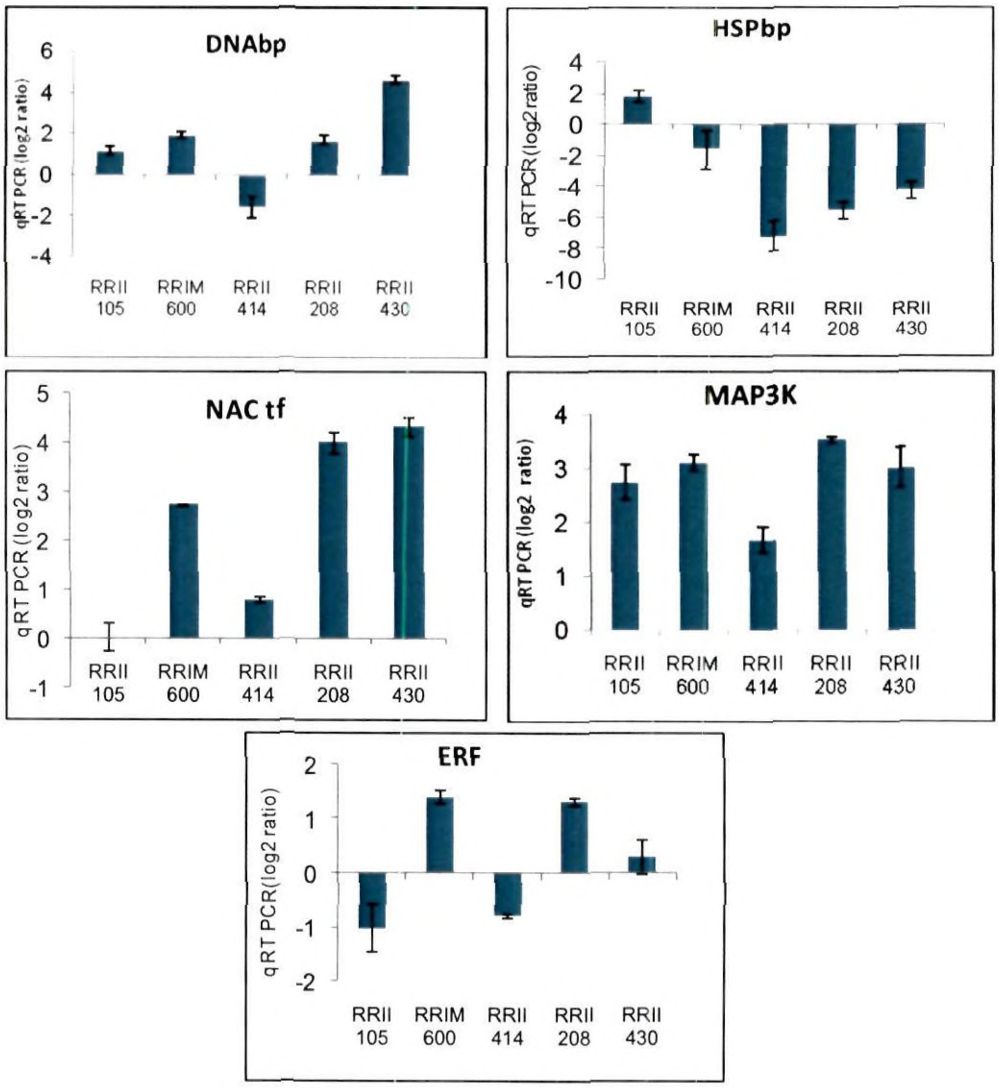
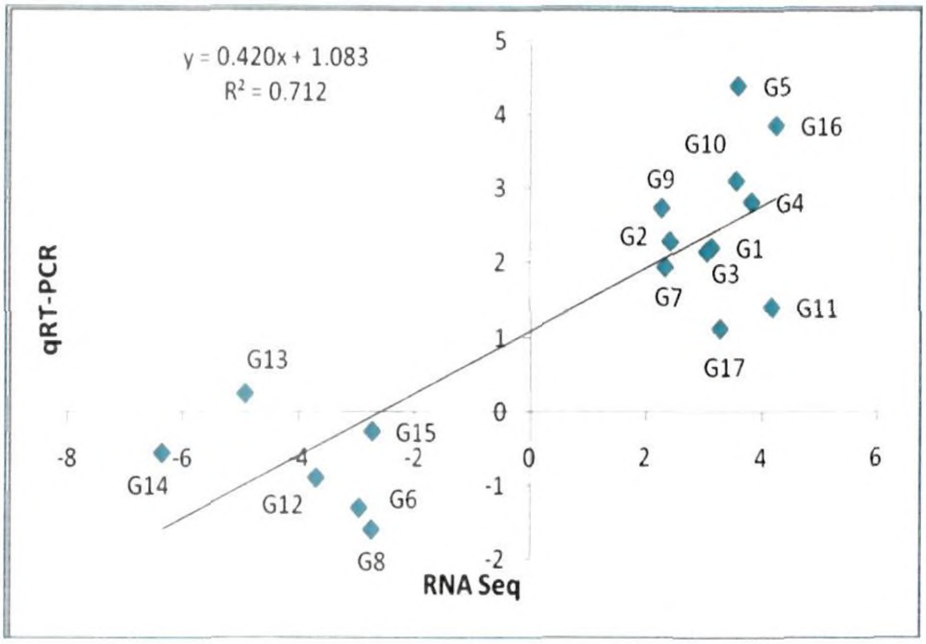
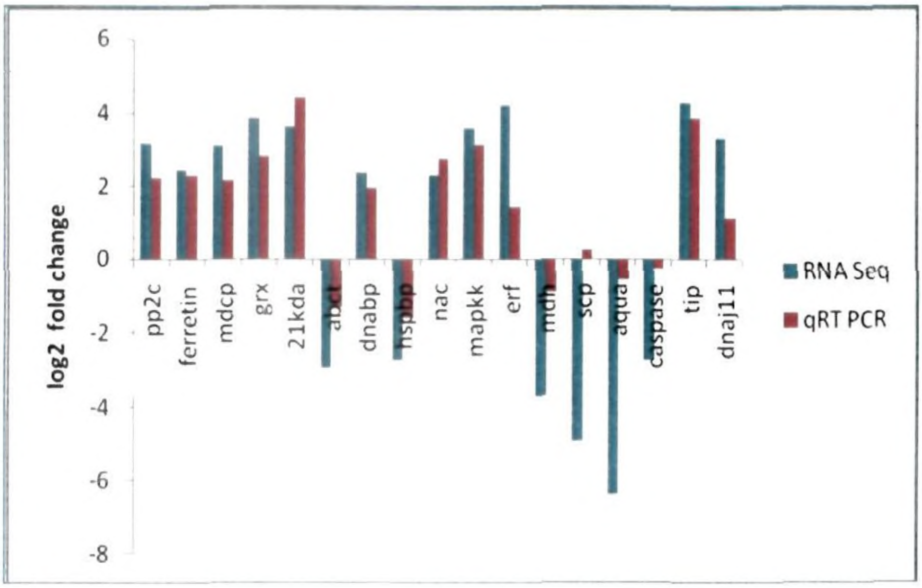


Fig 4.4. Expression analysis of seventeen genes in five clones of drought stress treated *Hevea* with its own control as calibrator (Fold change in log2 ratio). Log 2 ratio >0 are considered up-regulated; < 0 are considered down regulated. ± Error bars indicate SE of three biological replicates



a



b

Fig 4.5. qPCR validation of differentially expressed genes of *Hevea* clone RRIM 600.a. Correlation of fold change (log 2) analyzed by RNA-Seq (x axis) with data obtained using (y axis) b. Expression analysis of selected differentially expressed transcripts between RNA Seq and qRT- PCR

MAP kinase kinase kinase (MAP3K) and tonoplast intrinsic protein (TIP) were found up-regulated in all the clones studied, while their expression was significantly higher in tolerant clones. Interestingly, expression of aquaporin (Pip2.1) was found significantly lower in tolerant clones. The expression of glutaredoxin, 21 KDa protein and Dna J11 protein was found significantly higher in all the clones suggesting their drought responsiveness.

To confirm the accuracy and the reproducibility of the Illumina RNA-Seq results, quantitative PCR (qPCR) was performed on 17 selected transcripts with increased or decreased transcript abundance in clone RRIM 600. The correlation between RNA-Seq and qPCR was made using the log 2 fold change value determined between RNA-Seq and qPCR data by comparative Ct method ($2^{-\Delta\Delta C_t}$). qPCR results revealed the existence of similar trend in expression of these transcripts ($R^2 = 0.712$) with the RNA-Seq results as depicted in Fig 4.5 (a&b) confirming the accuracy and reproducibility of RNA-Seq results. Even though the exact fold change of the selected transcripts varied between RNA-Seq and qPCR analyses, all the seventeen transcripts exhibited similar trends in expression pattern with RNA-Seq data.

4.3.4.2. Expression analysis of cold responsive transcripts in *Hevea* clones

The plants grown under growth chamber conditions showed cold stress responsive syndrome after cold treatment at 8 °C during night and at 15 °C during day time for five days. Physiological parameters such as stomatal conductance (gs), net CO_2 assimilation rate (A) and quantum efficiency of PS II indicated the impact of stress in both the clones. Stomatal conductance in susceptible clone RRII 105 came down drastically from about 0.11 mol m^{-2} s^{-1} to near zero under cold stress while the tolerant clone RRIM 600 could maintain at about 0.04 mol m^{-2} s^{-1} (from 0.13 mol m^{-2} s^{-1} in control condition (Fig 4.6a).

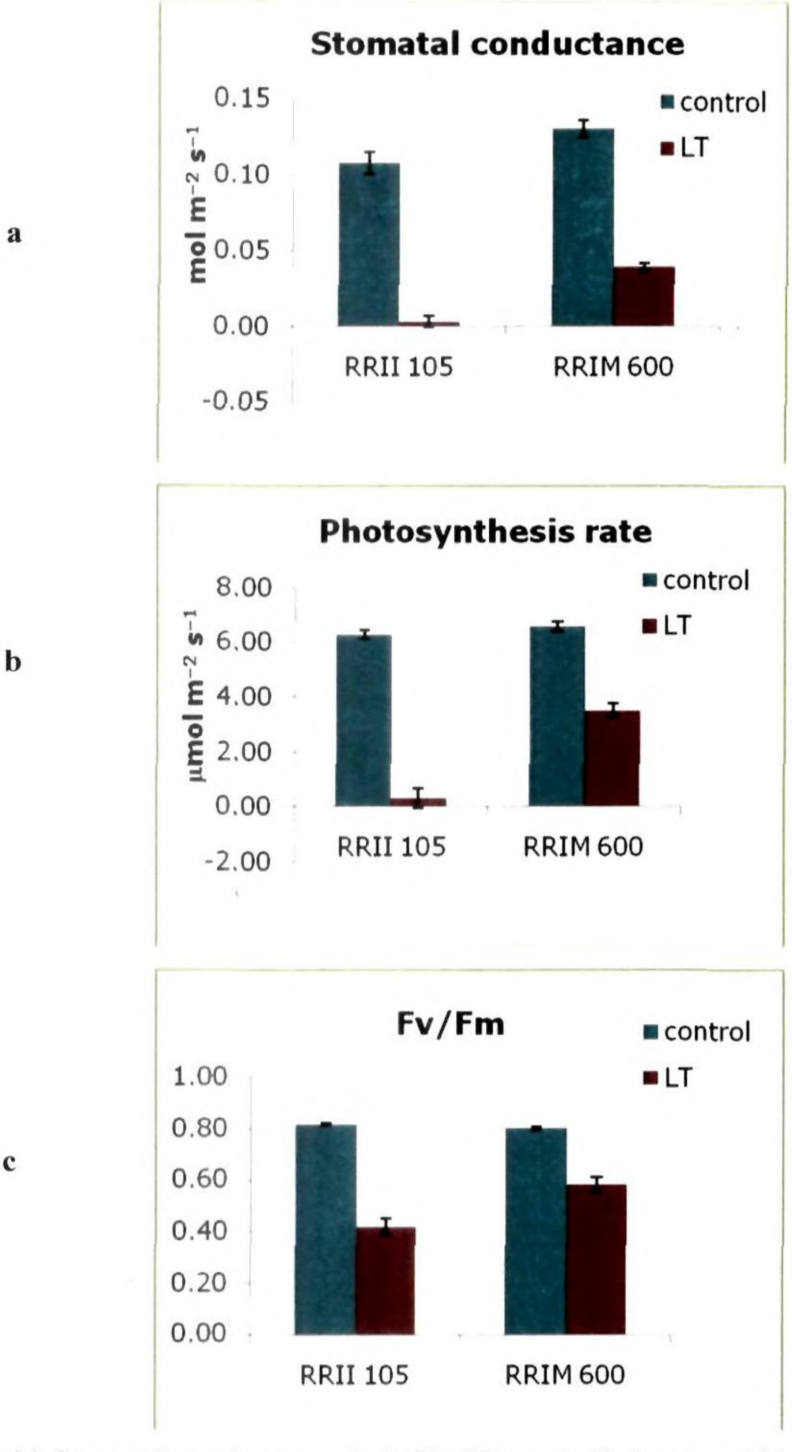


Fig 4.6. (a) Stomatal conductance (g_s), (b) CO_2 assimilation rate (A) and (c) Fv/Fm of control and low temperature (LT) treated plants of RRII 105 and RRIM 600

While both the clones maintained A at about 6 to 7 μmol m^{-2} s^{-1} in control conditions, RRIM 600maintained better A (3 μmol m^{-2} s^{-1}) than RRII 105 (near 0) upon exposure to cold stress (Fig 4.6 b). Similarly, the F_v/F_m ratio also was found better in RRIM 600 (0.6) than RRII 105 (0.3) under low temperature condition (Fig 4.6c).

Expression analysis was carried out for seven transcripts in the above *Hevea* clones which vary in their cold tolerance levels (Table 4.8; Fig.4.7). Among the transcripts investigated, an ethylene responsive transcription factor was found significantly up-regulated in RRIM 600, than RRII 105. Other transcripts like heat shock protein, drought responsive element binding protein, auxin/hydrogen symporter were found up-regulated in both the clones. The transcripts like oxygen evolving enhancer protein 1 and a transcription factor displayed a downward trend in both the clones.

Table 4.8. *Relative quantification of seven genes under cold stress in Hevea* clones RRII 105 and RRIM 600 with its own control as calibrator.

Gene	Transcript Id	RRII 105	RRIM 600
HSP	A_18065	52.555	45.385
OEP	A_27682	0.5302	0.329167
ERF d	A_20212	1.443333	0.878933
DREbp	C_16967	6.036667	4.67
ERFu	C_591	2.444	10.17
AUX/H sym	C_1073	3.524333	2.774333
TF	C_9761	0.663333	0.643667

Significant up-regulation (gene expression > 2 fold)

Significant down regulation (gene expression < 0.5 fold)

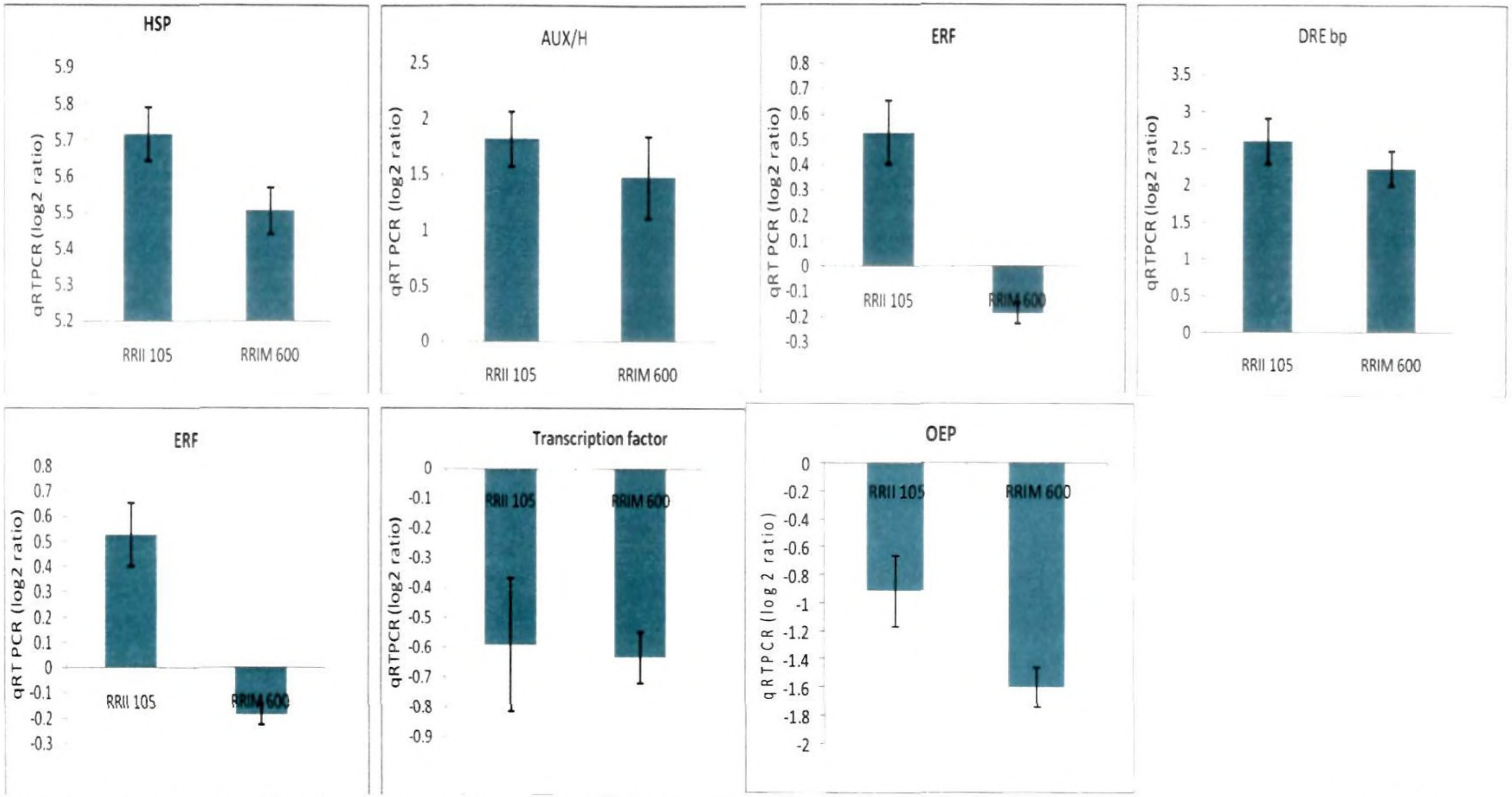


Fig 4.7. Relative quantification of seven genes in two clones of cold stressed *Hevea* (Fold change in log 2 ratio). Log 2 ratio >0 are considered as up-regulated; < 0 are considered as down regulated. ± Error bars indicate SE of three biological replicates.

4.4 Discussion

Next generation sequencing (NGS) technologies have wide-spread applications in crop improvement. In particular, RNA-Seq is widely used for profiling the transcriptome of any plant species. Applying RNA-Seq technology on plant breeding coupled with conventional approaches is a great combination to understand the molecular basis of complex traits. (Pérez-de-Castro *et al.*, 2012). During the past decade many novel assembly methods have been developed to assemble the sequencing reads from multiple next generation sequencing technologies. Such methods have been successfully employed in *de novo* assembly of transcriptome sequencing data from non-model organisms.

In this study, gene expression analysis of *Hevea* under irrigated and drought stressed condition was made using RNA-Seq data from Illumina high-throughput sequencing platform. Whole transcriptome *de novo* assembly is a complex process which requires high-quality reads with adequate sequencing depth. Therefore, more than 60 million high-quality sequencing reads from Illumina sequencing platform were generated. On average, over 89% percentage of these sequencing reads were successfully assembled using de-bruijn graph based *de novo* assembler. The transcriptome assembly statistics suggests effective assembly of the sequencing reads. Around 2 Kb of N50 statistics and more than 1 Kb of average transcript length indicated higher standard of assembled transcripts.

The functional annotation of transcripts was carried out using blast homology search methods with *Ricinus communis* as reference. Protein domain was identified using Pfam database. For over 30,000 transcript sequences were found to have at least one pfam domain hit in assembled transcripts. Protein domains such as Pentatricopeptide repeat, Leucine Rich repeats, Tetratricopeptide repeat, WD domain and Protein kinase were highly abundant in Pfam results. Full length open reading frames were predicted and over 18000 complete ORFs were filtered using in-house methods.

Further, in order to carry out comparative analysis among the samples, two sets (control with drought and control with cold) of clustered references were generated. The clustered transcripts were subsequently used for differential gene expression (DGE) analysis. Comparison of transcriptome profile of control and drought stressed plants resulted in 834 differentially expressed (DE) transcripts. Similarly, 1070 DE transcripts were identified between control and cold stressed samples.

The DE transcripts were annotated and classified based on Gene Ontology (GO) terms. Further, GO term enrichment analysis was carried out for screening transcripts that were involved in important biological processes during drought stress in *Hevea*. Metabolic process, cellular process and carbohydrate metabolic process were found significantly enriched among the biological processes in the GO term enrichment analysis. Transcripts enriched under various GO terms were extracted and used for further analysis.

Based on the results of differential gene expression (DGE) analysis, the transcripts responsive to drought and cold stresses were identified (Appendix Table 1-6) and transcripts with much higher fold of expressions (both up and down regulation) were shortlisted for further analysis There were transcripts that specifically expressed either in treated plants or in irrigated plants or in both the treatments. Accordingly, qPCR primers were synthesized for about two dozen transcripts. The primers were initially screened for their amplifiability and primer efficiency by preparing serial dilution of the primers and by estimating the slope. A total of 17 primer pairs that were found suitable for the validation analysis were employed for the expression analysis under drought stress. For this purpose, five clones with different levels of drought tolerance were included in this study. Based on field performance, the clones RRII 430, RRIM 600 and RRII 208 have been reported as tolerant clones whereas clones RRII 105 is known as moderately tolerant one. The clone RRII 414 is known as drought susceptible clone. Based on the expression analysis data, transcripts such as ferritin, DNA binding protein RAV1, NAC tf were found significantly up-regulated in the tolerant clones *viz.* RRIM 600, RRII 208 and RRII 430 when compared to RRII 105 and RRII 414. In general, expression of transcripts such as MAP3K and TIP has been found triggered in all the clones while it was much higher in tolerant clones.

In the case of ferritin, significant up-regulation was observed in clones RRIM 600, RRII 430 and RRII 208 when compared to their respective control plants. While in RRII 105 which is a moderately tolerant clone, there was a downward trend in clone RRII 414, a known drought susceptible clone it got significantly down-regulated. Ferritin family of genes are drought responsive genes which are strongly and uniformly up-regulated under drought. They play crucial role in removal of free Fe, which catalyzes the Fenton reaction that produces highly reactive hydroxyl radicals (Becana *et al.*, 1998; Briat *et al.*, 2010; Kang and Udvardi, 2012). Under stress free conditions, ferritin level is maintained at lower levels, but it shoots up to higher levels when exposed to water stressed conditions. Several reports indicate that ferritin expression in plants is induced by many environmental factors such as drought, salinity, cold, light intensity, pathogen attack, NO and ozone (De Laat *et al.*, 2014) that stimulate ROS production. Similarly, the DNA binding protein *RAV 1* also exhibited the same trend of ferritin. RAV (related to ABI3/VP1) protein is known to positively regulate leaf maturation and senescence, control flowering and cold response. In *Capsicum annuum*, *RAV1* has been reported to increase plant tolerance to drought and salt stresses (Sohn *et al.*, 2006). RAV proteins with AP2 domain in the N-terminal region are included in AP2 transcription factor family. The significant up-regulation of putative RAV1 DNA binding protein in tolerant clones indicates its strong association with drought tolerance.

NAC transcription factors which belong to one of the largest families of plant specific transcriptional regulators (NAC gene family) play important roles in the regulation of transcriptional reprogramming associated with plant stress responses. Genes in the NAC family have been shown to regulate a wide range of developmental processes of plants (Duval *et al.*, 2002., Kim *et al.*, 2006; Kim *et al.*, 2007., Ko *et al.*, 2007; Sperotto *et al.*, 2009). The over expression of *ANAC019*, *ANAC055* and *RD26* (*ANAC072*) in *Arabidopsis* resulted in up-regulation of stress-inducible genes which enhanced drought and salt tolerance in plants. *ATAF1* and *ATAF2* in *Arabidopsis* and *HvNAC6* in barley have been found to play important roles in response to drought and pathogen stresses (Mao *et al.*, 2014).

The other two transcripts which got triggered in all the clones irrespective of drought tolerance/susceptibility are MAP3K and putative TIP. Mitogen-activated protein kinases play essential roles in plant growth, development and in signalling response to various stresses such as pathogen infection, wounding, drought, salinity, UV irradiation, ozone and free radicals (Wang, 2015). In plants, MAPKs are rapidly activated when exposed to multiple abiotic stress stimuli (Hirt, 2000). MAPK cascades relay and amplify signals through the phosphorylation of substrate proteins, mediating a wide array of responses, including changes in gene expression. Higher levels of H_2O_2 which is generated during severe stress conditions in plants act as messengers in activating the expression of MAPK cascades that in turn trigger expression of transcription factors to cope up with the stress. The level of expression of MAP3K in the clones studied indicates its association with drought stress irrespective of whether the plants are stress tolerant or susceptible. But the levels were much higher in tolerant clones when compared to moderate and susceptible clones. The other transcript, tonoplast intrinsic protein (TIP) was found drought stress responsive and was found expressed at higher levels in tolerant clones. TIP is a subfamily of aquaporin which regulates water movement across vacuolar membranes and TIPs are reported to be associated with drought tolerance in many plants (Wang *et al*, 2011). Expression analysis indicated TIP as drought responsive though not strictly associated with only tolerant clones. In contrast to this, aquaporin displayed a negative trend. Its expression was significantly higher in susceptible and moderate clones when compared to tolerant clones. Aquaporins are integral membrane proteins that are involved in regulating the rapid movement of water as well as molecules like CO_2, H_2O_2, urea, ammonia, silicic acid, arsenite and wide range of small uncharged solutes. They have also been reported to modulate abiotic stress-induced signalling in plants (Srivastava *et al.*, 2014). In this study, aquaporin was found up-regulated in susceptible/moderate clones indicating its relevance as marker for drought susceptibility.

Interestingly, two transcripts viz. Glutaredoxins (GRX) and 21 KDa protein were found up-regulated in drought stressed samples of all the clones irrespective of their tolerance/susceptibility. GRXs are small, heat-stable disulfide oxido-reductases that play important role in plant development and in response to oxidative stress. In response to oxidative stress, GRXs are known to limit ROS production, participate in redox signalling and play antioxidant role, thereby protecting plants from cellular oxidative damage. Plant GRX genes have been reported to function in stress and pathogen response (Ndamukong *et al.*, 2007; Cheng, 2008; Guo *et al.*, 2010) as well as in jasmonic acid, gibberellic acid and auxin signaling (Hou *et al.*, 2008; Cheng *et al.*, 2011; Zander *et al.*, 2012). A glutaredoxin gene, (*SlGRX1*) from tomato was found induced by oxidative, drought and salt stresses and its over-expression in *Arabidopsis* significantly increased resistance to oxidative, drought, and salt stresses (Guo *et al.,* 2010). Transcript encoding 21 KDa protein (function unknown) was also found up-regulated and drought responsive.

The transcript homologous to Ethylene response factor (ERF) was found up-regulated in tolerant clones viz. RRIM 600 and RRII 208 and was found lesser in RRII 105 and RRII 414 while there was no significant change in RRII 430. ERFs belong to the transcription factor family APETALA2/ERF are associated with plant development and are reported to express in response to biotic and abiotic stresses (Mizoi *et al.*, 2012). Several ERFs also bind to dehydration responsive elements (DRE) and play regulatory role in plants under abiotic stress situations (Cheng *et al.*, 2013). They act as key regulatory hub, integrating ethylene, abscisic acid, jasmonate, and redox signaling in the plants when exposed to abiotic stresses (Muller and Bosch, 2015). In this attempt, though ERF was found expressed at significantly higher levels in the tolerant clones RRIM 600 and RRII 208, it was not so in the tolerant clone RRII 430.

Transcripts like malate dehydrogenase (MDH), serine carboxy peptidase and ABC transporter protein got either down regulated or did not show any change across the clones investigated. Malate dehydrogenase (MDH) catalyzes a reversible NAD$^+$ dependent dehydrogenase reaction (oxaloacetate to malate conversion) involved in central metabolism and redox homeostasis between organelle compartments using either NAD/H or NADP/H as oxidant/reductant, respectively (Scheibe *et al.*, 2005., Hebbelmann *et al.*, 2011). Serine carboxypeptidases (SCPs) are members of the α/β hydrolase family of proteins that play roles in multiple cellular processes by making use of a Ser-Asp-His catalytic triad to cleave the carboxy terminal peptide bonds of their protein or peptide substrates. *OsBISCPL1,* a serine carboxy peptidase-like gene is reported to be involved in regulation of defense responses against biotic and oxidative stress in rice (Liu *et al.*, 2008). ABC transporters are one of the largest protein families which are present in all organisms ranging from bacteria to humans (Henikoff *et al.*, 1997) and in most cases, functional ABC transporters act as ATP-driven pumps. In plants, apart from detoxification, ABC transporters have been reported to be involved in diverse processes such as pathogen response, surface lipid deposition, phytate accumulation in seeds, and in transport of the hormones like auxin and abscisic acid (Martinoia *et al.*, 1993).They are also involved in overall plant growth and development, response to abiotic stress and interaction of the plant with its environment (Kang *et al.*, 2011). Owing to down-regulation or no change in expression of these three transcripts in the experiment, they are ruled out as candidate genes for stress tolerance. Transcripts such as DnaJ 11 (involved in protein folding/unfolding), caspase (which cleave aspartate residues), mitochondrial dicarboxylate carrier protein (transports molecules across membranes and ATP/ADP and Pi) and protein phosphatase 2c (mediates abiotic stress triggered signals), did not show any trend in its expression pattern in any of the clones studied.

In the gene expression study of cold responsive transcripts, seven transcripts were analysed among which only one transcript coding for ERF(1) was found significantly up-regulated in cold tolerant clone RRIM 600 while it was not much induced in clone RRII 105. There were two transcripts *viz.* HSP and DRE bp which got significantly up-regulated in both the clones while transcripts viz. OEP, ERF (2) and TF (putative) were found significantly down regulated in both the clones. The transcript encoding AUX/H symporter displayed an upward trend in both the clones.

ERF (tr id: C_591) was the only transcript which displayed a significant up-regulation in clone RRIM 600 which is known as cold tolerant clone in the North East Regions of India. ERFs are known to function as regulators of stress responsive genes by binding to sequences containing AGCCGCC motifs (GCC box) and also by binding to DREs (Cheng *et al.*, 2013). Stresses like drought, cold, high salinity and osmotic stress are also known to induce ERF genes (Xu *et al.*, 2008) and its over-expression was found to enhance salt, drought, light stress and cold and heat tolerance and disease resistance in many plants (Muller and Bosch, 2015). Up-regulation of ERF (tr id: C_591) in tolerant clone indicates its possible involvement in stress regulation. In contrast to this, another ERF (tr id: 20212) was found down regulated in both the clones indicating its non-association with cold tolerance. As per the literature available, there are as high as 120 and 140 ERFs reported in *Arabidopsis* and rice respectively which are involved in stress regulation and in signalling parthways of ethylene and jasmonic acid. They are also known to regulate ROS-responsive gene expression.

Two transcripts which were found up-regulated in both the clones were DREbp1A and HSP. Dehydration-responsive element-binding protein 1A which was found up-regulated in both the clones is a transcriptional activator that binds specifically to the DNA sequence 5'-AGCCGAC-3' and bind to the C-repeat/DRE element that mediates cold-inducible transcription. Similarly, a cold induced *DREB1/CBF* (*C-repeat binding factor*)-like gene, belonging to the A-1 subgroup, was identified by Sakuma *et al.* (2002). CBF/DREB1 factors play key role in freezing tolerance and cold acclimation in many plants (Nakashima and Yamaguchi-Shinozaki, 2006). Recent study on cold stress influenced expression of C-repeat binding factor 1 (isolated from *Hevea brasiliensis*) indicated that it is involved in enhancing cold tolerance in *Arabidopsis* (Cheng *et al.*, 2015). Heat shock protein (HSP) was also found up-regulated in both the clones. HSP or stress proteins are a family of proteins that are expressed in response to stressful conditions (Morimoto *et al.*, 1994; Gupta *et al.*, 2010) and are induced by all types of stresses (De Maio, 1999). In *Arabidopsis* and some other plant species, various stresses like low temperature, osmotic, salinity, oxidative, desiccation, high intensity irradiations, wounding, and heavy metals stresses were found to induce production of Hsps (Swindell *et al.*, 2007). In the context of cold stress, HSPs are involved in cryoprotection of membrane by facilitating refolding of denatured proteins and by preventing aggregation of proteins (Renaut *et al.*, 2006).

Transcript of auxin/H symporter was found up-regulated in both the clones. Cold stress affects plant growth and development and its regulation is closely related to the intracellular auxin gradient controlled by polar localization and intracellular trafficking of auxin transporters (Rahman, 2013). Many auxin related genes affected by cold stress had been reported in *Arabidopsis* including auxin transporter genes (Lee *et al.*, 2005). Oxygen evolving enhancer protein (OEP) was found down regulated under cold stress in both the clones. According to Peng *et al.* (2015), chloroplast and photosynthesis are affected by low temperature stress resulting in reduction in the expression levels of oxygen enhancer protein in paper mulberry. Gao *et al.* (2009) reported differential regulation of many photosynthesis related proteins including oxygen evolving enhancer protein under cold stress in *Thellungiella halophila*, a chilling-tolerant plant. Down regulation of oxygen enhancer protein in both the clones indicates reduction in photosynthetic activity under cold stress in *Hevea* which is quite common. Another transcript found down-regulated was a putative transcription factor (TF). As its identity is not known, it may be presumed that this cold responsive transcription factor may be involved in suppression of its associated genes to prevent the plants from cold injury. This study indicate the existence genes like ferritin, DNA binding protein, NAC tf and aquaporin to have stronger association with drought tolerance while ERF exhibited better association with cold tolerance.

4.5 Conclusion

Using RNA-Seq technology, abiotic stress responsive transcripts of *Hevea* were identified. After sequence clustering, 58581 and 68482 transcripts were obtained from control and drought stressed samples respectively and 66019 transcripts from cold stressed samples. The digital gene expression analysis indicated up regulation of 268 transcripts and down regulation of 566 transcripts under drought stress while between control and cold stressed samples, 961 and 109 transcripts were found up and down regulated respectively. Further quantitative gene expression analysis of 17 prominent drought responsive transcripts identified from transcriptome sequencing data revealed the existence of stronger association between transcripts such as ferritin, DNA binding protein, NAC tf and aquaporin with drought tolerance. An ethylene responsive transcription factor was found associated with cold tolerance. Further experimental validation of more number of transcripts is necessary to mine larger quantity of candidate genes exhibiting stronger association with drought/cold tolerance.

Chapter 5

Gene expression in *Hevea* under intermittent drought and watered conditions

5.1 Introduction

Drought is one of the most devastating abiotic stresses, which negatively influences plant growth and development in general (Deikman *et al.*, 2012) and it is the most important factor that restricts the expansion of *Hevea brasiliensis* cultivation to newer areas in several rubber growing countries (Sethuraj, 1986). Soil and atmospheric drought, high atmospheric temperature, high light and low relative humidity occurring at the same time severely affect the growth and yield of natural rubber (Chandrasekhar *et al.*, 1990; Jacob *et al.*, 1999; Devakumar *et al.*, 1998). Different genotypes adopt different survival mechanisms to acclimatize to extreme climatic conditions.

Plants have evolved various survival strategies to overcome water deficit conditions and at the molecular level, several transcription factors are triggered which function as central regulators and molecular switches for gene expression in stress signaling and adaptation networks (Zhang *et al.*, 2011). Transcription factors (Tfs) play important roles in plant stress responses by regulating various signalling pathways through their binding to the *cis*-acting elements located in promoter region of downstream target genes, thereby activating or repressing them. Many Tfs *viz.* WRKY (Rushton *et al.*, 2012), zinc finger (Huang *et al.*, 2009), *AP2/ERF2* (Sakuma *et al.*, 2002), *MYB* (Abe *et al.*, 1997), *ZmDREB2A* (Qin *et al.*, 2007) and *NAC* (Tran *et al.*, 2004) have been characterized as drought-responsive.

NAC transcription factors comprise one of the largest gene families, which are only found in plants. NAM (no apical meristem), ATAF

(Arabidopsis transcription activation factor), CUC (cup-shaped cotyledon) or NAC domain proteins possess a highly conserved N-terminal DNA binding domain (NAC) and a variable C-terminal transcription regulation region (TRR) which are known to activate or suppress transcription of many target genes (Ernst *et al.,* 2004). The C-terminal regions of some *NAC tfs* also contain transmembrane motifs (TMs) which anchor to the plasma membrane (Tran *et al.,* 2004; Nakashima *et al.,* 2007). NAC genes have been reported to be involved in organ development and boundary maintenance, cell division, secondary wall synthesis, senescence, defence against pathogens and also act as master regulators in abiotic stress responses (Takada *et al.,* 2001; Guo and Gan 2006; Kim *et al.,* 2006; Uauy *et al.,* 2006; Zhong *et al.,* 2006; Puranik *et al.,* 2012). In *Hevea brasiliensis*, along with expression of several drought responsive transcripts (Thomas *et al.,* 2005; 2011; 2012; Sathik *et al.,* 2012; Luke *et al.,* 2015), differential expression of *NAC tf* also was observed under drought stress. Further under drought stress, it was found significantly up-regulated in relatively drought tolerant *Hevea* clones while its expression was at minimal in the relatively susceptible clone (Thomas *et al.,* 2011).

Even though molecular effects of drought stress on plants are well documented, responses in plants to intermittent drought and re-watering are relatively unknown. The information generated on gene expression pattern under drought stress as well as associated with recovery from stress during re-hydration would provide a degree of cross verification of genes regulated by drought stress (Huang *et al.,* 2008). To evaluate the level of expression of drought responsive transcripts in *Hevea* under drought and re-watered conditions, *NAC tf* expression was taken as a candidate gene. Identification of drought responsive genes and validation of their association with drought tolerance are pre-requisites to establish if they can be employed in crop improvement programmes. Even though reports on gene response to drought stress and subsequent re-watering are available in other plants (Filippou *et al.,* 2011), no such studies were reported in *Hevea.* Hence this study was conducted in *Hevea* with an aim to assess the molecular response to drought stress and re-watering with reference to a specific drought responsive gene *viz NAC tf.* Prior to validation of expression in different clones of *Hevea* with varying levels of drought tolerance, their copy number in clone RRII 105 and RRIM 600 was assessed. Expression of *NAC tf* under drought and during re-watering was measured and the results are discussed.

5.2 Materials and methods

5.2.1. Plant material and stress induction

Two *Hevea* clones, RRII 105 (moderately drought tolerant) and RRIM 600 (relatively drought tolerant) were chosen for the present study. The plants were produced by bud-grafting of seedlings with clonal buds collected from *Hevea* budwood nursery maintained at Rubber Research Institute of India (RRII) farm at Kottayam. The budded stumps were later transferred to polythene bags (size, 65x 35 cm) and were grown in open field conditions at RRII as per the recommended package of practices (Mercykutty, 2008). After growing for six months (two to three whorl stage) in open field conditions, the plants were transferred to glass house for treatment. One group of plants was subjected to drought stress for five days and another group for ten days and both the groups were subsequently re-watered for another five days. This was followed by another similar cycle of drought stress and re-watering. The control plants were irrigated on alternate days to saturation level throughout the study period. Photosynthetic gas exchange parameters were recorded after each treatment (5th and 10th day and five days after re-watering) during the three cycles. The impact of drought stress on these plants was assessed by measuring net CO_2 assimilation rate

(A) and stomatal conductance (g_s) in leaves after each treatment and those leaf samples were collected in liquid N_2 for gene expression analysis.

5.2.2. Gene expression analysis

Total RNA from the leaf samples was extracted using Spectrum Plant Total RNA Kit (Sigma-Aldrich) followed by cDNA synthesis (4 µg of total RNA as starting material) using Superscript III reverse transcriptase (Invitrogen) following the manufacturer's instructions. Quantitative PCR (qPCR) primers were designed (amplicon size 130 bp) using Primer Express software (Table 5.1) followed by synthesis (M/s. Ocimum Biosolutions, Hyderabad). Quantitative gene expression analysis was eventually carried out using Light Cycler 480 II, Roche Real Time PCR System as described in 3.2.5.

5.2.3. Plant material and stress induction for copy number determination of *NAC tf*

Genomic DNA was isolated from leaf samples of RRII 105 and RRIM 600 as reported previously (Thomas *et al.,* 2001). Optimum concentration of DNA and primers required for obtaining Ct value in the range of 20-25 was standardised. *COI1*, the coronatine insensitive gene, a single copy gene in *Hevea* (Peng *et al.,* 2009) was used as reference and GAPDH was used as internal control.

Table 5.1. Genes and the corresponding primers used for qPCR analysis

Sl. No.	Gene	Forward primer (5'-3')	Reverse primer (5'-3')
1	HbDRT5b	TCAAACACTGTCATGTCCAAGAAA	GAATCAGGGCAACCTTTTAAACC
2	HbCOI1	AGGTATTTGTGGGTGCAAGGTT	GGCGAGCCATTGCTAGAAGA
3	GAPDH	GCCTGTGATAGTCTTCGGTGTTAG	GCAGCCTTATCCTTGTCAGTGAAC

5.3. Results

Plants of both the clones (RRII 105 and RRIM 600) before imposing drought treatment had an 'A' of about 10 and 11 μ mol m^{-1} s^{-1}, respectively (Fig 5.1). Upon undergoing water deficit stress for five days, the 'A' reduced to about 2.7 in clone RRII 105, while RRIM 600 had 3.4 μ mol m^{-1} s^{-1}. Upon drought treatment for ten days, the 'A' reduced further to about 0.8 μ mol m^{-1} s^{-1} in clone RRII 105 and 1.6 μ mol m^{-1} s^{-1} in clone RRIM 600. Though 'A' got reduced in both the clones, the clone RRIM 600 maintained better 'A' than clone RRII 105. After ten days of withholding water, the plants were watered daily for five days. On the sixth day, 'A' got improved to about 5.5 in RRII 105 and 7.3 μ mol m^{-1} s^{-1} in RRIM 600. When a second cycle of drought was imposed for five days on these plants, the 'A' got reduced to the levels of 3.3 μ mol m^{-1} s^{-1} in RRII 105 and 5 μ mol m^{-1} s^{-1} in RRIM 600, respectively. When compared to the drought for 5 days on the first cycle, the reduction in 'A' was lesser in the second cycle of drought. But when the drought was extended for ten days during the second cycle of drought, the A reduced to 0.6 μ mol m^{-1} s^{-1} in RRII 105 and 2.5 μ mol m^{-1} s^{-1} RRIM 600. This indicates that the reduction in 'A' in susceptible clone was much more than the tolerant clone. When this was followed by another round of re-watering, 'A' improved to about 5.1 μmol m^{-1} s^{-1} in both the clones. When a third round of drought stress was imposed, the plants exhibited about 3.6 and 4.4 μ mol m^{-1} s^{-1} on the fifth day and about 0.17 and 1.2 μmol m^{-1} s^{-1} on the tenth day in RRII 105 and RRIM 600, respectively. Throughout the treatments (of intermittent watering and three rounds of drought treatment), clone RRIM 600 maintained better 'A' indicating its drought tolerance nature. When 'A' of clone RRII 105 during the third round of drought treatment reached near zero, RRIM 600 maintained A to the level of 1.2 μ mol m^{-1} s^{-1}. This indicates that RRIM 600 may perform well by maintaining better 'A' under drought stress.

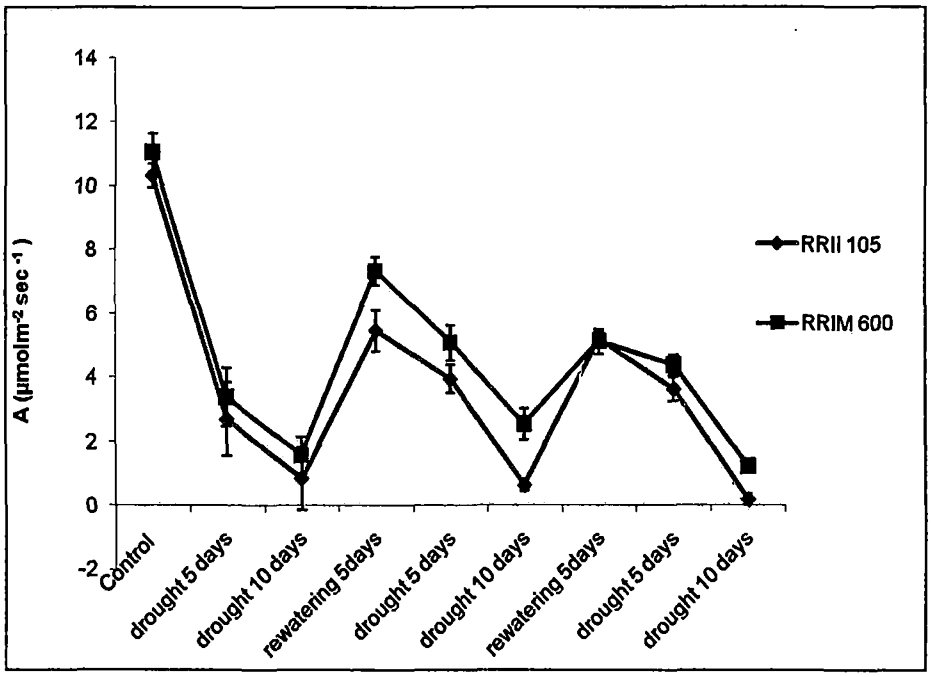


Fig.5.1. CO_2 assimilation rate (A) in two clones of *Hevea brasiliensis* under intermittent drought and watering cycles

In order to find if the copy number of *NAC tf* in the genome of the two clones was the same, a PCR was performed using specific primers of *NAC tf* and a single copy gene *COI1* (coronatine insensitive gene 1) as reference gene (Peng *et al.*, 2009). The Ct value of *NAC tf* in both the clones was found similar indicating that the copy number of this particular gene in both the clones did not differ (Table 5.2).

Table 5.2. Ct values of NAC transcription factor in RRII 105 and RRIM 600

Gene	RRII 105	RRIM 600
*HbCOI*1	21.53	21.18
HbDRT5b (*NAC tf*)	21.69	21.74

Expression of *NAC tf* was analyzed in irrigated and drought treated leaf samples of the two clones (Table 5.3; Fig 5.2). On the fifth day (during the first cycle of drought), its expression in clone RRIM 600 was about 5.5 fold higher while it was only 1.4 fold in RRII 105 than the respective watered controls. Interestingly, its expression got highly triggered to about 37 fold in RRIM 600 on the 10th day of drought while this was about only 2.7 fold in RRII 105. During the subsequent watering treatment, the expression level was much lower (between 0.3 and 0.2 fold) in both the clones. While no much change could be observed after the 5th day of drought in both the clones, it got about 9 and 1.5 fold up-regulated in clones RRIM 600 and RRII 105 respectively on the 10th day of the second cycle of drought. Again during the second cycle of re-watering, while there was not much change in RRIM 600, it was 0.5 fold down regulated in clone RRII 105. During the third round of drought cycle, there was slight increase in both the clones (1.7 and 1.9 fold increase in RRIM 600 and RRI 105, respectively) on the 5th day while there was about 9.8 and 5.7 fold increase in RRIM 600 and RRII 105, respectively on the 10th day. It was interesting to note that the levels of *NAC tf* expression shot up to about 37 fold in clone RRIM 600 on the 10th day of continuous drought treatment while it was only at meagre levels in clone RRII 105. Up-regulation of *NAC tf* only in the relatively drought tolerant clone (RRIM 600) during the subsequent drought cycles indicates its stronger association with drought tolerance. The up-regulation of *NAC tf* in RRII 105 in the third cycle of drought (5.7 fold increase) indicates that its up-regulation in this clone is much slower than that of RRIM 600 under repeated drought/watering cycles.

Table 5.3. Relative quantification of *NAC tf* in two clones of *Hevea* under intermittent drought and watering cycles

	RRII 105	**RRIM 600**
control	1	1
5 days drought	1.42997	5.5465
10 days drought	2.68675	37.04
Re-watering for 5 days	0.239325	0.31755
5 days drought	0.664275	1.01375
10 days drought	1.4739	8.983
Re-watering for 5 days	0.532775	1.14675
5 days drought	1.94625	1.76175
10 days drought	5.70025	9.793

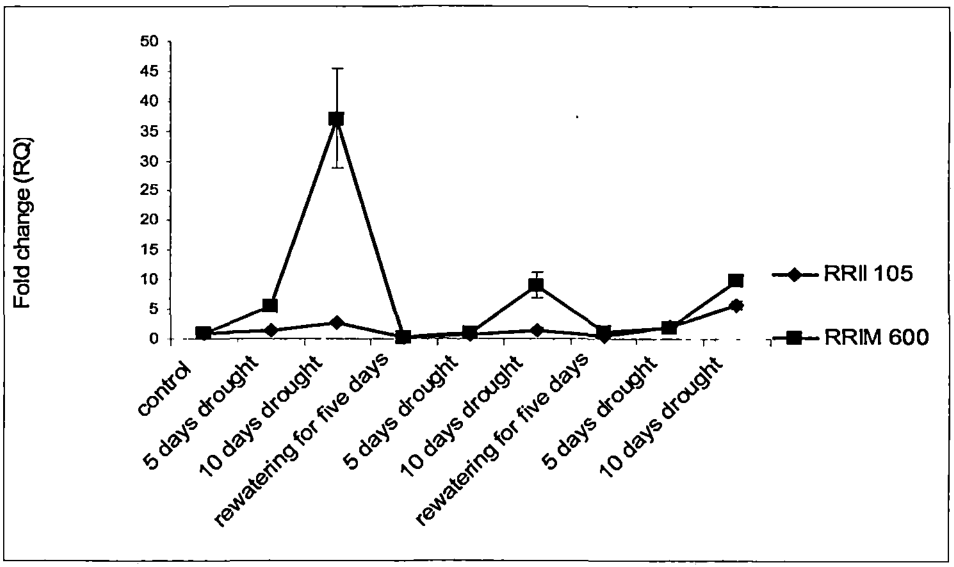


Fig.5.2. Expression analysis of *NAC tf* in two clones of *Hevea brasiliensis* under intermittent drought and watering cycles. ± Error bars indicate standard error of three biological replicates.

5.4 Discussion

During summer season in India, the agroclimatic regions like North Konkan, Maharashtra, Madhya Pradesh, Orissa which are prone to drought do not get summer showers. But the traditional regions often get intermittent summer showers which come as a boon thus saving crop plants from acute drought stress. Though it could be presumed that the summer shower helps the plants to recover from the severity of the drought, very few reports are available on its impact on the physiological and molecular aspects of rubber plants. Hence, this experiment was designed to study the effect of alternate cycles of drought and watering on photosynthesis (CO_2 assimilation rate) and expression of NAC transcription factor (*NAC tf*). *NAC tf* has been established as stress responsive and as strongly associated with stress tolerance in *Hevea* from our previous studies (Thomas *et al.,* 2011).

In the Indian rubber scenario, drought in both the traditional and non-traditional regions is severe during summer except for the fact that non-traditional regions are relatively warmer. The growth and productivity of *Hevea* plants are negatively influenced by drought (Sethuraj 1986, 1989; Chandrasekhar, 1990) while the photosynthetic mechanisms get severely affected under drought and high light thus leading to photoinhibition and photodamage (Devakumar *et al.,* 2002, 1998; Jacob *et al.*, 1999). Irrigation during drought season resulted in better growth, leaf area index and photosynthesis (Vijayakumar *et al.,* 1998, Devakumar *et al.,* 1998, 1999). CO_2 assimilation during stress free seasons generally ranges between 13 and 16 μ mol m^{-1} s^{-1} while under drought stress conditions it reaches near zero. Sometimes, it even reaches the negative side indicating respiratory loss of assimilated carbon (Sathik *et al.,* 1998b). While the optimum temperature for photosynthesis in *Hevea* ranges between 27 and 33°C (Zongdao and Xuequin 1983), the temperature go beyond 33°C in both traditional and non-traditional regions during summer. Light use efficiency was also reported to be severely affected by drought and high light under drought stress conditions in *Hevea* (Jacob *et al.,* 1999). Under such reduced photosynthetic efficiency and high light conditions, production of various active oxygen species such as super oxide, hydrogen peroxide and singlet oxygen increases drastically leading to the breakdown of cellular constituents and cell death (Nataraja and Jacob, 1998; Jacob *et al.,* 1999; Long *et al.,* 1994). It is a common phenomenon that the leaves of *Hevea* under such prolonged water deficit conditions turn yellow and necrotic eventually leading to the death of the plants.

Gas exchange parameters have been proven to be good indicators for evaluating the impact of stress on plants. But, the effect of long term drought with intermittent watering cycle on rubber had not been investigated earlier. Studies (Kang *et al.,* 2011) on two contrasting alfalfa (*Medicago sativa*) varieties indicated that they employ same strategies to cope up with drought. But qualitative differences in response among various genotypes have been observed that may have direct impact on drought tolerance in the tolerant variety of Alfalfa. The stomatal density and conductance were lesser in the tolerant variety. Delayed leaf senescence, greater root growth higher levels of accumulation of osmolytes were the other features found in the tolerant variety during drought. Both the varieties had similar shoot and root growth levels under watered conditions but the root mass of the susceptible variety got reduced to half of the tolerant variety under subsequent drought condition. In the case of oak (*Quercus robur*), although the re-watered plants displayed strong growth, they could not fully compensate for the reduced growth under previous drought situations (Spiess *et al.,* 2012).

In this study, both the clones maintained an optimum 'A' at 10-11 μ mol m^{-1} s^{-1} under optimum soil moisture conditions. Though 'A' got reduced in the first days of drought treatment to near 3 μ mol m^{-1} s^{-1} in both the clones, it went further down to less than 2 in RRIM 600 and below 1 in RRII 105. However, RRIM 600 maintained better tolerance than RRII 105 throughout the course of the treatment. Though 'A' improved during the subsequent irrigation cycles in both the clones, it never regained its original level indicating the severity of the damage inflicted upon the photosynthetic apparatus. Interestingly, the levels of *NAC tf* also showed the same trend.

In rice, many genes involved in growth, cell wall modification and lignin biosynthesis were found up-regulated in addition to photosynthesis and re-hydration related genes upon re-watering (Zhou *et al.,* 2007). The genes involved in stress protection mechanisms such as production of Early light inducible protein (ELIP) or LEA proteins and in detoxifying systems (thioredoxins) were found repressed under re-watered conditions (Spiess *et al.,* 2012). Role of transcription factors (tfs) during drought stress has been established in many plants. For e.g. MYB, DREB, bZIP and WRKY have been reported to be directly or indirectly involved in plant response to drought stress (Golldack *et al.,* 2014). Investigations on Chinese fir (*Cunninghamia lanceolata*), an evergreen conifer indicated that four genes of MYB tf were found significantly up-regulated in response to drought stress while they got down-regulated during the subsequent watering. Expression of another set of three MYB tf genes was found negatively correlated to drought tolerance (Hu *et al.,* 2015).

Prior to the selection of a stress responsive gene for the case study, the copy number of the same has to be ensured the same in both the clones. Difference in the copy number of *NAC tf* in their genome may end up in drastic change in their expression levels. For this purpose, a PCR was performed by using a single copy gene *COI1* (coronatine insensitive gene) as reference gene (Peng *et al.,* 2009). The results indicated that the copy number of *NAC tf* was same in both the clones (Table 2). Hence, *NAC tf* was employed further in the drought and intermittent re-watering experiment as reference gene. When the expression pattern of *NAC tf* under drought stress and subsequent re-watering was evaluated, *NAC tf* was found up-regulated in the relatively tolerant clone RRIM 600 under drought stress and the expression was much higher after 10 days of drought imposition (37 fold) when compared to the irrigated plants whereas the level of expression was relatively lesser in RRII 105. In the second and third cycle of drought also, expression of *NAC tf* was found highly up-regulated (8.9 and 9.7 fold) after 10 days of drought imposition in clone RRIM 600. Thus, the significant up-regulation in the levels of *NAC tf* in RRIM 600 under drought stress might be associated with its inherent drought tolerance nature. Upon re-watering, the expression of *NAC tf* got repressed in both the clones followed by a gradual increase during the subsequent drought stress cycles. After second re-watering, the level of expression went back to levels similar to control in RRIM 600 and 0.5 fold in RRII 105 indicating the recovery to the normal levels.

Though both the clones exhibited a similar trend in expression of *NAC tf* both under drought stress and re-watering, its expression was found relatively higher in clone RRIM 600 conforming to our previous results as well as results of physiological parameters in this study. From these results, it can be understood that the quantitative differences in the responses at the physiological and gene expression levels might have contributed to the increased drought tolerance observed in RRIM 600. The copy number of *NAC tf* when estimated did confirm the fact that higher levels of *NAC tf* found in clone RRIM 600 was not due to any difference in the copy number of the same in both the clones but due to the inherent mechanism of up-regulation existing in clone RRIM 600. Another interesting feature is the response in the expression levels of *NAC tf* to drought at the first cycle was at very high when compared to its level in the subsequent drought cycles. This indicates that the rate of response decreases gradually over the subsequent cycles of drought and re-watering. But among the clones studied, the tolerant ones always displayed better response to the drought/re-watering cycle.

5.5 Conclusion

The physiological parameters indicated that drought stress leads to reduction in CO_2 assimilation rate as well as poor crop performance while sub-sequent watering cycles help the plants to recover from stress though there was difference in the response among the clones studied. This study also observed that the copy number of *NAC tf* was same in both the clones. The quantitative expression studies performed after confirming the similarity in copy number of *NAC tf* in both the clones revealed that expression of *NAC tf* is triggered as a response to drought in both the clones though at different levels. The level of *NAC tf* in tolerant clone RRIM 600 was many folds higher than in the moderately tolerant clone RRII 105. This study also indicates the positive correlation between *NAC tf* and the drought tolerance trait. Above all, the study could establish beyond doubt that both the physiological and molecular level responses in *Hevea* got influenced by drought and subsequent re-watering cycles. The *NAC tf* which has been reported as one of the drought responsive Tf's in *Hevea* also has been reaffirmed for its drought responsiveness and its association with drought tolerance. The study reiterates the relevance of *NAC tf* in drought response and in drought tolerance while opening up the possibility of employing this particular transcription factor in crop improvement programmes of *Hevea brasiliensis*.

Chapter 6

Summary and Conclusion

Cultivation of *Hevea brasiliensis* in India is mainly carried out in Kerala and Kanyakumari District of Tamil Nadu which is almost saturated and for want of more area and to meet the demand of NR, of late the cultivation has been extended to non-traditional regions of India. As these regions are affected by abiotic stress factors such as drought and cold during summer and winter respectively, it is necessary to evolve or identify genotypes/cultivars that can with-stand such adverse environmental conditions prevailing in these regions. Traditional breeding methods are time consuming and cumbersome owing to long time required for field evaluations to release a new cultivar. But with the development of early evaluation screening tools with physiological, biochemical and molecular markers, it will be easier and more efficient to identify genotypes with desired characters. It is also imperative to identify genes/factors associated with drought/cold tolerance that can be utilized in the marker assisted selection for desired traits. This study is an attempt to identify genes or transcripts that can be used as markers for screening *Hevea* clones for drought or cold tolerance. Hence this study was envisaged to select candidate genes through molecular approaches like transcriptome sequencing and validation using quantitative expression analysis in clones of *Hevea* with varying levels of stress (drought/cold) tolerance.

The quantitative gene expression analysis carried out in different *Hevea* clones with varying levels of drought tolerance indicated the existence of strong association between transcripts such as *NAC tf*, *LEA 5* protein, *MAPK* and peroxidase with drought tolerance.

The attempt made on copy number determination of the *NAC tf* gene in two clones indicated similarity in its copy number among the clones studied (RRII 105 and RRIM 600). From the investigations on response of *NAC tf* during intermittent drought and re-watering cycles, *NAC tf* was found highly drought responsive as well as strongly associated with drought tolerance. Its expression got up-regulated many fold in tolerant clone RRIM 600 than clone RRII 105 under intermittent drought stress conditions.

Using NGS technology (Illumina HiSeq), abiotic stress responsive transcripts of *Hevea* were identified. After sequence clustering, 58581 transcripts from control and 68482 transcripts from drought stressed samples were obtained and 66019 transcripts were obtained from cold stressed samples. The differential gene expression analysis indicated up regulation of 268 transcripts and down regulation of 566 transcripts under drought stress while between control and cold stressed samples, 961 and 109 transcripts were found up and down regulated respectively. Further through quantitative gene expression analysis, transcripts such as ferritin, DNA binding protein, *NAC tf* and aquaporin were found to have stronger association with drought tolerance. From the quantitative expression study of cold stress, Ethylene Responsive Factor (ERF) was found to have stronger association with cold tolerance.

From the findings of this study, the transcripts such as *NAC tf*, *LEA 5*, peroxidase, MAP kinase, ferretin, DNA binding protein, *ERF* and aquaporin were found to have stronger association with stress tolerance and can be effectively employed in the crop improvement programmes of *Hevea* to develop stress tolerant varieties or to identify drought/cold tolerant genotypes from large collections of germplasm.

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APPENDICES

Appendix Table 1. DGE analysis derived transcripts found up-regulated in drought stressed samples

Transcript ID	log2 Fold Change	Regulation	Desription	E-value
B_Locus_23049_Transcript_3/3_Confidence_0.667_Length_876	6.167757165	UP	Putative uncharacterized protein F8M21_80 OS=Arabidopsis thaliana GN=F8M21_80 PE=4 SV=1	1.6655e-05
B_Locus_15945_Transcript_3/8_Confidence_0.526_Length_829	5.787726115	UP	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	9.7805e-134
B_Locus_23262_Transcript_2/2_Confidence_0.750_Length_1073	5.528638894	UP	At5g21940 OS=Arabidopsis thaliana GN=At5g21940 PE=2 SV=1	8.52282e-14
B_Locus_23262_Transcript_1/2_Confidence_0.750_Length_986	5.504792152	UP	At5g21940 OS=Arabidopsis thaliana GN=At5g21940 PE=2 SV=1	2.36073e-19
B_Locus_23049_Transcript_2/3_Confidence_0.667_Length_1471	5.270326898	UP	Putative uncharacterized protein F8M21_80 OS=Arabidopsis thaliana GN=F8M21_80 PE=4 SV=1	3.0973e-05
B_Locus_30839_Transcript_2/2_Confidence_1.000_Length_523	5.121463513	UP	At2g45360 OS=Arabidopsis thaliana GN=At2g45360 PE=2 SV=1	8.16184e-10
B_Locus_15945_Transcript_5/8_Confidence_0.526_Length_972	5.024166311	UP	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	9.13488e-101
B_Locus_14249_Transcript_1/4_Confidence_0.700_Length_1459	5.007292493	UP	F-box protein OS=Arabidopsis thaliana GN=At1g61340 PE=4 SV=1	1.14397e-30
B_Locus_22972_Transcript_1/1_Confidence_1.000_Length_446	4.990218979	UP	At1g29640 OS=Arabidopsis thaliana GN=F15D2.20 PE=2 SV=1	1.90627e-12
B_Locus_39373_Transcript_1/1_Confidence_1.000_Length_809	4.990218979	UP	Hin1 homolog OS=Arabidopsis thaliana GN=YLS9 PE=2 SV=1	2.94362e-52
B_Locus_30839_Transcript_1/2_Confidence_1.000_Length_808	4.955453561	UP	At2g45360 OS=Arabidopsis thaliana GN=At2g45360 PE=2 SV=1	2.50506e-09

B_Locus_14249_Transcript_2/4_Confidence_0.400_Length_936	4.901682305	UP	F-box protein At1g61340 OS=Arabidopsis thaliana GN=At1g61340 PE=2 SV=1	3.93842e-21
B_Locus_16830_Transcript_1/4_Confidence_0.545_Length_1247	4.787726115	UP	Xylulose kinase OS=Arabidopsis thaliana PE=4 SV=1	6.10502e-35
B_Locus_23223_Transcript_2/2_Confidence_1.000_Length_1575	4.455882552	UP	60S ribosomal protein L24-1 OS=Arabidopsis thaliana GN=RPL24A PE=1 SV=2	2.18958e-16
B_Locus_16271_Transcript_4/4_Confidence_0.667_Length_704	4.352789059	UP	Heavy-metal-associated domain-containing protein OS=Arabidopsis thaliana GN=MLP3.5 PE=4 SV=1	5.59348e-11
B_Locus_11432_Transcript_2/2_Confidence_0.750_Length_705	4.279725596	UP	At3g03280 OS=Arabidopsis thaliana GN=T17B22.3 PE=2 SV=1	3.92172e-08
B_Locus_24758_Transcript_1/1_Confidence_1.000_Length_1006	4.270326898	UP	Probable aquaporin TIP3-2 OS=Arabidopsis thaliana GN=TIP3-2 PE=2 SV=1	6.81161e-145
B_Locus_324_Transcript_2/2_Confidence_1.000_Length_1660	4.204699858	UP	Ethylene-responsive transcription factor 6 OS=Arabidopsis thaliana GN=ERF6 PE=2 SV=2	4.32155e-54
B_Locus_3079_Transcript_1/10_Confidence_0.607_Length_1533	4.044157786	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	0
B_Locus_42628_Transcript_1/1_Confidence_1.000_Length_1120	4.035306869	UP	E3 ubiquitin-protein ligase PUB22 OS=Arabidopsis thaliana GN=PUB22 PE=1 SV=1	5.15132e-118
B_Locus_13028_Transcript_1/3_Confidence_0.667_Length_1085	4.012939056	UP	GEM-like protein 5 OS=Arabidopsis thaliana GN=At5g13200 PE=1 SV=1	1.54812e-84
B_Locus_5055_Transcript_3/9_Confidence_0.591_Length_1102	3.955453561	UP	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	1.37014e-112
B_Locus_6380_Transcript_1/1_Confidence_1.000_Length_691	3.919829651	UP	Emb|CAB72159.1 OS=Arabidopsis thaliana GN=At5g60680 PE=2 SV=1	2.16565e-40
B_Locus_3403_Transcript_3/11_Confidence_0.575_Length_2216	3.85153304	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0
B_Locus_13302_Transcript_2/2_Confidence_1.000_Length_1552	3.84582907	UP	Late embryogenesis abundant domain-containing protein OS=Arabidopsis thaliana GN=F28P5.13 PE=1 SV=1	3.69755e-56
B_Locus_30709_Transcript_1/1_Confidence_1.000_Length_758	3.84582907	UP	Glutaredoxin-C9 OS=Arabidopsis thaliana GN=GRXC9 PE=1 SV=1	4.8908e-28
B_Locus_3079_Transcript_6/10_Confidence_0.536_Length_1726	3.797573901	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	0
B_Locus_3403_Transcript_5/11_Confidence_0.625_Length_2341	3.777275621	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0

B_Locus_23159_Transcript_1/2_Confidence_1.000_Length_655	3.744742945	UP	BRH1 RING finger protein OS=Arabidopsis thaliana GN=F2A19.60 PE=2 SV=1	1.08856e-79
B_Locus_3403_Transcript_10/11_Confidence_0.625_Length_2944	3.739468003	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0
B_Locus_20222_Transcript_1/1_Confidence_1.000_Length_566	3.73540508	UP	At4g10262 OS=Arabidopsis thaliana GN=At4g10265 PE=4 SV=1	2.05873e-25
B_Locus_3403_Transcript_4/11_Confidence_0.625_Length_2346	3.724433962	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0
A_Locus_6202_Transcript_5/10_Confidence_0.452_Length_2451	3.719487024	UP	DPP6 N-terminal domain-like protein OS=Arabidopsis thaliana GN=F8K7.10 PE=2 SV=1	2.73607e-101
B_Locus_3079_Transcript_4/10_Confidence_0.179_Length_490	3.673189684	UP	Galactinol synthase OS=Arabidopsis thaliana GN=AtGolS1 PE=2 SV=1	6.52995e-46
B_Locus_3403_Transcript_7/11_Confidence_0.650_Length_2350	3.655307164	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0
B_Locus_33419_Transcript_1/3_Confidence_0.714_Length_1466	3.625382293	UP	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 SV=1	6.48469e-92
B_Locus_31435_Transcript_1/1_Confidence_1.000_Length_1383	3.615823464	UP	AT-hook motif nuclear localized protein 17 OS=Arabidopsis thaliana GN=AHL17 PE=2 SV=1	2.56535e-64
A_Locus_6202_Transcript_1/10_Confidence_0.516_Length_2631	3.615194133	UP	DPP6 N-terminal domain-like protein OS=Arabidopsis thaliana GN=F8K7.10 PE=2 SV=1	2.0596e-99
B_Locus_3403_Transcript_8/11_Confidence_0.650_Length_2323	3.610346323	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0
B_Locus_2197_Transcript_1/3_Confidence_0.667_Length_1039	3.609978783	UP	Probable calcium-binding protein CML45 OS=Arabidopsis thaliana GN=CML45 PE=1 SV=1	1.49385e-47
B_Locus_19881_Transcript_1/2_Confidence_1.000_Length_1148	3.604327826	UP	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 SV=1	3.26406e-56
A_Locus_21661_Transcript_1/3_Confidence_0.714_Length_809	3.591156827	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	1.37339e-66
B_Locus_3477_Transcript_7/7_Confidence_0.684_Length_2467	3.590948796	UP	Transmembrane amino acid transporter-like protein OS=Arabidopsis thaliana GN=At2g39130 PE=4 SV=1	0
B_Locus_42413_Transcript_1/1_Confidence_1.000_Length_454	3.584962501	UP	AT4g05070 protein OS=Arabidopsis thaliana GN=T32N4.14 PE=4 SV=1	1.32901e-05
B_Locus_31589_Transcript_1/1_Confidence_1.000_Length_657	3.57975421	UP	Mitogen-activated protein kinase kinasekinase 15 OS=Arabidopsis thaliana GN=MAPKKK15 PE=4 SV=1	3.61901e-34

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A_Locus_34353_Transcript_1/1_Confidence_1.000_Length_1053	3.565608153	UP	Probable CCR4-associated factor 1 homolog 9 OS=Arabidopsis thaliana GN=CAF1-9 PE=2 SV=1	3.25217e-143
B_Locus_3477_Transcript_6/7_Confidence_0.684_Length_2718	3.528638894	UP	Transmembrane amino acid transporter-like protein OS=Arabidopsis thaliana GN=At2g39130 PE=4 SV=1	0
B_Locus_1715_Transcript_4/5_Confidence_0.667_Length_1344	3.520099405	UP	Jasmonate O-methyltransferase OS=Arabidopsis thaliana GN=JMT PE=1 SV=3	1.0545e-95
A_Locus_21661_Transcript_2/3_Confidence_0.714_Length_833	3.51925817	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	2.69973e-65
B_Locus_16830_Transcript_3/4_Confidence_0.727_Length_2206	3.504792152	UP	Putative xylulose kinase OS=Arabidopsis thaliana GN=XK-2 PE=2 SV=1	1.36263e-31
A_Locus_33181_Transcript_1/1_Confidence_1.000_Length_509	3.504792152	UP	At2g27080/T20P8.13 OS=Arabidopsis thaliana GN=At2g27080/T20P8.13 PE=2 SV=1	6.93425e-66
B_Locus_10396_Transcript_5/11_Confidence_0.473_Length_1402	3.482362238	UP	DPP6 N-terminal domain-like protein OS=Arabidopsis thaliana GN=F8K7.10 PE=2 SV=1	3.89774e-65
B_Locus_30552_Transcript_1/1_Confidence_1.000_Length_1520	3.477664974	UP	Emb|CAB62340.1 OS=Arabidopsis thaliana GN=At5g63350 PE=2 SV=1	2.17018e-76
B_Locus_23900_Transcript_1/1_Confidence_1.000_Length_1149	3.477047162	UP	Thioredoxin-like 1-1, chloroplastic OS=Arabidopsis thaliana GN=At1g08570 PE=2 SV=1	1.13391e-82
B_Locus_18628_Transcript_1/1_Confidence_1.000_Length_984	3.468681859	UP	Ethylene-responsive transcription factor 9 OS=Arabidopsis thaliana GN=ERF9 PE=2 SV=1	1.93879e-33
B_Locus_12507_Transcript_1/1_Confidence_1.000_Length_1184	3.465180296	UP	EID1-like F-box protein 3 OS=Arabidopsis thaliana GN=EDL3 PE=2 SV=1	2.80613e-114
B_Locus_11776_Transcript_1/3_Confidence_0.667_Length_1115	3.462971976	UP	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	1.97806e-141
B_Locus_36088_Transcript_1/2_Confidence_0.889_Length_630	3.458643691	UP	DPP6 N-terminal domain-like protein OS=Arabidopsis thaliana GN=F8K7.10 PE=2 SV=1	0.000350452
B_Locus_17162_Transcript_4/6_Confidence_0.722_Length_2010	3.455268247	UP	WRKY transcription factor 18 OS=Arabidopsis thaliana GN=WRKY18 PE=4 SV=1	1.28419e-44
B_Locus_2719_Transcript_1/1_Confidence_1.000_Length_1328	3.450699449	UP	Calcium-binding protein CML37 OS=Arabidopsis thaliana GN=CML37 PE=2 SV=1	1.00206e-35
B_Locus_3403_Transcript_11/11_Confidence_0.625_Length_2242	3.43920381	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	0
A_Locus_20483_Transcript_2/3_Confidence_0.333_Length_1047	3.438066038	UP	Asparagine synthetase OS=Arabidopsis thaliana GN=ASN1 PE=4 SV=1	1.97532e-08

B_Locus_30606_Transcript_1/1_Confidence_1.000_Length_565	3.405256479	UP	Putative uncharacterized protein At5g62860/MQB2_160 OS=Arabidopsis thaliana GN=At5g62860/MQB2_160 PE=2 SV=1	2.07138e-14
B_Locus_7715_Transcript_20/21_Confidence_0.060_Length_821	3.405256479	UP	Methionine sulfoxidereductase (MSS4-like) protein OS=Arabidopsis thaliana GN=At3g05540 PE=4 SV=1	7.35894e-16
A_Locus_16078_Transcript_1/1_Confidence_1.000_Length_1004	3.390459477	UP	Ankyrin repeat-containing protein OS=Arabidopsis thaliana GN=At5g51160 PE=2 SV=1	6.63818e-18
A_Locus_6202_Transcript_9/10_Confidence_0.484_Length_2522	3.353385954	UP	DPP6 N-terminal domain-like protein OS=Arabidopsis thaliana GN=F8K7.10 PE=2 SV=1	3.25668e-102
B_Locus_19655_Transcript_2/3_Confidence_0.600_Length_1027	3.349325203	UP	MLP-like protein 423 OS=Arabidopsis thaliana GN=MLP423 PE=1 SV=1	2.64239e-10
A_Locus_21661_Transcript_3/3_Confidence_0.714_Length_701	3.348112571	UP	Asparagine synthetase [glutamine-hydrolyzing] OS=Arabidopsis thaliana GN=ASN1 PE=2 SV=2	4.43806e-74
B_Locus_7852_Transcript_2/3_Confidence_0.667_Length_3260	3.343328729	UP	ATPase 8, plasma membrane-type OS=Arabidopsis thaliana GN=AHA8 PE=1 SV=1	0
B_Locus_30224_Transcript_1/1_Confidence_1.000_Length_1054	3.326519808	UP	Probable CCR4-associated factor 1 homolog 11 OS=Arabidopsis thaliana GN=CAF1-11 PE=2 SV=1	1.17361e-85
B_Locus_831_Transcript_3/5_Confidence_0.583_Length_1988	3.323003329	UP	AT4g27280/M4I22_90 OS=Arabidopsis thaliana GN=AT4g27280 PE=2 SV=1	2.22149e-44
B_Locus_3477_Transcript_4/7_Confidence_0.684_Length_2537	3.298341275	UP	Transmembrane amino acid transporter-like protein OS=Arabidopsis thaliana GN=At2g39130 PE=4 SV=1	0
B_Locus_32804_Transcript_1/1_Confidence_1.000_Length_638	3.293508121	UP	At4g36040 OS=Arabidopsis thaliana GN=At4g36040 PE=2 SV=1	2.63723e-30
B_Locus_17162_Transcript_6/6_Confidence_0.722_Length_1883	3.283467998	UP	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=1	1.50245e-70
B_Locus_26326_Transcript_1/1_Confidence_1.000_Length_807	3.27802129	UP	Secretory carrier-associated membrane protein 1 OS=Arabidopsis thaliana GN=SCAMP1 PE=1 SV=1	4.00867e-30
B_Locus_3079_Transcript_2/10_Confidence_0.464_Length_1102	3.270326898	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	2.15309e-151
B_Locus_4612_Transcript_1/1_Confidence_1.000_Length_584	3.262090748	UP	At1g67920 OS=Arabidopsis thaliana GN=T23K23.23 PE=4 SV=1	3.12918e-08
B_Locus_17162_Transcript_5/6_Confidence_0.722_Length_2180	3.258512795	UP	WRKY like transcription factor OS=Arabidopsis thaliana GN=At4g31795 PE=2 SV=1	5.90151e-41
B_Locus_13028_Transcript_2/3_Confidence_0.667_Length_1006	3.236502021	UP	GEM-like protein 5 OS=Arabidopsis thaliana GN=At5g13200 PE=1 SV=1	1.06177e-111

B_Locus_2612_Transcript_1/5_Confidence_0.333_Length_800	3.209336269	UP	RING-H2 finger protein ATL2 OS=Arabidopsis thaliana GN=ATL2 PE=2 SV=2	2.72633e-56
B_Locus_22526_Transcript_1/1_Confidence_1.000_Length_845	3.201242587	UP	At1g72510 OS=Arabidopsis thaliana GN=T10D10.2 PE=2 SV=1	2.51016e-37
B_Locus_20745_Transcript_1/1_Confidence_1.000_Length_1831	3.196266762	UP	Nematode resistance protein-like HSPRO2 OS=Arabidopsis thaliana GN=HSPRO2 PE=1 SV=1	0
B_Locus_1286_Transcript_3/3_Confidence_0.714_Length_1454	3.185533244	UP	Pathogenesis-related thaumatin-like protein OS=Arabidopsis thaliana GN=T20H2.19 PE=4 SV=1	1.13175e-144
A_Locus_6202_Transcript_8/10_Confidence_0.419_Length_2556	3.168714539	UP	DPP6 N-terminal domain-like protein OS=Arabidopsis thaliana GN=F8K7.10 PE=2 SV=1	1.60218e-109
B_Locus_17162_Transcript_3/6_Confidence_0.722_Length_1989	3.160143981	UP	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=1	3.07202e-70
B_Locus_23441_Transcript_3/4_Confidence_0.727_Length_2000	3.156985425	UP	PP2C-type phosphatase AP2C1 OS=Arabidopsis thaliana GN=AP2C1 PE=3 SV=1	1.60684e-89
B_Locus_16184_Transcript_2/2_Confidence_0.750_Length_1082	3.153420279	UP	Putative uncharacterized protein At5g17350 OS=Arabidopsis thaliana GN=T10B6_10 PE=2 SV=1	4.72107e-12
B_Locus_10245_Transcript_1/1_Confidence_1.000_Length_1161	3.149697193	UP	AT4g02340 protein OS=Arabidopsis thaliana GN=T14P8.15 PE=2 SV=1	2.14331e-168
B_Locus_23441_Transcript_1/4_Confidence_0.727_Length_1975	3.145542431	UP	PP2C-type phosphatase AP2C1 OS=Arabidopsis thaliana GN=AP2C1 PE=3 SV=1	1.73225e-88
B_Locus_7506_Transcript_1/1_Confidence_1.000_Length_1212	3.114477082	UP	AT5g64260/MSJ1_10 OS=Arabidopsis thaliana GN=EXL2 PE=2 SV=1	1.90815e-60
B_Locus_1715_Transcript_2/5_Confidence_0.667_Length_1724	3.09870361	UP	At5g38020 OS=Arabidopsis thaliana GN=At5g38020 PE=2 SV=1	1.13103e-42
A_Locus_37309_Transcript_1/1_Confidence_1.000_Length_1151	3.095036411	UP	EID1-like F-box protein 3 OS=Arabidopsis thaliana GN=EDL3 PE=2 SV=1	5.44332e-114
B_Locus_4440_Transcript_7/8_Confidence_0.091_Length_1406	3.086837169	UP	Putative uncharacterized protein At1g78070 OS=Arabidopsis thaliana GN=At1g78070 PE=2 SV=1	3.37773e-43
B_Locus_3721_Transcript_8/15_Confidence_0.170_Length_1374	3.084971856	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	2.57822e-131
B_Locus_825_Transcript_1/3_Confidence_0.333_Length_850	3.083328384	UP	Putative uncharacterized protein At2g23810 (Fragment) OS=Arabidopsis thaliana GN=At2g23810 PE=4 SV=1	3.54588e-13
B_Locus_20721_Transcript_2/7_Confidence_0.389_Length_3047	3.077370928	UP	At4g32030 OS=Arabidopsis thaliana GN=F10N7.160 PE=2 SV=2	7.45503e-07

A_Locus_29734_Transcript_1/1_Confidence_1.000_Length_945	3.074715253	UP	Putative mitochondrial dicarboxylate carrier protein OS=Arabidopsis thaliana GN=At2g22500 PE=2 SV=1	1.50617e-80
B_Locus_6660_Transcript_1/2_Confidence_1.000_Length_952	3.069770278	UP	At1g52250 OS=Arabidopsis thaliana GN=F9I5.13 PE=4 SV=1	3.23293e-43
B_Locus_11776_Transcript_2/3_Confidence_0.667_Length_1025	3.069207275	UP	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	3.0831e-147
B_Locus_23735_Transcript_1/1_Confidence_1.000_Length_769	3.061302077	UP	Sigma factor binding protein 1, chloroplastic OS=Arabidopsis thaliana GN=SIB1 PE=1 SV=1	6.61475e-11
B_Locus_33050_Transcript_1/1_Confidence_1.000_Length_1401	3.055980437	UP	RING-H2 finger protein ATL2 OS=Arabidopsis thaliana GN=ATL2 PE=2 SV=2	5.1778e-67
B_Locus_20205_Transcript_1/1_Confidence_1.000_Length_995	3.054050591	UP	Zinc finger protein ZAT10 OS=Arabidopsis thaliana GN=ZAT10 PE=2 SV=1	5.4364e-66
B_Locus_12024_Transcript_2/2_Confidence_1.000_Length_616	3.05246742	UP	At4g12735 OS=Arabidopsis thaliana GN=At4g12735 PE=4 SV=1	0.000977573
B_Locus_21838_Transcript_1/2_Confidence_0.750_Length_964	3.049597526	UP	Hin1 homolog OS=Arabidopsis thaliana GN=YLS9 PE=2 SV=1	3.83976e-66
B_Locus_14249_Transcript_3/4_Confidence_0.700_Length_1264	3.047934477	UP	F-box protein At1g61340 OS=Arabidopsis thaliana GN=At1g61340 PE=2 SV=1	4.95052e-36
B_Locus_6975_Transcript_1/1_Confidence_1.000_Length_997	3.046362057	UP	Indole-3-acetate O-methyltransferase 1 OS=Arabidopsis thaliana GN=IAMT1 PE=1 SV=1	3.05738e-51
B_Locus_3477_Transcript_5/7_Confidence_0.684_Length_2347	3.033287701	UP	Transmembrane amino acid transporter-like protein OS=Arabidopsis thaliana GN=At2g39130 PE=4 SV=1	0
B_Locus_27929_Transcript_2/3_Confidence_0.714_Length_952	3.026744855	UP	Protein MOTHER of FT and TF 1 OS=Arabidopsis thaliana GN=MFT PE=1 SV=1	3.98393e-62
B_Locus_12260_Transcript_5/7_Confidence_0.609_Length_1710	3.024166311	UP	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g42430 PE=4 SV=1	0
B_Locus_25602_Transcript_2/2_Confidence_1.000_Length_1525	3.018233355	UP	At5g43150 OS=Arabidopsis thaliana GN=At5g43150 PE=4 SV=1	0.00032865
B_Locus_2953_Transcript_2/5_Confidence_0.583_Length_1874	3.017939737	UP	Probable protein phosphatase 2C 24 OS=Arabidopsis thaliana GN=At2g29380 PE=2 SV=1	1.60489e-106
B_Locus_23445_Transcript_1/4_Confidence_0.600_Length_682	3.005986295	UP	MYB transcription factor OS=Arabidopsis thaliana GN=F6F3.18 PE=2 SV=1	1.87085e-11
A_Locus_10802_Transcript_1/1_Confidence_1.000_Length_870	3.003282086	UP	Zinc finger protein ZAT10 OS=Arabidopsis thaliana GN=ZAT10 PE=2 SV=1	8.46717e-60

B_Locus_4440_Transcript_8/8_Confidence_0.545_Length_1969	3.002885391	UP	At1g78070/F28K19_28 OS=Arabidopsis thaliana GN=At1g78070 PE=2 SV=1	1.18528e-167
A_Locus_18261_Transcript_1/1_Confidence_1.000_Length_835	2.990949643	UP	AT4g27280/M4I22_90 OS=Arabidopsis thaliana GN=AT4g27280 PE=2 SV=1	1.12957e-46
B_Locus_3456_Transcript_1/5_Confidence_0.500_Length_2917	2.98563171	UP	Sucrose synthase 6 OS=Arabidopsis thaliana GN=T9L24.42 PE=4 SV=1	0
B_Locus_16595_Transcript_1/1_Confidence_1.000_Length_1876	2.982441629	UP	At3g61880 OS=Arabidopsis thaliana GN=CYP78A9 PE=2 SV=1	0
B_Locus_1995_Transcript_4/7_Confidence_0.600_Length_1652	2.978325301	UP	AT5g22920/MRN17_15 OS=Arabidopsis thaliana GN=At5g22920 PE=2 SV=1	3.15519e-73
B_Locus_5217_Transcript_2/2_Confidence_1.000_Length_2051	2.973963088	UP	Putative uncharacterized protein At3g11760 (Fragment) OS=Arabidopsis thaliana GN=At3g11760 PE=2 SV=1	1.28525e-89
B_Locus_1286_Transcript_1/3_Confidence_0.714_Length_1546	2.971090667	UP	Pathogenesis-related thaumatin-like protein OS=Arabidopsis thaliana GN=T20H2.19 PE=4 SV=1	1.32453e-143
B_Locus_23441_Transcript_4/4_Confidence_0.727_Length_1866	2.969970295	UP	PP2C-type phosphatase AP2C1 OS=Arabidopsis thaliana GN=AP2C1 PE=3 SV=1	4.69807e-112
B_Locus_7880_Transcript_1/1_Confidence_1.000_Length_1294	2.967135366	UP	Putative mitochondrial dicarboxylate carrier protein OS=Arabidopsis thaliana GN=At2g22500 PE=2 SV=1	1.3852e-126
B_Locus_8471_Transcript_7/9_Confidence_0.200_Length_1310	2.967135366	UP	AT4G05040 protein OS=Arabidopsis thaliana GN=AT4G05040 PE=2 SV=1	2.00391e-21
A_Locus_17789_Transcript_1/1_Confidence_1.000_Length_1490	2.964640073	UP	Ethylene-responsive transcription factor ERF105 OS=Arabidopsis thaliana GN=ERF105 PE=2 SV=1	5.51404e-44
B_Locus_10145_Transcript_2/2_Confidence_0.833_Length_2203	2.940634267	UP	Putative uncharacterized protein At2g23810 OS=Arabidopsis thaliana GN=TET8 PE=4 SV=1	2.01233e-64
B_Locus_4904_Transcript_2/7_Confidence_0.188_Length_1108	2.932885804	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	1.90692e-38
B_Locus_14803_Transcript_1/2_Confidence_1.000_Length_2037	2.913988773	UP	Hypoxia-responsive family protein OS=Arabidopsis thaliana GN=At5g27760 PE=2 SV=1	3.83232e-07
B_Locus_5243_Transcript_4/5_Confidence_0.600_Length_1525	2.911792332	UP	NAC domain containing protein 47 OS=Arabidopsis thaliana GN=T11I18.18 PE=4 SV=1	2.25659e-104
B_Locus_19881_Transcript_2/2_Confidence_1.000_Length_1064	2.911318152	UP	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 SV=1	1.50124e-56
A_Locus_12936_Transcript_1/1_Confidence_1.000_Length_700	2.888897792	UP	Putative uncharacterized protein At5g17350 OS=Arabidopsis thaliana GN=T10B6_10 PE=2 SV=1	4.25037e-30

B_Locus_3079_Transcript_9/10_Confidence_0.536_Length_1460	2.886662787	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	0
B_Locus_1715_Transcript_1/5_Confidence_0.667_Length_1547	2.883303775	UP	Jasmonate O-methyltransferase OS=Arabidopsis thaliana GN=JMT PE=1 SV=3	3.47843e-40
B_Locus_11401_Transcript_5/5_Confidence_0.643_Length_2231	2.880659054	UP	AT5G48150 protein OS=Arabidopsis thaliana GN=AT5G48150 PE=2 SV=1	3.97226e-166
B_Locus_34731_Transcript_1/1_Confidence_1.000_Length_889	2.856635825	UP	F17L21.7 OS=Arabidopsis thaliana PE=4 SV=1	5.24422e-27
B_Locus_2701_Transcript_6/14_Confidence_0.585_Length_2437	2.852142844	UP	Thioredoxin-like protein Clot OS=Arabidopsis thaliana GN=At5g42850 PE=2 SV=1	6.63248e-31
B_Locus_13136_Transcript_6/6_Confidence_0.375_Length_1866	2.851194748	UP	Cytochrome P450 82C2 OS=Arabidopsis thaliana GN=CYP82C2 PE=2 SV=2	5.73099e-176
B_Locus_20721_Transcript_7/7_Confidence_0.500_Length_3249	2.850288718	UP	At4g32030 OS=Arabidopsis thaliana GN=F10N7.160 PE=2 SV=2	8.0305e-07
B_Locus_1715_Transcript_5/5_Confidence_0.667_Length_1628	2.838909656	UP	At5g38020 OS=Arabidopsis thaliana GN=At5g38020 PE=2 SV=1	7.0907e-43
B_Locus_4653_Transcript_1/1_Confidence_1.000_Length_1109	2.834278207	UP	Ankyrin repeat-containing protein OS=Arabidopsis thaliana GN=At5g51160 PE=2 SV=1	1.03315e-17
B_Locus_20721_Transcript_4/7_Confidence_0.389_Length_1822	2.833118018	UP	At4g32030 OS=Arabidopsis thaliana GN=F10N7.160 PE=2 SV=2	9.61188e-15
B_Locus_3477_Transcript_1/7_Confidence_0.684_Length_2147	2.83236681	UP	Transmembrane amino acid transporter-like protein OS=Arabidopsis thaliana GN=At2g39130 PE=4 SV=1	0
B_Locus_3721_Transcript_9/15_Confidence_0.208_Length_1392	2.829848285	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	8.47791e-154
B_Locus_3721_Transcript_7/15_Confidence_0.208_Length_1384	2.827769098	UP	At1g56600/F25P12_16 OS=Arabidopsis thaliana GN=F25P12.95 PE=2 SV=1	3.21485e-149
B_Locus_17065_Transcript_1/2_Confidence_1.000_Length_629	2.823969636	UP	At3g13227 OS=Arabidopsis thaliana GN=At3g13227 PE=4 SV=1	1.02298e-07
A_Locus_6202_Transcript_2/10_Confidence_0.226_Length_1881	2.823461257	UP	F8K7.9 OS=Arabidopsis thaliana GN=F8K7.9 PE=2 SV=1	7.3733e-32
B_Locus_6386_Transcript_4/5_Confidence_0.400_Length_1107	2.805059464	UP	At3g48240 OS=Arabidopsis thaliana GN=T29H11_240 PE=2 SV=1	1.84875e-47
B_Locus_17547_Transcript_2/2_Confidence_1.000_Length_1357	2.802195854	UP	Emb|CAB83157.1 OS=Arabidopsis thaliana GN=OXS3 PE=2 SV=1	3.98734e-22

B_Locus_206_Transcript_3/3_Confidence_0.400_Length_941	2.792780485	UP	ATP synthase subunit 9, mitochondrial OS=Arabidopsis thaliana GN=ATP9 PE=2 SV=2	2.76528e-13
B_Locus_7757_Transcript_1/1_Confidence_1.000_Length_766	2.781698733	UP	At2g27080/T20P8.13 OS=Arabidopsis thaliana GN=At2g27080/T20P8.13 PE=2 SV=1	1.23404e-71
B_Locus_12260_Transcript_1/7_Confidence_0.609_Length_1828	2.778839551	UP	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g42430 PE=4 SV=1	0
A_Locus_5058_Transcript_1/1_Confidence_1.000_Length_726	2.761485031	UP	AT4g27280/M4I22_90 OS=Arabidopsis thaliana GN=AT4g27280 PE=2 SV=1	4.37046e-46
B_Locus_29706_Transcript_2/3_Confidence_0.667_Length_851	2.760521757	UP	At2g17880 OS=Arabidopsis thaliana GN=At2g17880 PE=2 SV=1	7.18965e-32
B_Locus_10145_Transcript_1/2_Confidence_0.833_Length_1590	2.749410492	UP	Putative uncharacterized protein At2g23810 OS=Arabidopsis thaliana GN=TET8 PE=4 SV=1	7.19287e-112
B_Locus_23441_Transcript_2/4_Confidence_0.727_Length_1585	2.742515469	UP	PP2C-type phosphatase AP2C1 OS=Arabidopsis thaliana GN=AP2C1 PE=3 SV=1	3.40057e-147
B_Locus_10890_Transcript_1/2_Confidence_1.000_Length_2462	2.730926975	UP	Protein phosphatase 2C 7 OS=Arabidopsis thaliana GN=HAB2 PE=2 SV=2	5.52848e-116
B_Locus_2953_Transcript_1/5_Confidence_0.667_Length_1926	2.727184573	UP	Probable protein phosphatase 2C 78 OS=Arabidopsis thaliana GN=At5g59220 PE=2 SV=1	1.22199e-125
B_Locus_13136_Transcript_4/6_Confidence_0.312_Length_1535	2.709262665	UP	Cytochrome P450 82C2 OS=Arabidopsis thaliana GN=CYP82C2 PE=2 SV=2	1.31368e-81
A_Locus_26211_Transcript_1/1_Confidence_1.000_Length_1214	2.704523976	UP	Ethylene-responsive transcription factor 6 OS=Arabidopsis thaliana GN=ERF6 PE=2 SV=2	4.67067e-65
B_Locus_30376_Transcript_1/1_Confidence_1.000_Length_712	2.700712362	UP	AT3g10020/T22K18_16 OS=Arabidopsis thaliana GN=T22K18.16 PE=2 SV=1	1.47054e-29
B_Locus_27895_Transcript_4/5_Confidence_0.667_Length_1270	2.69639142	UP	Glycolipid transfer protein (GLTP) family protein OS=Arabidopsis thaliana GN=At3g21260 PE=4 SV=1	2.26748e-69
A_Locus_2726_Transcript_5/5_Confidence_0.733_Length_1930	2.679241184	UP	AT5g22920/MRN17_15 OS=Arabidopsis thaliana GN=At5g22920 PE=2 SV=1	6.83149e-158
B_Locus_12260_Transcript_4/7_Confidence_0.609_Length_1748	2.677628749	UP	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g42430 PE=4 SV=1	0
B_Locus_8382_Transcript_3/5_Confidence_0.643_Length_1223	2.67385041	UP	AT1G56220 protein OS=Arabidopsis thaliana GN=AT1G56220 PE=2 SV=1	3.359e-19
B_Locus_20721_Transcript_1/7_Confidence_0.500_Length_3190	2.671902138	UP	At4g32030 OS=Arabidopsis thaliana GN=F10N7.160 PE=2 SV=2	7.85872e-07

B_Locus_10344_Transcript_1/1_Confidence_1.000_Length_1330	2.660911354	UP	At5g21170 OS=Arabidopsis thaliana PE=2 SV=1	6.10119e-118
A_Locus_5921_Transcript_5/6_Confidence_0.357_Length_1291	2.659962525	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	1.59266e-108
B_Locus_435_Transcript_2/4_Confidence_0.727_Length_3006	2.651768428	UP	Leucine-rich repeat receptor-like protein kinase (Fragment) OS=Arabidopsis thaliana GN=LRR-RLK PE=2 SV=1	0
B_Locus_9308_Transcript_1/5_Confidence_0.667_Length_1841	2.645654688	UP	Serine carboxypeptidase-like 25 OS=Arabidopsis thaliana GN=SCPL25 PE=2 SV=2	0
B_Locus_33419_Transcript_3/3_Confidence_0.714_Length_1556	2.643416216	UP	F21O3.6 protein OS=Arabidopsis thaliana GN=F21O3.6 PE=2 SV=1	7.00711e-102
B_Locus_17714_Transcript_1/1_Confidence_1.000_Length_589	2.628613913	UP	Putative uncharacterized protein At5g10695 OS=Arabidopsis thaliana GN=At5g10695 PE=2 SV=1	1.98276e-32
B_Locus_4904_Transcript_5/7_Confidence_0.438_Length_1438	2.6276489	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	8.13203e-108
A_Locus_2726_Transcript_3/5_Confidence_0.733_Length_2033	2.623664653	UP	AT5g22920/MRN17_15 OS=Arabidopsis thaliana GN=At5g22920 PE=2 SV=1	1.78974e-123
B_Locus_12260_Transcript_6/7_Confidence_0.522_Length_2449	2.62026937	UP	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g42430 PE=4 SV=1	1.61712e-153
A_Locus_21002_Transcript_2/2_Confidence_1.000_Length_545	2.613498412	UP	At1g52565 OS=Arabidopsis thaliana GN=At1g52565 PE=2 SV=1	6.55192e-16
B_Locus_22251_Transcript_1/9_Confidence_0.500_Length_2585	2.609874198	UP	Zinc finger CCCH domain-containing protein 29 OS=Arabidopsis thaliana GN=At2g40140 PE=2 SV=1	2.80751e-133
B_Locus_17557_Transcript_2/2_Confidence_1.000_Length_1525	2.603526106	UP	Putative uncharacterized protein F8F6_200 OS=Arabidopsis thaliana GN=F8F6_200 PE=4 SV=1	1.16997e-45
A_Locus_17478_Transcript_1/1_Confidence_1.000_Length_1015	2.601653691	UP	Zinc finger protein ZAT10 OS=Arabidopsis thaliana GN=ZAT10 PE=2 SV=1	3.746e-68
B_Locus_17547_Transcript_1/2_Confidence_1.000_Length_1455	2.59594004	UP	Emb|CAB83157.1 OS=Arabidopsis thaliana GN=OXS3 PE=2 SV=1	1.29387e-14
B_Locus_2493_Transcript_14/17_Confidence_0.231_Length_1066	2.591206904	UP	Vacuolar protein sorting-associated protein 2 homolog 1 OS=Arabidopsis thaliana GN=VPS2.1 PE=1 SV=2	2.71456e-37
B_Locus_1286_Transcript_2/3_Confidence_0.714_Length_2096	2.588968432	UP	Thaumatin-like protein OS=Arabidopsis thaliana GN=TL1 PE=4 SV=1	1.47886e-132
A_Locus_20360_Transcript_1/1_Confidence_1.000_Length_1169	2.577469657	UP	Putative non-LTR retroelement reverse transcriptase OS=Arabidopsis thaliana GN=At2g22350 PE=2 SV=1	1.47844e-18

B_Locus_14919_Transcript_1/2_Confidence_1.000_Length_3929	2.570934509	UP	Putative disease resistance protein (TMV N-like) OS=Arabidopsis thaliana GN=F16J13.80 PE=4 SV=1	0
B_Locus_2918_Transcript_25/26_Confidence_0.119_Length_3303	2.560875699	UP	Transposon protein-like OS=Arabidopsis thaliana PE=4 SV=1	7.15936e-15
B_Locus_4270_Transcript_17/20_Confidence_0.239_Length_3623	2.551107345	UP	Riboflavin biosynthesis protein ribBA, chloroplastic OS=Arabidopsis thaliana GN=RIBBA PE=2 SV=2	3.59986e-119
B_Locus_22015_Transcript_1/3_Confidence_0.667_Length_1377	2.546747469	UP	Globulin-like protein OS=Arabidopsis thaliana PE=2 SV=1	1.17096e-172
B_Locus_27895_Transcript_3/5_Confidence_0.722_Length_1165	2.543453772	UP	At3g21260 OS=Arabidopsis thaliana GN=At3g21260 PE=2 SV=1	2.63541e-39
B_Locus_249_Transcript_3/4_Confidence_0.667_Length_2630	2.53955757	UP	Probable protein phosphatase 2C 63 OS=Arabidopsis *thaliana* GN=At4g33920 PE=1 SV=1	6.06938e-101
B_Locus_3595_Transcript_3/3_Confidence_0.714_Length_1905	2.521665971	UP	Probable protein phosphatase 2C 24 OS=Arabidopsis thaliana GN=At2g29380 PE=2 SV=1	4.16198e-139
B_Locus_4904_Transcript_3/7_Confidence_0.375_Length_1441	2.521280275	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	9.48156e-71
A_Locus_2726_Transcript_4/5_Confidence_0.467_Length_1311	2.518829985	UP	CHY and CTCHY and RING-type zinc finger protein OS=Arabidopsis thaliana GN=At5g25560 PE=4 SV=1	5.94391e-71
B_Locus_22015_Transcript_3/3_Confidence_0.667_Length_1292	2.513985506	UP	Cupin family protein OS=Arabidopsis thaliana GN=At2g28680 PE=2 SV=1	0
B_Locus_28633_Transcript_3/5_Confidence_0.571_Length_1666	2.513509369	UP	AT5g25280/F18G18_20 OS=Arabidopsis thaliana GN=At5g25280 PE=2 SV=1	2.49021e-38
B_Locus_1995_Transcript_2/7_Confidence_0.600_Length_1647	2.504792152	UP	AT5g22920/MRN17_15 OS=Arabidopsis thaliana GN=At5g22920 PE=2 SV=1	7.94486e-82
B_Locus_17557_Transcript_1/2_Confidence_1.000_Length_1565	2.504792152	UP	Putative uncharacterized protein At5g03990; F8F6_200 OS=Arabidopsis thaliana GN=F8F6_200 PE=2 SV=1	9.66607e-49
B_Locus_16489_Transcript_2/6_Confidence_0.500_Length_1577	2.499123402	UP	Probable WRKY transcription factor 46 OS=Arabidopsis thaliana GN=WRKY46 PE=2 SV=1	1.59449e-16
A_Locus_26707_Transcript_1/1_Confidence_1.000_Length_779	2.490145376	UP	Polyprotein OS=Arabidopsis thaliana PE=4 SV=1	2.50527e-48
B_Locus_36167_Transcript_1/1_Confidence_1.000_Length_1433	2.489918876	UP	Predicted GPI-anchored protein OS=Arabidopsis thaliana GN=At1g70170 PE=2 SV=1	2.32613e-140
B_Locus_4440_Transcript_6/8_Confidence_0.591_Length_2626	2.487718639	UP	At1g78070/F28K19_28 OS=Arabidopsis thaliana GN=At1g78070 PE=2 SV=1	3.65817e-165

A_Locus_13096_Transcript_1/1_Confidence_1.000_Length_1993	2.476009251	UP	DNA-binding protein-like (Fragment) OS=Arabidopsis thaliana GN=At5g47430 PE=2 SV=1	7.92393e-105
B_Locus_4440_Transcript_4/8_Confidence_0.545_Length_2071	2.474243089	UP	At1g78070/F28K19_28 OS=Arabidopsis thaliana GN=At1g78070 PE=2 SV=1	2.1827e-160
B_Locus_16489_Transcript_4/6_Confidence_0.688_Length_2335	2.470183115	UP	Probable WRKY transcription factor 53 OS=Arabidopsis thaliana GN=WRKY53 PE=1 SV=1	3.92992e-27
B_Locus_2953_Transcript_3/5_Confidence_0.583_Length_1859	2.469168242	UP	Protein phosphatase 2C 3 OS=Arabidopsis thaliana GN=AIP1 PE=1 SV=1	1.26919e-81
B_Locus_10890_Transcript_2/2_Confidence_1.000_Length_2362	2.46861854	UP	Protein phosphatase 2C 7 OS=Arabidopsis thaliana GN=HAB2 PE=2 SV=2	4.26054e-144
B_Locus_4904_Transcript_1/7_Confidence_0.375_Length_1543	2.46404581	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	2.02004e-100
A_Locus_4907_Transcript_8/11_Confidence_0.107_Length_556	2.462087701	UP	Hypersensitive-induced response protein 2 OS=Arabidopsis thaliana GN=HIR2 PE=1 SV=1	2.92395e-97
B_Locus_23081_Transcript_3/6_Confidence_0.667_Length_2316	2.460751591	UP	Nitrate transporter 1.3 OS=Arabidopsis thaliana GN=NRT1.3 PE=1 SV=1	0
B_Locus_27578_Transcript_1/1_Confidence_1.000_Length_962	2.453261851	UP	Heavy-metal-associated domain-containing protein OS=Arabidopsis thaliana GN=T15F16.6 PE=2 SV=1	2.00407e-57
B_Locus_3595_Transcript_2/3_Confidence_0.714_Length_2162	2.450344368	UP	Protein phosphatase 2C 3 OS=Arabidopsis thaliana GN=AIP1 PE=1 SV=1	1.83724e-65
A_Locus_9688_Transcript_1/2_Confidence_1.000_Length_1267	2.448649074	UP	Cytochrome P450 82C2 OS=Arabidopsis thaliana GN=CYP82C2 PE=2 SV=2	3.61002e-78
B_Locus_24210_Transcript_3/3_Confidence_0.750_Length_1980	2.448006126	UP	AT5g59550/f2o15_210 OS=Arabidopsis thaliana GN=At5g59550 PE=2 SV=1	4.26674e-70
B_Locus_2953_Transcript_5/5_Confidence_0.583_Length_1779	2.43920381	UP	Probable protein phosphatase 2C 78 OS=Arabidopsis thaliana GN=At5g59220 PE=2 SV=1	2.58157e-135
B_Locus_5266_Transcript_2/2_Confidence_0.909_Length_653	2.435694212	UP	Ferritin-4, chloroplastic OS=Arabidopsis thaliana GN=FER4 PE=2 SV=1	7.01201e-33
B_Locus_23445_Transcript_4/4_Confidence_0.600_Length_585	2.432679174	UP	MYB transcription factor OS=Arabidopsis thaliana GN=F6F3.18 PE=2 SV=1	1.10507e-11
B_Locus_16196_Transcript_4/4_Confidence_0.625_Length_1681	2.432358623	UP	Cytochrome P450 71B36 OS=Arabidopsis thaliana GN=CYP71B36 PE=2 SV=1	2.76357e-170
B_Locus_1423_Transcript_3/5_Confidence_0.688_Length_1370	2.430791571	UP	Protein ALUMINUM SENSITIVE 3 OS=Arabidopsis thaliana GN=ALS3 PE=1 SV=1	5.26361e-112

B_Locus_1423_Transcript_5/5_Confidence_0.688_Length_1257	2.428294015	UP	Protein ALUMINUM SENSITIVE 3 OS=Arabidopsis thaliana GN=ALS3 PE=1 SV=1	8.28375e-147
B_Locus_4270_Transcript_18/20_Confidence_0.296_Length_4346	2.412345903	UP	Riboflavin biosynthesis protein ribBA, chloroplastic OS=Arabidopsis thaliana GN=RIBBA PE=2 SV=2	5.30418e-117
B_Locus_7164_Transcript_1/1_Confidence_1.000_Length_1075	2.410669975	UP	Ethylene-responsive transcription factor 1B OS=Arabidopsis thaliana GN=ERF1B PE=1 SV=2	7.37834e-49
B_Locus_7616_Transcript_1/1_Confidence_1.000_Length_2141	2.409729955	UP	Lysin motif receptor-like kinase OS=Arabidopsis thaliana GN=At2g23770 PE=2 SV=1	1.3095e-175
B_Locus_2880_Transcript_6/7_Confidence_0.500_Length_1918	2.409068092	UP	At2g38470 OS=Arabidopsis thaliana PE=2 SV=1	1.84689e-113
B_Locus_1835_Transcript_3/3_Confidence_0.583_Length_1529	2.405256479	UP	Probable WRKY transcription factor 53 OS=Arabidopsis thaliana GN=WRKY53 PE=1 SV=1	9.3582e-72
B_Locus_22216_Transcript_2/3_Confidence_0.714_Length_1946	2.402964667	UP	AT2G41430 protein OS=Arabidopsis thaliana GN=ERD15 PE=2 SV=1	1.20345e-39
B_Locus_23114_Transcript_3/7_Confidence_0.714_Length_621	2.396988863	UP	At2g28570 OS=Arabidopsis thaliana GN=At2g28570 PE=2 SV=1	0.000216218
B_Locus_8382_Transcript_4/5_Confidence_0.714_Length_2097	2.392582649	UP	Dormancy/auxin associated protein OS=Arabidopsis thaliana GN=At1g56220 PE=4 SV=1	7.08068e-38
B_Locus_439_Transcript_1/1_Confidence_1.000_Length_1632	2.391450679	UP	Syntaxin-121 OS=Arabidopsis thaliana GN=SYP121 PE=3 SV=1	7.04185e-158
B_Locus_8382_Transcript_5/5_Confidence_0.643_Length_844	2.390459477	UP	AT1G56220 protein OS=Arabidopsis thaliana GN=AT1G56220 PE=2 SV=1	4.36223e-43
B_Locus_22251_Transcript_2/9_Confidence_0.500_Length_3246	2.385804116	UP	Zinc finger CCCH domain-containing protein 29 OS=Arabidopsis thaliana GN=At2g40140 PE=2 SV=1	1.79895e-127
B_Locus_22251_Transcript_4/9_Confidence_0.500_Length_2623	2.384122265	UP	Zinc finger CCCH domain-containing protein 29 OS=Arabidopsis thaliana GN=At2g40140 PE=2 SV=1	1.47864e-129
B_Locus_23081_Transcript_1/6_Confidence_0.667_Length_2293	2.372835001	UP	Nitrate transporter 1.3 OS=Arabidopsis thaliana GN=NRT1.3 PE=1 SV=1	0
B_Locus_18741_Transcript_4/6_Confidence_0.706_Length_1714	2.357638458	UP	Ferritin-4, chloroplastic OS=Arabidopsis thaliana GN=FER4 PE=2 SV=1	8.76007e-16
B_Locus_11741_Transcript_1/1_Confidence_1.000_Length_1443	2.351883128	UP	AP2/ERF and B3 domain-containing transcription repressor TEM1 OS=Arabidopsis thaliana GN=TEM1 PE=1 SV=1	7.52955e-151
B_Locus_30992_Transcript_3/4_Confidence_0.700_Length_1037	2.342062652	UP	F18O14.29 OS=Arabidopsis thaliana PE=4 SV=1	4.29085e-08

B_Locus_18741_Transcript_2/6_Confidence_0.706_Length_1648	2.341495889	UP	Ferritin (Fragment) OS=Arabidopsis thaliana GN=At3g11050 PE=2 SV=1	3.81472e-22
A_Locus_7906_Transcript_7/7_Confidence_0.700_Length_2390	2.328141287	UP	Thioredoxin-like protein Clot OS=Arabidopsis thaliana GN=At5g42850 PE=2 SV=1	2.55273e-31
B_Locus_12316_Transcript_3/6_Confidence_0.684_Length_2264	2.32404012	UP	NAC domain-containing protein 62 OS=Arabidopsis thaliana GN=T9C5.120 PE=2 SV=1	1.54788e-13
B_Locus_3138_Transcript_10/11_Confidence_0.633_Length_3423	2.303454198	UP	Probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 9 OS=Arabidopsis thaliana GN=TPS9 PE=1 SV=1	0
A_Locus_5921_Transcript_2/6_Confidence_0.571_Length_1479	2.286734655	UP	NAM protein, putative OS=Arabidopsis thaliana PE=2 SV=1	1.73094e-58
B_Locus_4904_Transcript_4/7_Confidence_0.438_Length_1443	2.282125689	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	6.05512e-85
A_Locus_5921_Transcript_3/6_Confidence_0.571_Length_1455	2.281662533	UP	NAC domain-containing protein 29 OS=Arabidopsis thaliana GN=NAC029 PE=2 SV=1	2.42125e-88
B_Locus_19578_Transcript_2/3_Confidence_0.800_Length_2077	2.281017177	UP	Homeobox-leucine zipper protein ATHB-7 OS=Arabidopsis thaliana GN=HB-7 PE=3 SV=1	7.29165e-37
B_Locus_20231_Transcript_1/4_Confidence_0.500_Length_1558	2.278344366	UP	Protein IQ-domain 3 OS=Arabidopsis thaliana GN=T25B15_60 PE=2 SV=1	7.29964e-49

Appendix Table 2 DGE analysis derived transcripts found only in irrigated samples

Transcript ID	**Description**	**E-value**
A_Locus_23591_Transcript_1/1_Confidence_1.000_Length_1133	Putative disease resistance RPP13-like protein 1 OS=Arabidopsis thaliana GN=RPPL1 PE=2 SV=1	7.41822e-27
A_Locus_10861_Transcript_1/1_Confidence_1.000_Length_776	AT1G49750 protein OS=Arabidopsis thaliana GN=F14J22.4 PE=2 SV=1	4.72838e-97
A_Locus_18411_Transcript_1/1_Confidence_1.000_Length_560	Uncharacterized protein OS=Arabidopsis thaliana GN=At3g09430 PE=2 SV=1	8.58301e-68
A_Locus_8609_Transcript_1/1_Confidence_1.000_Length_564	NA	NA
A_Locus_4321_Transcript_14/26_Confidence_1.000_Length_741	AT3g28670/MZN14_13 OS=Arabidopsis thaliana GN=At3g28670 PE=2 SV=1	9.45348e-21
A_Locus_14040_Transcript_1/1_Confidence_1.000_Length_826	Putative subtilisin serine protease OS=Arabidopsis thaliana GN=At2g05920 PE=2 SV=1	2.30008e-100
A_Locus_16282_Transcript_1/1_Confidence_1.000_Length_827	E3 ubiquitin-protein ligase RGLG1 OS=Arabidopsis thaliana GN=RGLG1 PE=1 SV=1	1.29835e-61
A_Locus_17660_Transcript_1/2_Confidence_1.000_Length_610	NA	NA
A_Locus_21567_Transcript_1/1_Confidence_1.000_Length_611	NA	NA
A_Locus_30563_Transcript_1/1_Confidence_1.000_Length_518	Beta-glucosidase 31 OS=Arabidopsis thaliana GN=BGLU31 PE=2 SV=1	7.39778e-35
A_Locus_28495_Transcript_1/1_Confidence_1.000_Length_521	Endonuclease 5 OS=Arabidopsis thaliana GN=ENDO5 PE=2 SV=1	1.50892e-32
A_Locus_12910_Transcript_1/1_Confidence_1.000_Length_1066	BAG family molecular chaperone regulator 3 OS=Arabidopsis thaliana GN=BAG3 PE=1 SV=1	2.37214e-57

A_Locus_25409_Transcript_1/1_Confidence_1.000_Length_979	RPS2 OS=Arabidopsis thaliana GN=rps2 PE=4 SV=1	1.27853e-09
A_Locus_26080_Transcript_1/1_Confidence_1.000_Length_566	Uncharacterized protein OS=Arabidopsis thaliana GN=At4g13630 PE=4 SV=1	9.47818e-28
A_Locus_8309_Transcript_3/7_Confidence_0.389_Length_566	Putative disease resistance protein At1g50180 OS=Arabidopsis thaliana GN=At1g50180 PE=2 SV=2	1.489e-07
A_Locus_12018_Transcript_1/1_Confidence_1.000_Length_1106	F15O4.13 OS=Arabidopsis thaliana PE=4 SV=1	9.21075e-61
A_Locus_32468_Transcript_1/1_Confidence_1.000_Length_750	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 SV=1	6.58757e-31
A_Locus_32682_Transcript_2/2_Confidence_1.000_Length_521	*Beta-myrcene/(E)-beta-ocimene synthase 2, chloroplastic* OS=Arabidopsis thaliana GN=TPS24 PE=1 SV=1	5.20389e-06
A_Locus_29264_Transcript_1/1_Confidence_1.000_Length_931	Pre-mRNA-processing-splicing factor OS=Arabidopsis thaliana GN=At4g38780 PE=4 SV=1	1.97326e-14
A_Locus_1431_Transcript_6/8_Confidence_0.250_Length_931	Peptidyl-prolylcis-trans isomerase FKBP20-1 OS=Arabidopsis thaliana GN=FKBP20-1 PE=2 SV=1	0.000458
A_Locus_9900_Transcript_1/1_Confidence_1.000_Length_932	Alpha-L-fucosidase 1 OS=Arabidopsis thaliana GN=FUC1 PE=1 SV=2	2.52669e-79
A_Locus_15366_Transcript_3/3_Confidence_0.333_Length_786	NA	NA
A_Locus_20074_Transcript_1/2_Confidence_1.000_Length_1361	Accelerated cell death 6 (Fragment) OS=Arabidopsis thaliana GN=At4g14400 PE=4 SV=1	8.0151e-13
A_Locus_16321_Transcript_1/1_Confidence_1.000_Length_1069	Arabidopsis thaliana gl1 homolog OS=Arabidopsis thaliana PE=2 SV=1	5.52933e-105
A_Locus_10441_Transcript_1/1_Confidence_1.000_Length_383	NA	NA

A_Locus_22793_Transcript_1/1_Confidence_1.000_Length_385	NA	NA
A_Locus_22975_Transcript_4/4_Confidence_0.500_Length_805	Impaired sucrose induction 1 OS=Arabidopsis thaliana GN=isi1 PE=2 SV=1	1.45417e-99
A_Locus_24461_Transcript_1/1_Confidence_1.000_Length_588	Putative senescence-specific cysteine protease SAG12 OS=Arabidopsis thaliana GN=SAG12 PE=2 SV=1	2.52516e-82
A_Locus_19342_Transcript_3/4_Confidence_0.250_Length_588	NA	NA
A_Locus_17660_Transcript_2/2_Confidence_1.000_Length_855	NA	NA
A_Locus_23787_Transcript_1/1_Confidence_1.000_Length_757	Lipoxygenase 4, chloroplastic OS=Arabidopsis thaliana GN=LOX4 PE=2 SV=1	5.69817e-104
A_Locus_30337_Transcript_1/1_Confidence_1.000_Length_758	NA	NA
A_Locus_36926_Transcript_2/2_Confidence_1.000_Length_254	NA	NA
A_Locus_1897_Transcript_1/1_Confidence_1.000_Length_366	NA	NA
A_Locus_6635_Transcript_8/9_Confidence_0.250_Length_625	NOL1/NOP2/sun family protein OS=Arabidopsis thaliana GN=At5g26180/T19G15_30 PE=2 SV=1	1.67632e-16
A_Locus_24507_Transcript_1/1_Confidence_1.000_Length_667	NA	NA
A_Locus_13479_Transcript_1/1_Confidence_1.000_Length_592	Sulfate transporter 3.1 OS=Arabidopsis thaliana GN=SULTR3;1 PE=2 SV=1	1.52773e-103
A_Locus_38026_Transcript_1/1_Confidence_1.000_Length_668	BRH1 RING finger protein OS=Arabidopsis thaliana GN=F2A19.60 PE=2 SV=1	4.33058e-16

A_Locus_18665_Transcript_1/1_Confidence_1.000_Length_359	CASP-like protein At2g39530 OS=Arabidopsis thaliana GN=At2g39530 PE=2 SV=1	2.55848e-34
A_Locus_4195_Transcript_1/1_Confidence_1.000_Length_717	Putative uncharacterized protein At3g14360 OS=Arabidopsis thaliana GN=At3g14360 PE=2 SV=1	1.42119e-98
A_Locus_17758_Transcript_1/1_Confidence_1.000_Length_639	AT3g26590/MFE16_11 OS=Arabidopsis thaliana GN=At3g26590 PE=2 SV=1	3.90496e-65
A_Locus_30748_Transcript_1/1_Confidence_1.000_Length_1433	Retroelement pol polyprotein-like OS=Arabidopsis thaliana PE=4 SV=1	1.88857e-24
A_Locus_23402_Transcript_1/1_Confidence_1.000_Length_1089	AT3g13690/MMM17_12 OS=Arabidopsis thaliana PE=2 SV=1	9.80135e-14
A_Locus_28018_Transcript_1/5_Confidence_0.300_Length_673	Beta-glucosidase 40 OS=Arabidopsis thaliana GN=BGLU40 PE=2 SV=1	1.32308e-39
A_Locus_18876_Transcript_1/1_Confidence_1.000_Length_675	Putative uncharacterized protein T5C23.170 OS=Arabidopsis thaliana GN=T5C23.170 PE=2 SV=1	6.36869e-14
A_Locus_24714_Transcript_1/1_Confidence_1.000_Length_310	Water channel-like protein (Fragment) OS=Arabidopsis thaliana GN=At4g23400 PE=2 SV=1	2.6202e-08
A_Locus_22790_Transcript_1/1_Confidence_1.000_Length_219	Putative endomembrane protein EMP70 precusorisolog (Fragment) OS=Arabidopsis thaliana GN=At1g10950 PE=2 SV=1	3.43246e-08
A_Locus_22229_Transcript_1/1_Confidence_1.000_Length_509	At1g53500/F22G10_13 OS=Arabidopsis thaliana PE=2 SV=1	1.4003e-82
A_Locus_12148_Transcript_1/1_Confidence_1.000_Length_510	NA	NA
A_Locus_8309_Transcript_2/7_Confidence_0.444_Length_722	Putative disease resistance protein At1g50180 OS=Arabidopsis thaliana GN=At1g50180 PE=2 SV=2	3.63079e-07
A_Locus_19364_Transcript_1/1_Confidence_1.000_Length_643	At4g00752 OS=Arabidopsis thaliana GN=At4g00752 PE=2 SV=1	6.73686e-09

A_Locus_20074_Transcript_2/2_Confidence_1.000_Length_1438	Accelerated cell death 6 (Fragment) OS=Arabidopsis thaliana GN=At4g14400 PE=4 SV=1	9.09144e-13
A_Locus_18299_Transcript_1/1_Confidence_1.000_Length_636	NA	NA
A_Locus_22632_Transcript_1/1_Confidence_1.000_Length_407	NA	NA
A_Locus_9461_Transcript_8/13_Confidence_0.091_Length_871	NA	NA
A_Locus_22843_Transcript_1/1_Confidence_1.000_Length_958	NA	NA
A_Locus_5895_Transcript_10/46_Confidence_1.000_Length_513	NA	NA

Appendix Table 3. DGE analysis derived transcripts found only in drought stressed samples

Transcript ID	Description	E-value
B_Locus_15945_Transcript_4/8_Confidence_0.474_Length_949	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	2.15065e-99
B_Locus_25512_Transcript_1/1_Confidence_1.000_Length_993	LEA protein in group 3 OS=Arabidopsis thaliana GN=AtECP63 PE=2 SV=1	1.62513e-05
B_Locus_18397_Transcript_3/3_Confidence_0.750_Length_520	NA	NA
B_Locus_18940_Transcript_1/1_Confidence_1.000_Length_548	At5g42290 OS=Arabidopsis thaliana GN=At5g42290 PE=4 SV=1	7.26318e-22
B_Locus_19845_Transcript_2/3_Confidence_0.600_Length_768	NA	NA
B_Locus_16155_Transcript_5/10_Confidence_0.065_Length_806	NA	NA
B_Locus_10304_Transcript_1/1_Confidence_1.000_Length_642	17.6 kDa class I heat shock protein 3 OS=Arabidopsis thaliana GN=HSP17.6C PE=2 SV=2	8.31144e-51
B_Locus_17015_Transcript_1/1_Confidence_1.000_Length_703	DRE/CRT-binding factor 1 OS=Arabidopsis thaliana GN=CBF1/DREB1b PE=4 SV=1	3.5969e-49
B_Locus_19845_Transcript_3/3_Confidence_0.400_Length_707	NA	NA
B_Locus_15945_Transcript_2/8_Confidence_0.526_Length_1004	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	7.45174e-95
B_Locus_14494_Transcript_1/2_Confidence_1.000_Length_817	Ethylene-responsive transcription factor ERF109 OS=Arabidopsis thaliana GN=ERF109 PE=1 SV=1	3.32996e-35
B_Locus_23049_Transcript_1/3_Confidence_0.500_Length_914	Putative uncharacterized protein F8M21_80 OS=Arabidopsis thaliana GN=F8M21_80 PE=4 SV=1	1.76307e-05

Appendix Table 4 .DGE Analysis derived transcripts found down regulated under cold stress

Transcript ID	log2FoldChange	Description	E-value
A_Locus_858_Transcript_1/1_Confidence_1.000_Length_6779	-11.63594266	NA	NA
A_Locus_2088_Transcript_3/3_Confidence_0.714_Length_888	-11.40864768	17.6 kDa class I heat shock protein 2 OS=Arabidopsis thaliana GN=HSP17.6B PE=2 SV=1	1.27963e-71
A_Locus_11934_Transcript_7/7_Confidence_0.667_Length_818	-10.02902672	17.4 kDa class I heat shock protein OS=Arabidopsis thaliana GN=HSP17.4A PE=1 SV=2	4.01932e-17
A_Locus_18065_Transcript_1/3_Confidence_0.750_Length_375	-8.613989219	25.3 kDa heat shock protein, chloroplastic OS=Arabidopsis thaliana GN=HSP25.3 PE=2 SV=1	2.72866e-12
A_Locus_7818_Transcript_3/3_Confidence_0.714_Length_1053	-8.2726958	17.6 kDa class I heat shock protein 2 OS=Arabidopsis thaliana GN=HSP17.6B PE=2 SV=1	2.37018e-58
A_Locus_100_Transcript_6/10_Confidence_0.379_Length_2560	-8.164430249	(E)-beta-ocimene synthase, chloroplastic OS=Arabidopsis thaliana GN=TPS02 PE=2 SV=1	4.78387e-99
C_Locus_13329_Transcript_1/1_Confidence_1.000_Length_418	-8.096665436	17.6 kDa class I heat shock protein 2 OS=Arabidopsis thaliana GN=HSP17.6B PE=2 SV=1	1.24114e-49
A_Locus_19342_Transcript_2/4_Confidence_0.625_Length_2237	-7.951985683	Fatty acyl-CoA reductase 2 OS=Arabidopsis thaliana GN=FAR2 PE=2 SV=2	0
A_Locus_2137_Transcript_4/5_Confidence_0.600_Length_982	-7.916032006	Peptidyl-prolylcis-trans isomerase FKBP62 OS=Arabidopsis thaliana GN=FKBP62 PE=1 SV=2	2.23694e-157
A_Locus_27837_Transcript_1/1_Confidence_1.000_Length_869	-7.513078896	22.0 kDa heat shock protein OS=Arabidopsis thaliana GN=HSP22.0 PE=2 SV=1	1.05454e-49
A_Locus_20212_Transcript_3/3_Confidence_0.714_Length_1576	-7.407538342	Ethylene-responsive transcription factor CRF3 OS=Arabidopsis thaliana GN=CRF3 PE=1 SV=1	4.14106e-13

A_Locus_20217_Transcript_1/1_Confidence_1.000_Length_814	-7.376125389	NA	NA
A_Locus_12630_Transcript_2/4_Confidence_0.824_Length_563	-6.952855038	Peptidyl-prolylcis-trans isomerase FKBP65 OS=Arabidopsis thaliana GN=FKBP65 PE=1 SV=1	8.40276e-34
A_Locus_9284_Transcript_1/1_Confidence_1.000_Length_1783	-6.929491045	Probable pectinesterase/pectinesterase inhibitor 35 OS=Arabidopsis thaliana GN=PME35 PE=2 SV=1	0
A_Locus_2630_Transcript_1/2_Confidence_0.900_Length_543	-6.806634297	Heat shock 70 kDa protein 5 OS=Arabidopsis thaliana GN=HSP70-5 PE=2 SV=1	5.80093e-41
A_Locus_17972_Transcript_1/1_Confidence_1.000_Length_1341	-6.791162889	AT3g06200/F28L1_14 OS=Arabidopsis thaliana GN=At3g06200 PE=2 SV=1	1.30991e-96
A_Locus_12915_Transcript_1/1_Confidence_1.000_Length_1338	-6.714294125	BAG family molecular chaperone regulator 5, mitochondrial OS=Arabidopsis thaliana GN=BAG5 PE=1 SV=1	1.96536e-21
A_Locus_1793_Transcript_7/7_Confidence_0.385_Length_372	-6.707098624	Chlorophyll A-B-binding protein 2, 5' partial; 1-750 (Fragment) OS=Arabidopsis thaliana GN=T1P2.1 PE=4 SV=1	9.77644e-05
A_Locus_17218_Transcript_1/1_Confidence_1.000_Length_534	-6.675004994	NA	NA
A_Locus_27682_Transcript_5/5_Confidence_0.632_Length_1036	-6.663155276	Oxygen-evolving enhancer protein 1-2, chloroplastic OS=Arabidopsis thaliana GN=PSBO2 PE=1 SV=1	6.76425e-39
A_Locus_817_Transcript_1/1_Confidence_1.000_Length_275	-6.615911532	Peptidyl-prolylcis-trans isomerase FKBP65 OS=Arabidopsis thaliana GN=FKBP65 PE=1 SV=1	0.000207741
A_Locus_18472_Transcript_2/2_Confidence_1.000_Length_1067	-6.602401245	Ribosomal protein L37 OS=Arabidopsis thaliana GN=AT1G52300 PE=3 SV=1	1.34139e-07
A_Locus_2137_Transcript_5/5_Confidence_0.650_Length_1160	-6.587726535	Peptidyl-prolylcis-trans isomerase FKBP62 OS=Arabidopsis thaliana GN=FKBP62 PE=1 SV=2	2.37441e-158
A_Locus_25387_Transcript_4/6_Confidence_0.667_Length_1560	-6.522674053	At5g19440 OS=Arabidopsis thaliana GN=At5g19440 PE=2 SV=1	1.91276e-123

A_Locus_20638_Transcript_2/3_Confidence_0.667_Length_1528	-6.522674053	NA	NA
A_Locus_2137_Transcript_3/5_Confidence_0.650_Length_1243	-6.490797037	Rotamase FKBP 1 OS=Arabidopsis thaliana GN=ROF1 PE=4 SV=1	6.2483e-126
A_Locus_1350_Transcript_1/3_Confidence_0.769_Length_579	-6.399270726	Heat stress transcription factor A-2 OS=Arabidopsis thaliana GN=HSFA2 PE=1 SV=1	6.17733e-40
A_Locus_30410_Transcript_1/1_Confidence_1.000_Length_2672	-6.339366839	Isp4-like protein OS=Arabidopsis thaliana GN=At5g64410 PE=2 SV=1	0
C_Locus_33984_Transcript_1/1_Confidence_1.000_Length_416	-6.314617249	Heat shock protein 90-1 OS=Arabidopsis thaliana GN=HSP90-1 PE=1 SV=3	2.48456e-49
A_Locus_3911_Transcript_2/6_Confidence_0.471_Length_2418	-6.282410955	Cellulose synthase-like protein E1 OS=Arabidopsis thaliana GN=CSLE1 PE=1 SV=1	0
A_Locus_20212_Transcript_1/3_Confidence_0.714_Length_1652	-6.2726958	Ethylene-responsive transcription factor CRF3 OS=Arabidopsis thaliana GN=CRF3 PE=1 SV=1	1.13017e-14
A_Locus_4731_Transcript_4/7_Confidence_0.650_Length_2531	-6.241729449	At5g38510 OS=Arabidopsis thaliana GN=At5g38510 PE=2 SV=1	1.52978e-24
A_Locus_18065_Transcript_2/3_Confidence_0.833_Length_428	-6.227431066	25.3 kDa heat shock protein, chloroplastic OS=Arabidopsis thaliana GN=HSP25.3 PE=2 SV=1	4.61124e-12
A_Locus_5241_Transcript_5/5_Confidence_0.692_Length_1401	-6.212989554	NA	NA
A_Locus_8620_Transcript_4/5_Confidence_0.667_Length_1405	-6.212989554	GDSL esterase/lipase EXL3 OS=Arabidopsis thaliana GN=EXL3 PE=2 SV=1	6.80056e-107
A_Locus_5241_Transcript_1/3_Confidence_0.692_Length_1315	-6.202793786	Putative uncharacterized protein OS=Arabidopsis thaliana GN=At5g41470 PE=2 SV=1	1.5245e-06
A_Locus_16011_Transcript_3/3_Confidence_0.611_Length_737	-6.150705275	NA	NA

C_Locus_28870_Transcript_1/1_Confidence_1.000_Length_1371	-6.110366346	BAG family molecular chaperone regulator 6 OS=Arabidopsis thaliana GN=BAG6 PE=1 SV=1	2.03477e-07
A_Locus_3416_Transcript_50/54_Confidence_0.059_Length_1211	-6.107636553	Oxysterol-binding protein-related protein 4B OS=Arabidopsis thaliana GN=ORP4B PE=2 SV=2	1.41746e-40
A_Locus_2085_Transcript_5/10_Confidence_0.542_Length_1686	-6.09390957	Putative retroelement pol polyprotein OS=Arabidopsis thaliana GN=At2g11140 PE=2 SV=1	1.85607e-38
A_Locus_3205_Transcript_7/7_Confidence_0.471_Length_645	-6.048135542	23.6 kDa heat shock protein, mitochondrial OS=Arabidopsis thaliana GN=HSP23.6 PE=2 SV=1	7.13835e-31
A_Locus_492_Transcript_3/4_Confidence_0.273_Length_426	-6.017438744	NA	NA
A_Locus_12630_Transcript_3/4_Confidence_0.824_Length_542	-5.988054938	Peptidyl-prolylcis-trans isomerase FKBP62 OS=Arabidopsis thaliana GN=FKBP62 PE=1 SV=2	1.75118e-45
A_Locus_3911_Transcript_1/6_Confidence_0.471_Length_2593	-5.987206543	Cellulose synthase-like protein E1 OS=Arabidopsis thaliana GN=CSLE1 PE=1 SV=1	2.46263e-142
C_Locus_36212_Transcript_1/1_Confidence_1.000_Length_384	-5.96108789	NA	NA
A_Locus_4731_Transcript_6/7_Confidence_0.450_Length_2131	-5.937711552	At5g38510 OS=Arabidopsis thaliana GN=At5g38510 PE=2 SV=1	7.58815e-27
A_Locus_4731_Transcript_2/7_Confidence_0.650_Length_2515	-5.92432934	At5g38510 OS=Arabidopsis thaliana GN=At5g38510 PE=2 SV=1	3.1651e-27
C_Locus_1776_Transcript_2/5_Confidence_0.214_Length_358	-5.912908161	NA	NA
A_Locus_3415_Transcript_5/7_Confidence_0.250_Length_1113	-5.889790921	NA	NA
A_Locus_20020_Transcript_3/4_Confidence_0.625_Length_1657	-5.848344549	Cytochrome P450 OS=Arabidopsis thaliana GN=CYP93D1 PE=3 SV=1	3.122e-116

A_Locus_16795_Transcript_1/1_Confidence_1.000_Length_779	-5.838029494	Putative uncharacterized protein At1g09310 OS=Arabidopsis thaliana GN=T31J12.3 PE=1 SV=1	3.66559e-58
A_Locus_478_Transcript_2/7_Confidence_0.389_Length_1383	-5.809196811	At1g25440 OS=Arabidopsis thaliana GN=At1g25440 PE=2 SV=1	2.44172e-45
A_Locus_4731_Transcript_5/7_Confidence_0.650_Length_2143	-5.80838196	At5g38510 OS=Arabidopsis thaliana GN=At5g38510 PE=2 SV=1	9.42842e-39
A_Locus_4597_Transcript_5/5_Confidence_0.600_Length_1162	-5.759050703	L-ascorbate peroxidase 2, cytosolic OS=Arabidopsis thaliana GN=APX2 PE=2 SV=3	1.14779e-145
A_Locus_9300_Transcript_4/5_Confidence_0.400_Length_1477	-5.735667776	Gibberellin 20 oxidase 2 OS=Arabidopsis thaliana GN=20ox2 PE=2 SV=1	4.12684e-137
A_Locus_3205_Transcript_6/7_Confidence_0.235_Length_629	-5.70624973	23.5 kDa heat shock protein, mitochondrial OS=Arabidopsis thaliana GN=HSP23.5 PE=2 SV=1	9.82411e-16
A_Locus_13668_Transcript_1/7_Confidence_0.500_Length_1513	-5.698831008	At3g21690 OS=Arabidopsis thaliana GN=At3g21690 PE=2 SV=1	0
A_Locus_1007_Transcript_6/8_Confidence_0.286_Length_1356	-5.677952278	Thioredoxin F2, chloroplastic OS=Arabidopsis thaliana GN=At5g16400 PE=2 SV=1	1.53821e-14
A_Locus_8620_Transcript_3/5_Confidence_0.667_Length_1556	-5.63815127	GDSL esterase/lipase EXL3 OS=Arabidopsis thaliana GN=EXL3 PE=2 SV=1	5.91785e-80
C_Locus_23472_Transcript_1/1_Confidence_1.000_Length_445	-5.637835961	NA	NA
A_Locus_1793_Transcript_3/7_Confidence_0.115_Length_352	-5.60018342	At2g34420/T31E10.24 OS=Arabidopsis thaliana GN=Lhb1B2 PE=2 SV=1	1.49169e-10
A_Locus_504_Transcript_2/10_Confidence_0.333_Length_316	-5.58680439	Dormancy/auxin associated protein OS=Arabidopsis thaliana GN=At2g33830 PE=2 SV=1	0.000776492
A_Locus_3415_Transcript_4/7_Confidence_0.611_Length_827	-5.547953461	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase OS=Arabidopsis thaliana GN=TPS21 PE=1 SV=2	3.32854e-40

A_Locus_5980_Transcript_2/8_Confidence_0.750_Length_1385	-5.541289731	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase OS=Arabidopsis thaliana GN=TPS21 PE=1 SV=2	1.0761e-72
A_Locus_100_Transcript_9/10_Confidence_0.138_Length_736	-5.514453546	Tricyclene synthase, chloroplastic OS=Arabidopsis thaliana GN=TPS03 PE=2 SV=1	3.29817e-20
A_Locus_23243_Transcript_3/6_Confidence_0.643_Length_1755	-5.481094949	Transcription factor L2 OS=Arabidopsis thaliana GN=atl2 PE=2 SV=1	1.4381e-40
A_Locus_2085_Transcript_6/10_Confidence_0.542_Length_1661	-5.43353355	Putative retroelement pol polyprotein OS=Arabidopsis thaliana GN=At2g11140 PE=2 SV=1	1.87897e-27
A_Locus_17320_Transcript_1/2_Confidence_1.000_Length_465	-5.420794439	NA	NA
A_Locus_8319_Transcript_1/2_Confidence_1.000_Length_1653	-5.356024183	Prolylcarboxypeptidase-like protein OS=Arabidopsis thaliana GN=At5g22860 PE=4 SV=1	0
A_Locus_4597_Transcript_3/5_Confidence_0.600_Length_1273	-5.356024183	L-ascorbate peroxidase 2, cytosolic OS=Arabidopsis thaliana GN=APX2 PE=2 SV=3	4.15688e-143
C_Locus_7775_Transcript_1/2_Confidence_1.000_Length_636	-5.348644653	17.6 kDa class I heat shock protein 2 OS=Arabidopsis thaliana GN=HSP17.6B PE=2 SV=1	1.03308e-69
A_Locus_25212_Transcript_1/1_Confidence_1.000_Length_1250	-5.3406959	At4g19950 OS=Arabidopsis thaliana GN=At4g19950 PE=2 SV=1	1.47914e-56
A_Locus_2993_Transcript_5/5_Confidence_0.400_Length_267	-5.311169947	18.1 kDa class I heat shock protein OS=Arabidopsis thaliana GN=HSP18.1 PE=2 SV=1	8.29298e-31
A_Locus_5990_Transcript_17/18_Confidence_0.200_Length_2437	-5.293808787	Disease resistance protein-like OS=Arabidopsis thaliana GN=At5g17680 PE=4 SV=1	5.25719e-72
A_Locus_662_Transcript_4/9_Confidence_0.421_Length_1471	-5.287244108	Beta-galactosidase 3 OS=Arabidopsis thaliana GN=BGAL3 PE=1 SV=1	4.11805e-130
A_Locus_27682_Transcript_4/5_Confidence_0.368_Length_1377	-5.278839294	Oxygen-evolving enhancer protein 1-2, chloroplastic OS=Arabidopsis thaliana GN=PSBO2 PE=1 SV=1	3.02265e-91

A_Locus_1350_Transcript_2/3_Confidence_0.846_Length_1295	-5.278418517	Heat stress transcription factor A-2 OS=Arabidopsis thaliana GN=HSFA2 PE=1 SV=1	3.08049e-37
A_Locus_22521_Transcript_1/1_Confidence_1.000_Length_2151	-5.2726958	Putative retroelement pol polyprotein OS=Arabidopsis thaliana GN=At2g23330 PE=2 SV=1	2.01684e-106
A_Locus_1392_Transcript_2/2_Confidence_0.958_Length_368	-5.257014177	Chlorophyll a-b binding protein 1, chloroplastic OS=Arabidopsis thaliana GN=LHCB1.3 PE=1 SV=1	1.03887e-13
A_Locus_3416_Transcript_53/54_Confidence_0.054_Length_696	-5.246464258	Oxysterol-binding protein-related protein 4B OS=Arabidopsis thaliana GN=ORP4B PE=2 SV=2	9.26861e-72
C_Locus_33597_Transcript_1/1_Confidence_1.000_Length_585	-5.243151524	At5g38510 OS=Arabidopsis thaliana GN=At5g38510 PE=2 SV=1	4.4088e-51
A_Locus_22096_Transcript_1/3_Confidence_0.600_Length_557	-5.215889444	17.6 kDa class I heat shock protein 2 OS=Arabidopsis thaliana GN=HSP17.6B PE=2 SV=1	4.42547e-62
A_Locus_478_Transcript_7/7_Confidence_0.444_Length_1395	-5.215637511	At1g25440 OS=Arabidopsis thaliana GN=At1g25440 PE=2 SV=1	2.56326e-68
C_Locus_30281_Transcript_1/1_Confidence_1.000_Length_321	-5.202793786	L-ascorbate peroxidase 2, cytosolic OS=Arabidopsis thaliana GN=APX2 PE=2 SV=3	8.88601e-52
A_Locus_264_Transcript_9/12_Confidence_0.113_Length_1680	-5.185915153	NA	NA
A_Locus_6451_Transcript_4/9_Confidence_0.600_Length_1575	-5.185639065	UDP-glycosyltransferase 91A1 OS=Arabidopsis thaliana GN=UGT91A1 PE=2 SV=1	2.64061e-55
A_Locus_3069_Transcript_2/3_Confidence_0.333_Length_979	-5.181491401	Peroxidase 52 OS=Arabidopsis thaliana GN=PER52 PE=2 SV=1	4.70434e-64
A_Locus_18389_Transcript_7/7_Confidence_0.389_Length_1156	-5.174378104	Putative uncharacterized protein At2g04690 OS=Arabidopsis thaliana GN=At2g04690 PE=2 SV=1	3.59348e-30
A_Locus_3069_Transcript_1/3_Confidence_0.667_Length_1367	-5.167037846	Peroxidase 52 OS=Arabidopsis thaliana GN=PER52 PE=2 SV=1	2.75528e-119

A_Locus_11934_Transcript_6/7_Confidence_0.333_Length_493	-5.102161423	17.4 kDa class I heat shock protein OS=Arabidopsis thaliana GN=HSP17.4A PE=1 SV=2	3.1025e-16
A_Locus_12346_Transcript_1/2_Confidence_1.000_Length_1928	-5.092989777	Subtilase family protein OS=Arabidopsis thaliana GN=At5g59190 PE=4 SV=1	5.85631e-114
A_Locus_13668_Transcript_2/7_Confidence_0.667_Length_1703	-5.033964642	At3g21690 OS=Arabidopsis thaliana GN=At3g21690 PE=2 SV=1	3.60875e-169
A_Locus_9300_Transcript_3/5_Confidence_0.267_Length_1042	-4.93007978	Gibberellin 20 oxidase 2 OS=Arabidopsis thaliana GN=20ox2 PE=2 SV=1	1.9722e-67
A_Locus_167_Transcript_56/57_Confidence_0.056_Length_992	-4.921223429	Probable LRR receptor-like serine/threonine-protein kinase At4g08850 OS=Arabidopsis thaliana GN=At4g08850 PE=1 SV=3	4.92991e-44
A_Locus_264_Transcript_5/12_Confidence_0.113_Length_1733	-4.853319365	NA	NA
A_Locus_264_Transcript_6/12_Confidence_0.600_Length_1353	-4.849223705	Carbonic anhydrase 2, chloroplastic OS=Arabidopsis thaliana GN=CA2 PE=1 SV=2	1.51407e-19
A_Locus_5990_Transcript_10/18_Confidence_0.386_Length_3104	-4.842852366	Disease resistance protein-like OS=Arabidopsis thaliana GN=At5g17680 PE=4 SV=1	6.85661e-72
A_Locus_15140_Transcript_1/1_Confidence_1.000_Length_3787	-4.837785486	Putative uncharacterized protein F3A4.200 OS=Arabidopsis thaliana GN=F3A4.200 PE=2 SV=1	3.62338e-33
A_Locus_2085_Transcript_7/10_Confidence_0.542_Length_2000	-4.83447793	Retroelement pol polyprotein-like OS=Arabidopsis thaliana PE=4 SV=1	2.59344e-29
A_Locus_13312_Transcript_3/7_Confidence_0.522_Length_412	-4.827008088	Terpenoid synthase 15 OS=Arabidopsis thaliana GN=TPS15 PE=2 SV=2	1.01319e-16
A_Locus_18533_Transcript_3/3_Confidence_0.714_Length_1287	-4.814308225	Putative uncharacterized protein F19K16.25 OS=Arabidopsis thaliana GN=F19K16.25 PE=4 SV=1	8.75549e-44
A_Locus_19333_Transcript_4/4_Confidence_0.500_Length_268	-4.794561465	Calcyclin binding protein OS=Arabidopsis thaliana GN=T1P2.12 PE=2 SV=1	5.90973e-19

A_Locus_3415_Transcript_2/7_Confidence_0.583_Length_815	-4.785708459	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase OS=Arabidopsis thaliana GN=TPS21 PE=1 SV=2	5.17133e-41
A_Locus_5990_Transcript_12/18_Confidence_0.386_Length_2899	-4.774737341	Disease resistance protein-like OS=Arabidopsis thaliana GN=At5g41750 PE=4 SV=1	6.20481e-72
A_Locus_12696_Transcript_2/2_Confidence_1.000_Length_611	-4.759050703	UDP-glycosyltransferase 91A1 OS=Arabidopsis thaliana GN=UGT91A1 PE=2 SV=1	2.88922e-57
C_Locus_23049_Transcript_1/5_Confidence_0.273_Length_438	-4.746237018	AT5g54270/MDK4_9 OS=Arabidopsis thaliana GN=LHCB3*1 PE=2 SV=1	4.75057e-26
C_Locus_3920_Transcript_1/1_Confidence_1.000_Length_953	-4.702474597	Oxygen-evolving enhancer protein 1-2, chloroplastic OS=Arabidopsis thaliana GN=PSBO2 PE=1 SV=1	3.74888e-104
A_Locus_17091_Transcript_1/2_Confidence_1.000_Length_722	-4.69551065	18.1 kDa class I heat shock protein OS=Arabidopsis thaliana GN=HSP18.1 PE=2 SV=1	4.39193e-58
A_Locus_5990_Transcript_18/18_Confidence_0.386_Length_3003	-4.684480321	Disease resistance protein-like OS=Arabidopsis thaliana GN=At5g17680 PE=4 SV=1	6.60487e-72

Appendix Table 5. DGE derived data of transcripts found only in cold stressed samples

Transcript ID	Description	E-value
C_Locus_26756_Transcript_2/3_Confidence_0.714_Length_1493	AT1G08630 protein OS=Arabidopsis thaliana GN=THA1 PE=2 SV=1	1.10399e-152
C_Locus_3426_Transcript_5/5_Confidence_0.583_Length_2138	Calmodulin-binding heat-shock protein OS=Arabidopsis thaliana GN=At5g37710 PE=4 SV=1	3.51101e-162
C_Locus_6834_Transcript_3/4_Confidence_0.833_Length_3646	G-type lectin S-receptor-like serine/threonine-protein kinase At1g61390 OS=Arabidopsis thaliana GN=At1g61390 PE=2 SV=1	0
C_Locus_27279_Transcript_1/1_Confidence_1.000_Length_414	Putative uncharacterized protein (Fragment) OS=Arabidopsis thaliana PE=4 SV=1	7.70609e-20
C_Locus_8670_Transcript_3/4_Confidence_0.700_Length_1199	At3g23250 OS=Arabidopsis thaliana GN=MYB15 PE=2 SV=1	8.73636e-50
C_Locus_13729_Transcript_1/1_Confidence_1.000_Length_1373	AT5g24910/F6A4_120 OS=Arabidopsis thaliana GN=CYP714A1 PE=2 SV=1	3.35842e-137
C_Locus_1586_Transcript_3/10_Confidence_0.108_Length_671	AT5g14040/MUA22_4 OS=Arabidopsis thaliana GN=PHT3;1 PE=2 SV=1	1.62335e-19
C_Locus_32064_Transcript_1/1_Confidence_1.000_Length_1149	Putative non-LTR retroelement reverse transcriptase OS=Arabidopsis thaliana GN=At2g01840 PE=2 SV=1	2.54093e-07
C_Locus_17949_Transcript_3/4_Confidence_0.667_Length_1373	Transcription factor bHLH92 OS=Arabidopsis thaliana GN=BHLH92 PE=2 SV=1	6.70801e-34
C_Locus_11448_Transcript_3/4_Confidence_0.444_Length_870	60S ribosomal protein L19-2 OS=Arabidopsis thaliana GN=RPL19B PE=2 SV=1	2.26399e-17
C_Locus_1451_Transcript_9/11_Confidence_0.114_Length_1	NA	NA

152		
C_Locus_10289_Transcript_1/1_Confidence_1.000_Length_675	NA	NA
C_Locus_5458_Transcript_2/3_Confidence_0.714_Length_1084	Peroxidase 62 OS=Arabidopsis thaliana GN=PER62 PE=2 SV=1	2.22094e-136
C_Locus_5738_Transcript_1/4_Confidence_0.700_Length_1341	GDSL esterase/lipase At5g08460 OS=Arabidopsis thaliana GN=At5g08460 PE=2 SV=1	1.42214e-81
C_Locus_30628_Transcript_1/1_Confidence_1.000_Length_707	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 SV=1	6.18532e-09
C_Locus_3426_Transcript_1/5_Confidence_0.417_Length_2022	Calmodulin-binding heat-shock protein OS=Arabidopsis thaliana GN=At5g37710 PE=4 SV=1	0
C_Locus_22269_Transcript_1/1_Confidence_1.000_Length_1122	Ethylene-responsive transcription factor ERF026 OS=Arabidopsis thaliana GN=ERF026 PE=2 SV=1	7.49001e-43
C_Locus_11507_Transcript_1/1_Confidence_1.000_Length_2025	G-type lectin S-receptor-like serine/threonine-protein kinase RKS1 OS=Arabidopsis thaliana GN=RKS1 PE=3 SV=3	1.19978e-159
C_Locus_8127_Transcript_4/4_Confidence_0.625_Length_1191	Probable F-box protein At1g44080 OS=Arabidopsis thaliana GN=At1g44080 PE=4 SV=1	2.53603e-05
C_Locus_14771_Transcript_2/5_Confidence_0.273_Length_1663	Wall-associated receptor kinase 2 OS=Arabidopsis thaliana GN=WAK2 PE=1 SV=1	1.97958e-54
C_Locus_24679_Transcript_1/1_Confidence_1.000_Length_944	Putative uncharacterized protein At1g07860 OS=Arabidopsis thaliana GN=At1g07860 PE=2 SV=1	7.98624e-11
C_Locus_18090_Transcript_1/3_Confidence_0.333_Length_608	Alpha-hydroxynitrilelyase OS=Arabidopsis thaliana GN=HNL PE=1 SV=1	3.28177e-21
C_Locus_2703_Transcript_4/5_Confidence_0.733_Length_1456	Myb-like HTH transcriptional regulator-like protein OS=Arabidopsis thaliana GN=At2g01060/F23H14.3	2.00667e-41

	PE=2 SV=1	
C_Locus_13383_Transcript_2/4_Confidence_0.400_Length_1459	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana GN=WRKY75 PE=2 SV=1	5.88116e-16
C_Locus_4152_Transcript_2/4_Confidence_0.500_Length_1459	Lysine histidine transporter-like 8 OS=Arabidopsis thaliana GN=AATL1 PE=1 SV=1	3.68188e-111
C_Locus_22799_Transcript_4/5_Confidence_0.636_Length_1389	At5g61890 OS=Arabidopsis thaliana PE=2 SV=1	5.3809e-35
C_Locus_32695_Transcript_2/2_Confidence_1.000_Length_1166	Peroxidase 71 OS=Arabidopsis thaliana GN=PER71 PE=1 SV=1	6.2635e-150
C_Locus_22297_Transcript_1/2_Confidence_1.000_Length_837	NA	NA
C_Locus_16437_Transcript_1/1_Confidence_1.000_Length_1747	G-type lectin S-receptor-like serine/threonine-protein kinase RLK1 OS=Arabidopsis thaliana GN=RLK1 PE=2 SV=2	4.60381e-44
C_Locus_2682_Transcript_2/6_Confidence_0.533_Length_1576	At2g26190/T1D16.17 OS=Arabidopsis thaliana GN=At2g26190 PE=2 SV=1	2.05884e-142
C_Locus_1663_Transcript_21/22_Confidence_0.056_Length_291	Peroxidase like protein (Fragment) OS=Arabidopsis thaliana GN=At2g38380 PE=2 SV=1	2.08032e-30
C_Locus_9916_Transcript_1/1_Confidence_1.000_Length_1827	NA	NA
C_Locus_11426_Transcript_2/4_Confidence_0.700_Length_1577	Putative calcium-transporting ATPase 13, plasma membrane-type OS=Arabidopsis thaliana GN=ACA13 PE=2 SV=1	0
C_Locus_30309_Transcript_1/1_Confidence_1.000_Length_642	NA	NA
C_Locus_28764_Transcript_1/1_Confidence_1.000_Length_6	NA	NA

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C_Locus_18490_Transcript_2/3_Confidence_0.600_Length_1501	D-mannose binding lectin protein with Apple-like carbohydrate-binding domain OS=Arabidopsis thaliana GN=F9K20.10 PE=2 SV=1	3.57746e-156
C_Locus_5264_Transcript_2/2_Confidence_1.000_Length_1050	NA	NA
C_Locus_35183_Transcript_1/1_Confidence_1.000_Length_983	Expansin-like protein OS=Arabidopsis thaliana GN=At4g17030 PE=2 SV=1	1.13187e-113
C_Locus_7729_Transcript_2/4_Confidence_0.333_Length_1502	Cytochrome P-450-like protein OS=Arabidopsis thaliana GN=At5g52400 PE=3 SV=1	1.39698e-135
C_Locus_19208_Transcript_1/1_Confidence_1.000_Length_782	At2g47485 OS=Arabidopsis thaliana GN=At2g47485 PE=2 SV=1	2.04428e-10
C_Locus_21813_Transcript_1/2_Confidence_0.667_Length_436	At1g29290 OS=Arabidopsis thaliana GN=At1g29290 PE=4 SV=1	3.14883e-08
C_Locus_11755_Transcript_1/1_Confidence_1.000_Length_874	Carbonic anhydrase OS=Arabidopsis thaliana GN=ACA7 PE=2 SV=1	1.13855e-90
C_Locus_4695_Transcript_3/4_Confidence_0.286_Length_1878	Polyprotein OS=Arabidopsis thaliana PE=2 SV=1	3.84121e-137
C_Locus_25497_Transcript_1/1_Confidence_1.000_Length_812	Putative uncharacterized protein At2g36210/F2H17.18 OS=Arabidopsis thaliana GN=At2g36210/F2H17.18 PE=2 SV=1	4.87872e-42
C_Locus_3426_Transcript_4/5_Confidence_0.167_Length_1087	Lipase class 3 family protein / putative calmodulin-binding heat-shock protein OS=Arabidopsis thaliana GN=At5g37710 PE=4 SV=1	2.76781e-13
C_Locus_1397_Transcript_2/2_Confidence_1.000_Length_1088	Germin-like protein subfamily 1 member 13 OS=Arabidopsis thaliana GN=GLP6 PE=2 SV=2	6.00151e-88

C_Locus_8670_Transcript_4/4_Confidence_0.700_Length_1089	R2R3-MYB transcription factor OS=Arabidopsis thaliana GN=AtMYB15 PE=2 SV=1	1.51747e-65
C_Locus_4695_Transcript_4/4_Confidence_0.571_Length_1625	EDGP OS=Arabidopsis thaliana PE=2 SV=1	8.39828e-126
C_Locus_760_Transcript_7/10_Confidence_0.118_Length_518	NA	NA
C_Locus_5213_Transcript_1/3_Confidence_0.667_Length_910	Protein TIFY 5A OS=Arabidopsis thaliana GN=TIFY5A PE=1 SV=1	6.63745e-17
C_Locus_22529_Transcript_4/6_Confidence_0.526_Length_1347	Hydroxysteroid dehydrogenase 3 OS=Arabidopsis thaliana GN=T21L8.110 PE=3 SV=1	2.08127e-48
C_Locus_4900_Transcript_9/10_Confidence_0.346_Length_2755	ADP-ribosylation factor GTPase-activating protein AGD1 OS=Arabidopsis thaliana GN=AGD1 PE=2 SV=2	1.2915e-42
C_Locus_3635_Transcript_2/3_Confidence_0.400_Length_1349	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 SV=1	6.36122e-06
C_Locus_9906_Transcript_2/6_Confidence_0.667_Length_1196	Nudix hydrolase 12, mitochondrial OS=Arabidopsis thaliana GN=NUDT12 PE=2 SV=1	1.02863e-42
C_Locus_280_Transcript_4/10_Confidence_0.593_Length_1669	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=1 SV=2	5.39354e-163
C_Locus_1242_Transcript_2/5_Confidence_0.462_Length_1847	Wall-associated receptor kinase 3 OS=Arabidopsis thaliana GN=WAK3 PE=2 SV=2	1.32206e-121
C_Locus_28301_Transcript_1/1_Confidence_1.000_Length_948	NA	NA
C_Locus_18900_Transcript_1/2_Confidence_0.889_Length_1984	Probable WRKY transcription factor 72 OS=Arabidopsis thaliana GN=WRKY72 PE=2 SV=1	4.9374e-64
C_Locus_15206_Transcript_7/7_Confidence_0.409_Length_1984	Transcription factor bHLH92 OS=Arabidopsis thaliana GN=BHLH92 PE=2 SV=1	9.2576e-27

C_Locus_11556_Transcript_3/3_Confidence_0.600_Length_2090	Probable indole-3-acetic acid-amidosynthetase GH3.1 OS=Arabidopsis thaliana GN=GH3.1 PE=2 SV=1	0
C_Locus_26770_Transcript_1/2_Confidence_0.833_Length_686	NA	NA
C_Locus_16009_Transcript_3/3_Confidence_0.714_Length_1236	NA	NA
C_Locus_16996_Transcript_1/1_Confidence_1.000_Length_1714	Aromatic aldehyde synthase OS=Arabidopsis thaliana GN=AAS PE=2 SV=1	0
C_Locus_18182_Transcript_1/2_Confidence_1.000_Length_1168	Inorganic pyrophosphatase 1 OS=Arabidopsis thaliana GN=PS2 PE=1 SV=1	3.16543e-77
C_Locus_34691_Transcript_1/2_Confidence_0.750_Length_643	NA	NA
C_Locus_365_Transcript_3/6_Confidence_0.235_Length_842	NA	NA
C_Locus_10658_Transcript_1/1_Confidence_1.000_Length_1267	Mitogen-activated protein kinase kinasekinase 15 OS=Arabidopsis thaliana GN=MAPKKK15 PE=4 SV=1	4.55339e-125
C_Locus_8956_Transcript_3/4_Confidence_0.286_Length_842	WRKY transcription factor 22 OS=Arabidopsis thaliana GN=WRKY22 PE=2 SV=1	2.03158e-22
C_Locus_32127_Transcript_1/2_Confidence_1.000_Length_647	NA	NA
C_Locus_18112_Transcript_2/2_Confidence_1.000_Length_1505	Protein kinase APK1A, chloroplastic OS=Arabidopsis thaliana GN=APK1A PE=2 SV=1	1.24316e-43
C_Locus_3426_Transcript_3/5_Confidence_0.417_Length_1433	Lipase class 3 family protein / putative calmodulin-binding heat-shock protein OS=Arabidopsis thaliana GN=At5g37710 PE=4 SV=1	3.65435e-105

C_Locus_13753_Transcript_3/9_Confidence_0.571_Length_1505	Proline-rich receptor-like protein kinase PERK13 OS=Arabidopsis thaliana GN=PERK13 PE=2 SV=1	1.32893e-42
C_Locus_18490_Transcript_3/3_Confidence_0.600_Length_1508	D-mannose binding lectin protein with Apple-like carbohydrate-binding domain OS=Arabidopsis thaliana GN=F9K20.10 PE=2 SV=1	2.65769e-151
C_Locus_7914_Transcript_8/11_Confidence_0.152_Length_1436	NA	NA
C_Locus_2704_Transcript_2/22_Confidence_1.000_Length_876	NA	NA
C_Locus_2866_Transcript_2/10_Confidence_0.269_Length_814	Probable xyloglucanendotransglucosylase/hydrolase protein 23 OS=Arabidopsis thaliana GN=XTH23 PE=2 SV=1	1.67291e-82
C_Locus_30527_Transcript_1/3_Confidence_0.333_Length_620	Pentatricopeptide repeat-containing protein At5g61990, mitochondrial OS=Arabidopsis thaliana GN=At5g61990 PE=2 SV=1	4.22319e-47
C_Locus_23594_Transcript_3/3_Confidence_0.667_Length_878	Protein PLANT CADMIUM RESISTANCE 2 OS=Arabidopsis thaliana GN=PCR2 PE=1 SV=1	1.05879e-53
C_Locus_280_Transcript_10/10_Confidence_0.667_Length_1800	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=1 SV=2	0
C_Locus_18079_Transcript_3/3_Confidence_0.714_Length_1092	F18B13.24 protein OS=Arabidopsis thaliana GN=F18B13.24 PE=4 SV=1	7.14895e-78
C_Locus_36219_Transcript_1/1_Confidence_1.000_Length_313	NA	NA
C_Locus_280_Transcript_2/10_Confidence_0.667_Length_1886	Beta-glucosidase 45 OS=Arabidopsis thaliana GN=BGLU45 PE=1 SV=1	2.72282e-149

C_Locus_801_Transcript_7/15_Confidence_0.123_Length_1025	Putative transcription factor bHLH041 OS=Arabidopsis thaliana GN=BHLH41 PE=4 SV=1	6.05315e-11
C_Locus_2703_Transcript_3/5_Confidence_0.333_Length_1093	Myb-like HTH transcriptional regulator-like protein OS=Arabidopsis thaliana GN=At2g01060 PE=4 SV=1	2.04444e-11
C_Locus_27404_Transcript_1/1_Confidence_1.000_Length_522	Putative uncharacterized protein AT4g28460 OS=Arabidopsis thaliana GN=F20O9.140 PE=2 SV=1	2.73462e-06
C_Locus_643_Transcript_7/34_Confidence_1.000_Length_371	NA	NA
C_Locus_2067_Transcript_3/7_Confidence_0.250_Length_914	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 PE=1 SV=2	1.33042e-78
C_Locus_34738_Transcript_1/1_Confidence_1.000_Length_470	Uncharacterized protein OS=Arabidopsis thaliana GN=At4g28460 PE=4 SV=1	7.41548e-07
C_Locus_16647_Transcript_1/1_Confidence_1.000_Length_470	Putative uncharacterized protein OS=Arabidopsis thaliana GN=At4g01575 PE=2 SV=1	8.14269e-20
C_Locus_11787_Transcript_1/1_Confidence_1.000_Length_525	NA	NA
C_Locus_1030_Transcript_1/3_Confidence_0.667_Length_1352	At1g13520 OS=Arabidopsis thaliana GN=At1g13520 PE=2 SV=1	3.40564e-91
C_Locus_23459_Transcript_1/1_Confidence_1.000_Length_658	Zinc finger protein ZAT11 OS=Arabidopsis thaliana GN=ZAT11 PE=2 SV=1	6.11772e-49
C_Locus_2067_Transcript_4/7_Confidence_0.611_Length_2179	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 PE=1 SV=2	0
C_Locus_1839_Transcript_1/4_Confidence_0.400_Length_251	Osmotin OS=Arabidopsis thaliana PE=2 SV=1	9.35669e-31

C_Locus_22529_Transcript_1/6_Confidence_0.684_Length_1465	11-beta-hydroxysteroid dehydrogenase-like OS=Arabidopsis thaliana GN=HSD1 PE=1 SV=1	1.08718e-62
C_Locus_13888_Transcript_3/4_Confidence_0.455_Length_952	Protein SPIRAL1-like 2 OS=Arabidopsis thaliana GN=SP1L2 PE=2 SV=1	6.32097e-29
C_Locus_30149_Transcript_1/1_Confidence_1.000_Length_1466	U-box domain-containing protein 21 OS=Arabidopsis thaliana GN=PUB21 PE=2 SV=1	5.33102e-119
C_Locus_2067_Transcript_5/7_Confidence_0.333_Length_1467	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 PE=1 SV=2	7.43062e-166
C_Locus_23010_Transcript_3/4_Confidence_0.600_Length_1991	Class I glutamine amidotransferase domain-containing protein OS=Arabidopsis thaliana GN=At5g38200 PE=4 SV=1	0
C_Locus_4152_Transcript_4/4_Confidence_0.625_Length_1993	Lysine histidine transporter-like 8 OS=Arabidopsis thaliana GN=AATL1 PE=1 SV=1	0
C_Locus_31994_Transcript_1/2_Confidence_1.000_Length_751	NA	NA
C_Locus_4421_Transcript_7/19_Confidence_1.000_Length_1398	NA	NA
C_Locus_18285_Transcript_3/4_Confidence_0.667_Length_1721	NA	NA
C_Locus_22424_Transcript_1/1_Confidence_1.000_Length_1721	1-aminocyclopropane-1-carboxylate synthase-like protein 1 OS=Arabidopsis thaliana GN=ACS1 PE=1 SV=2	0
C_Locus_17581_Transcript_2/2_Confidence_1.000_Length_1643	PP2C-type phosphatase AP2C1 OS=Arabidopsis thaliana GN=AP2C1 PE=3 SV=1	3.4817e-84
C_Locus_17897_Transcript_2/3_Confidence_0.800_Length_1757	Probable WRKY transcription factor 72 OS=Arabidopsis thaliana GN=WRKY72 PE=2 SV=1	2.34634e-63
C_Locus_29706_Transcript_1/1_Confidence_1.000_Length_1758	UDP-glycosyltransferase 85A2 OS=Arabidopsis	5.29545e-175

	thaliana GN=UGT85A2 PE=2 SV=1	
C_Locus_5615_Transcript_5/7_Confidence_0.167_Length_591	Ammonium transporter 2 OS=Arabidopsis thaliana GN=AMT2 PE=1 SV=2	1.36207e-15
C_Locus_8380_Transcript_1/1_Confidence_1.000_Length_1056	Xanthoxin dehydrogenase OS=Arabidopsis thaliana GN=ABA2 PE=1 SV=1	1.0711e-71
C_Locus_2067_Transcript_6/7_Confidence_0.583_Length_2580	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 PE=1 SV=2	0
C_Locus_5890_Transcript_1/6_Confidence_0.688_Length_1214	GDSL esterase/lipase CPRD49 OS=Arabidopsis thaliana GN=CPRD49 PE=2 SV=1	8.5172e-44
C_Locus_11469_Transcript_1/1_Confidence_1.000_Length_730	NA	NA
C_Locus_10228_Transcript_2/4_Confidence_0.750_Length_1690	Glutamate dehydrogenase 2 OS=Arabidopsis thaliana GN=GDH2 PE=3 SV=1	0
C_Locus_1661_Transcript_2/17_Confidence_1.000_Length_880	NA	NA
C_Locus_22829_Transcript_2/2_Confidence_1.000_Length_1313	Putative transcription factor OS=Arabidopsis thaliana GN=MYB36 PE=2 SV=1	4.19113e-70
C_Locus_18572_Transcript_5/6_Confidence_0.316_Length_682	NA	NA
C_Locus_38035_Transcript_1/1_Confidence_1.000_Length_819	Putative uncharacterized protein AT4g17280 OS=Arabidopsis thaliana GN=dl4675c PE=2 SV=1	5.47115e-39
C_Locus_1566_Transcript_1/1_Confidence_1.000_Length_881	Calcium-binding protein CML42 OS=Arabidopsis thaliana GN=CML42 PE=1 SV=1	1.86401e-64
C_Locus_17798_Transcript_5/10_Confidence_0.528_Length_1889	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP2C4 PE=3 SV=1	2.16527e-126
C_Locus_8557_Transcript_2/4_Confidence_0.462_Length_684	Urophorphyrinmethylase 1 OS=Arabidopsis thaliana GN=UPM1 PE=3 SV=1	3.23527e-93

C_Locus_15148_Transcript_1/1_Confidence_1.000_Length_1805	At3g48520 OS=Arabidopsis thaliana GN=T8P19.30 PE=2 SV=1	0
C_Locus_7729_Transcript_4/4_Confidence_0.444_Length_685	Cytochrome P-450-like protein OS=Arabidopsis thaliana GN=At5g52400 PE=3 SV=1	2.88136e-71
C_Locus_471_Transcript_5/6_Confidence_0.208_Length_685	At1g22480 OS=Arabidopsis thaliana GN=F12K8.17 PE=2 SV=1	0.000235561
C_Locus_33329_Transcript_1/1_Confidence_1.000_Length_856	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana GN=WRKY75 PE=2 SV=1	1.36791e-53
C_Locus_24123_Transcript_2/2_Confidence_0.750_Length_720	C-repeat binding factor 1 OS=Arabidopsis thaliana GN=CBF1 PE=2 SV=1	9.74529e-39
C_Locus_34020_Transcript_1/1_Confidence_1.000_Length_1137	Probable F-box protein At4g22030 OS=Arabidopsis thaliana GN=At4g22030 PE=2 SV=1	2.86841e-101
C_Locus_11576_Transcript_4/5_Confidence_0.105_Length_302	NA	NA
C_Locus_24908_Transcript_2/2_Confidence_1.000_Length_1138	SPX domain-containing protein 3 OS=Arabidopsis thaliana GN=SPX3 PE=2 SV=1	2.09343e-90
C_Locus_6834_Transcript_1/4_Confidence_0.833_Length_3865	G-type lectin S-receptor-like serine/threonine-protein kinase At1g61390 OS=Arabidopsis thaliana GN=At1g61390 PE=2 SV=1	0
C_Locus_40635_Transcript_1/1_Confidence_1.000_Length_560	At2g28710 OS=Arabidopsis thaliana GN=At2g28710 PE=2 SV=1	7.25956e-34
C_Locus_5264_Transcript_1/2_Confidence_1.000_Length_1470	NA	NA
C_Locus_17940_Transcript_1/3_Confidence_0.571_Length_754	NA	NA

C_Locus_4534_Transcript_1/1_Confidence_1.000_Length_893	Putative uncharacterized protein OS=Arabidopsis thaliana GN=At5g12340 PE=4 SV=1	3.08771e-49
C_Locus_23115_Transcript_1/2_Confidence_1.000_Length_1905	Mate efflux domain-containing protein OS=Arabidopsis thaliana GN=F7H19.220 PE=2 SV=1	0
C_Locus_21560_Transcript_1/1_Confidence_1.000_Length_755	NA	NA
C_Locus_20690_Transcript_1/1_Confidence_1.000_Length_755	At2g22880 OS=Arabidopsis thaliana GN=At2g22880 PE=2 SV=1	0.000167234
C_Locus_17902_Transcript_1/2_Confidence_1.000_Length_895	Protein PHLOEM PROTEIN 2-LIKE A1 OS=Arabidopsis thaliana GN=PP2A1 PE=2 SV=1	1.26573e-36
C_Locus_280_Transcript_8/10_Confidence_0.667_Length_1910	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=1 SV=2	1.17723e-138
C_Locus_24028_Transcript_1/1_Confidence_1.000_Length_1059	At2g22880 OS=Arabidopsis thaliana GN=At2g22880 PE=2 SV=1	9.29268e-12
C_Locus_4974_Transcript_1/1_Confidence_1.000_Length_537	Putative uncharacterized protein AT4g21920 OS=Arabidopsis thaliana GN=T8O5.130 PE=4 SV=1	4.14638e-13
C_Locus_7202_Transcript_3/3_Confidence_0.667_Length_1278	NAC-domain protein-like OS=Arabidopsis thaliana GN=At5g22380 PE=2 SV=1	1.25758e-66
C_Locus_18490_Transcript_1/3_Confidence_0.600_Length_1444	D-mannose binding lectin protein with Apple-like carbohydrate-binding domain OS=Arabidopsis thaliana GN=F9K20.10 PE=2 SV=1	2.81605e-150
C_Locus_6684_Transcript_3/6_Confidence_0.741_Length_3055	Glutamate receptor 2.9 OS=Arabidopsis thaliana GN=GLR2.9 PE=2 SV=1	1.27232e-116

C_Locus_3129_Transcript_2/3_Confidence_0.714_Length_1893	At4g39830 OS=Arabidopsis thaliana GN=AT4g39830 PE=2 SV=1	0
C_Locus_9140_Transcript_1/1_Confidence_1.000_Length_1320	Myb domain protein 108 OS=Arabidopsis thaliana GN=MYB108 PE=2 SV=1	1.66511e-95
C_Locus_760_Transcript_9/10_Confidence_0.500_Length_1100	At4g24380 OS=Arabidopsis thaliana GN=At4g24380/T22A6_210 PE=2 SV=1	1.74833e-39
C_Locus_16628_Transcript_3/3_Confidence_0.818_Length_1896	Cytochrome P450 OS=Arabidopsis thaliana GN=CYP72A15 PE=2 SV=1	3.69648e-152
C_Locus_280_Transcript_3/10_Confidence_0.667_Length_1896	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=1 SV=2	0
C_Locus_4152_Transcript_3/4_Confidence_0.250_Length_1249	Lysine histidine transporter-like 8 OS=Arabidopsis thaliana GN=AATL1 PE=1 SV=1	3.58047e-70
C_Locus_17581_Transcript_1/2_Confidence_1.000_Length_1810	PP2C-type phosphatase AP2C1 OS=Arabidopsis thaliana GN=AP2C1 PE=3 SV=1	4.78017e-71
C_Locus_35805_Transcript_1/1_Confidence_1.000_Length_1810	Kinase-like protein OS=Arabidopsis thaliana GN=At2g05940 PE=2 SV=1	0
C_Locus_1030_Transcript_3/3_Confidence_0.778_Length_1250	At1g13520 OS=Arabidopsis thaliana GN=At1g13520 PE=2 SV=1	2.03163e-98
C_Locus_16674_Transcript_13/16_Confidence_0.047_Length_573	Alanine aminotransferase 2, mitochondrial OS=Arabidopsis thaliana GN=ALAAT2 PE=2 SV=1	1.1618e-06
C_Locus_9521_Transcript_2/4_Confidence_0.300_Length_475	Probable xyloglucanendotransglucosylase/hydrolase protein 25 OS=Arabidopsis thaliana GN=XTH25 PE=2 SV=2	1.418e-39
C_Locus_14275_Transcript_1/3_Confidence_0.714_Length_1361	Cysteine-rich receptor-like protein kinase 10 OS=Arabidopsis thaliana GN=CRK10 PE=1 SV=3	9.30826e-97

C_Locus_18716_Transcript_1/1_Confidence_1.000_Length_861	Putative uncharacterized protein At3g25780 OS=Arabidopsis thaliana GN=At3g25780 PE=2 SV=1	3.16845e-100
C_Locus_8435_Transcript_1/1_Confidence_1.000_Length_1140	Predicted by genscan and genefinder OS=Arabidopsis thaliana GN=At2g01300 PE=2 SV=1	7.00057e-37
C_Locus_7205_Transcript_5/10_Confidence_0.175_Length_863	NA	NA
C_Locus_4587_Transcript_1/2_Confidence_1.000_Length_2307	Calmodulin-binding protein-like protein OS=Arabidopsis thaliana GN=At3g13600 PE=4 SV=1	0
C_Locus_12181_Transcript_2/10_Confidence_0.346_Length_1864	Cysteine-rich receptor-like protein kinase 2 OS=Arabidopsis thaliana GN=CRK2 PE=2 SV=1	8.22553e-109
C_Locus_4900_Transcript_2/10_Confidence_0.538_Length_2621	ADP-ribosylation factor GTPase-activating protein AGD1 OS=Arabidopsis thaliana GN=AGD1 PE=2 SV=2	1.0527e-42
C_Locus_10228_Transcript_3/4_Confidence_0.750_Length_2309	Glutamate dehydrogenase (Fragment) OS=Arabidopsis thaliana GN=AT5G07440 PE=2 SV=1	2.46946e-165
C_Locus_22529_Transcript_6/6_Confidence_0.684_Length_1532	11-beta-hydroxysteroid dehydrogenase-like OS=Arabidopsis thaliana GN=HSD1 PE=1 SV=1	3.46627e-60
C_Locus_21310_Transcript_1/1_Confidence_1.000_Length_1533	At4g10500 OS=Arabidopsis thaliana GN=F3H7.16 PE=2 SV=1	4.46174e-159
C_Locus_22122_Transcript_1/2_Confidence_1.000_Length_896	Ethylene-responsive transcription factor ERF020 OS=Arabidopsis thaliana GN=ERF020 PE=2 SV=1	9.72058e-35
C_Locus_19115_Transcript_2/2_Confidence_1.000_Length_1406	At5g61890 OS=Arabidopsis thaliana PE=2 SV=1	5.81314e-34

C_Locus_6999_Transcript_2/2_Confidence_1.000_Length_698	DVL3 OS=Arabidopsis thaliana GN=RTFL21 PE=4 SV=1	0.000541101
C_Locus_13753_Transcript_6/9_Confidence_0.381_Length_1181	Proline-rich receptor-like protein kinase PERK14 OS=Arabidopsis thaliana GN=PERK14 PE=2 SV=1	7.93839e-19
C_Locus_1242_Transcript_4/5_Confidence_0.615_Length_2010	Wall-associated receptor kinase 3 OS=Arabidopsis thaliana GN=WAK3 PE=2 SV=2	5.73363e-138
C_Locus_12810_Transcript_1/5_Confidence_0.692_Length_1770	At2g15730 OS=Arabidopsis thaliana GN=At2g15730 PE=2 SV=1	7.99185e-131
C_Locus_18839_Transcript_3/10_Confidence_0.406_Length_427	NA	NA
C_Locus_14771_Transcript_1/5_Confidence_0.455_Length_2442	Wall-associated receptor kinase 1 OS=Arabidopsis thaliana GN=WAK1 PE=1 SV=2	8.67684e-128
C_Locus_17246_Transcript_1/2_Confidence_1.000_Length_998	At3g57830 OS=Arabidopsis thaliana GN=At3g57830/T10K17_40 PE=2 SV=1	7.6653e-81
C_Locus_610_Transcript_2/6_Confidence_0.579_Length_427	F-box protein At1g61340 OS=Arabidopsis thaliana GN=At1g61340 PE=2 SV=1	8.02938e-21
C_Locus_20216_Transcript_2/2_Confidence_1.000_Length_600	AT5G07440 protein (Fragment) OS=Arabidopsis thaliana GN=AT5G07440 PE=2 SV=1	8.14438e-55
C_Locus_19992_Transcript_1/1_Confidence_1.000_Length_999	Ethylene-responsive transcription factor ERF109 OS=Arabidopsis thaliana GN=ERF109 PE=1 SV=1	6.62596e-29
C_Locus_20216_Transcript_1/2_Confidence_1.000_Length_800	AT5G07440 protein (Fragment) OS=Arabidopsis thaliana GN=AT5G07440 PE=2 SV=1	1.84895e-32
C_Locus_16009_Transcript_1/3_Confidence_0.429_Length_1156	NA	NA
C_Locus_5955_Transcript_3/4_Confidence_0.364_Length_800	Calmodulin-like protein 11 OS=Arabidopsis thaliana GN=CML11 PE=2 SV=1	7.17731e-35
C_Locus_4375_Transcript_9/10_Confidence_0.172_Length_737	Coatomer subunit beta'-1 OS=Arabidopsis thaliana GN=At1g79990 PE=4 SV=1	2.8425e-15

C_Locus_16659_Transcript_8/10_Confidence_0.078_Length_449	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP707A1 PE=3 SV=1	1.70737e-37
C_Locus_33344_Transcript_1/1_Confidence_1.000_Length_1158	Rossmann-fold NAD(P)-binding domain-containing protein OS=Arabidopsis thaliana GN=T18N14.60 PE=3 SV=1	1.38552e-77
C_Locus_23010_Transcript_4/4_Confidence_0.600_Length_1702	Class I glutamine amidotransferase domain-containing protein OS=Arabidopsis thaliana GN=At5g38200 PE=4 SV=1	0
C_Locus_17599_Transcript_1/2_Confidence_1.000_Length_2338	Ethylene-responsive transcription factor ERF110 OS=Arabidopsis thaliana GN=ERF110 PE=2 SV=2	1.05953e-16
C_Locus_2703_Transcript_5/5_Confidence_0.733_Length_1324	Myb-like HTH transcriptional regulator-like protein OS=Arabidopsis thaliana GN=At2g01060/F23H14.3 PE=2 SV=1	2.50979e-48
C_Locus_10543_Transcript_2/3_Confidence_0.667_Length_1253	B3 domain-containing protein At2g36080 OS=Arabidopsis thaliana GN=ARF31 PE=2 SV=1	1.1224e-61
C_Locus_8760_Transcript_1/1_Confidence_1.000_Length_633	NA	NA
C_Locus_26856_Transcript_1/2_Confidence_0.800_Length_633	At2g23270 OS=Arabidopsis thaliana GN=At2g23270 PE=2 SV=1	8.4288e-09
C_Locus_34802_Transcript_1/1_Confidence_1.000_Length_1255	C-repeat binding factor 4 OS=Arabidopsis thaliana GN=CBF4 PE=2 SV=1	1.28986e-57
C_Locus_14576_Transcript_2/10_Confidence_0.289_Length_1107	Glutathione S-transferase T2 OS=Arabidopsis thaliana GN=GSTT2 PE=2 SV=1	4.4664e-07
C_Locus_1906_Transcript_5/9_Confidence_0.077_Length_635	NA	NA
C_Locus_12810_Transcript_2/5_Confidence_0.692_Length_1741	At2g15730 OS=Arabidopsis thaliana GN=At2g15730 PE=2 SV=1	2.31972e-162

C_Locus_111_Transcript_2/11_Confidence_0.382_Length_2134	NA	NA
C_Locus_26703_Transcript_1/2_Confidence_0.857_Length_1041	NA	NA
C_Locus_4498_Transcript_2/2_Confidence_1.000_Length_2028	Probable WRKY transcription factor 31 OS=Arabidopsis thaliana GN=WRKY31 PE=2 SV=1	1.18097e-117
C_Locus_3327_Transcript_1/6_Confidence_0.722_Length_1493	Probable inactive poly [ADP-ribose] polymerase SRO2 OS=Arabidopsis thaliana GN=SRO2 PE=1 SV=1	1.13506e-71
C_Locus_4790_Transcript_3/7_Confidence_0.421_Length_2031	F-box/LRR-repeat protein 17 OS=Arabidopsis thaliana GN=FBL17 PE=1 SV=1	0
C_Locus_1073_Transcript_9/10_Confidence_0.333_Length_480	Auxin efflux carrier family protein OS=Arabidopsis thaliana GN=T7H20_40 PE=4 SV=1	2.71338e-14
C_Locus_24290_Transcript_1/1_Confidence_1.000_Length_864	NAC-domain protein-like OS=Arabidopsis thaliana GN=At5g22380 PE=2 SV=1	2.93954e-70
C_Locus_2750_Transcript_1/2_Confidence_1.000_Length_667	Calcium-binding protein CML38 OS=Arabidopsis thaliana GN=CML38 PE=2 SV=1	2.65001e-45
C_Locus_2067_Transcript_1/7_Confidence_0.556_Length_1958	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 PE=1 SV=2	0
C_Locus_28124_Transcript_1/3_Confidence_0.667_Length_1077	At2g31180 OS=Arabidopsis thaliana GN=MYB14 PE=2 SV=1	4.81649e-77
C_Locus_2389_Transcript_1/3_Confidence_0.778_Length_1616	Temperature-sensitive omega-3 fatty acid desaturase, chloroplastic OS=Arabidopsis thaliana GN=FAD8 PE=2 SV=1	0
C_Locus_338_Transcript_5/18_Confidence_1.000_Length_569	NA	NA

C_Locus_216_Transcript_8/10_Confidence_0.176_Length_765	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=1	1.30171e-46
C_Locus_23975_Transcript_1/2_Confidence_1.000_Length_1657	F27F5.12 OS=Arabidopsis thaliana PE=4 SV=1	4.15318e-09
C_Locus_280_Transcript_9/10_Confidence_0.630_Length_1583	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=1 SV=2	5.73341e-168
C_Locus_2682_Transcript_4/6_Confidence_0.600_Length_2448	At2g26190/T1D16.17 OS=Arabidopsis thaliana GN=At2g26190 PE=2 SV=1	6.64504e-163
C_Locus_216_Transcript_3/10_Confidence_0.176_Length_1001	AT1G80840 protein OS=Arabidopsis thaliana GN=AT1G80840 PE=2 SV=1	1.4763e-43
C_Locus_18090_Transcript_2/3_Confidence_0.667_Length_1003	Alpha-hydroxynitrilelyase OS=Arabidopsis thaliana GN=HNL PE=1 SV=1	3.30007e-81
C_Locus_27214_Transcript_1/1_Confidence_1.000_Length_1005	Transcription repressor MYB5 OS=Arabidopsis thaliana GN=MYB5 PE=1 SV=1	5.99192e-60
C_Locus_16009_Transcript_2/3_Confidence_0.571_Length_1225	NA	NA
C_Locus_10228_Transcript_1/4_Confidence_0.750_Length_1454	Glutamate dehydrogenase (Fragment) OS=Arabidopsis thaliana GN=AT5G07440 PE=2 SV=1	0
C_Locus_12692_Transcript_3/5_Confidence_0.273_Length_1229	Leucine-rich repeat receptor-like protein kinase (Fragment) OS=Arabidopsis thaliana GN=LRR-RLK PE=2 SV=1	3.89493e-68
C_Locus_2682_Transcript_6/6_Confidence_0.600_Length_2344	At2g26190/T1D16.17 OS=Arabidopsis thaliana GN=At2g26190 PE=2 SV=1	0
C_Locus_17940_Transcript_3/3_Confidence_0.429_Length_830	NA	NA

Table 6: DGE analysis of genes up-regulated under cold stress

Transcript ID	log2 Fold Change	Description	E-value
C_Locus_6128_Tra	4.67	NA	NA
C_Locus_22259_T	4.68	Glutathione S-transferase U19 OS=Arabidopsis thaliana GN=GST	1.36004e-82
C_Locus_483_Tra	4.69	Protein kinase-like protein OS=Arabidopsis thaliana GN=At5g359	2.10985e-143
C_Locus_7078_Tra	4.69	NA	NA
C_Locus_5844_Tra	4.70	AT5G61520 protein OS=Arabidopsis thaliana GN=AT5G61520 PE	1.86149e-180
C_Locus_16839_T	4.70	AT4g32400/F8B4_100 OS=Arabidopsis thaliana GN=F8B4.100 P	1.19001e-71
C_Locus_22148_T	4.71	Phospholipase D p1 OS=Arabidopsis thaliana GN=PLDP1 PE=2	0
C_Locus_5906_Tra	4.72	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	3.94927e-111
A_Locus_16079_T	4.73	NA	NA
C_Locus_19053_T	4.73	Putative Myb family transcription factor At1g14600 OS=Arabidopsi	2.58923e-29
C_Locus_9058_Tra	4.74	Actin-like ATPase superfamily protein OS=Arabidopsis thaliana G	6.52238e-99
C_Locus_1210_Tra	4.74	Sulfite reductase [ferredoxin], chloroplastic OS=Arabidopsis thalia	4.49504e-80
C_Locus_9196_Tra	4.74	MYB transcription factor OS=Arabidopsis thaliana GN=T22E19.5	6.04147e-57
C_Locus_26704_T	4.75	At1g52565 OS=Arabidopsis thaliana GN=At1g52565 PE=2 SV=1	1.3463e-14
C_Locus_17625_T	4.75	NA	NA
C_Locus_22632_T	4.75	Wall-associated receptor kinase-like 20 OS=Arabidopsis thaliana	4.37427e-115
C_Locus_13520_T	4.76	Phosphoenolpyruvate/phosphate translocator 2, chloroplastic OS=	7.25657e-39
C_Locus_16839_T	4.76	AT4g32400/F8B4_100 OS=Arabidopsis thaliana GN=F8B4.100 P	3.56118e-118
C_Locus_1486_Tra	4.76	Protein WAX2 OS=Arabidopsis thaliana GN=WAX2 PE=1 SV=1	1.91906e-120
C_Locus_2060_Tra	4.76	NA	NA
C_Locus_4195_Tra	4.76	NA	NA
C_Locus_8102_Tra	4.77	Heavy metal P-type ATPase OS=Arabidopsis thaliana GN=HMA5	0
C_Locus_548_Tra	4.78	Adenine phosphoribosyltransferase 1, chloroplastic OS=Arabidops	1.67483e-29
C_Locus_17508_T	4.78	Phosphorylase-like protein protein OS=Arabidopsis thaliana GN=A	2.11313e-101

C_Locus_28170_T	4.79	AT5g13180/T19L5_140 OS=Arabidopsis thaliana GN=T19L5_140	5.8415e-49
C_Locus_5906_Tra	4.80	G-type lectin S-receptor-like serine/threonine-protein kinase At1g6	1.1227e-131
C_Locus_7576_Tra	4.81	G-type lectin S-receptor-like serine/threonine-protein kinase At1g6	2.07357e-104
C_Locus_1210_Tra	4.81	Sulfite reductase [ferredoxin], chloroplastic OS=Arabidopsis thalia	0
C_Locus_5906_Tra	4.81	G-type lectin S-receptor-like serine/threonine-protein kinase At1g6	2.14508e-150
C_Locus_21929_T	4.81	At2g17040 OS=Arabidopsis thaliana GN=At2g17040 PE=2 SV=1	1.94462e-55
C_Locus_1057_Tra	4.81	At5g07400 OS=Arabidopsis thaliana GN=At5g07400 PE=2 SV=1	0
C_Locus_4320_Tra	4.82	Serine/threonine-protein kinase At3g07070 OS=Arabidopsis thalia	4.52248e-137
A_Locus_30158_T	4.83	1-aminocyclopropane-1-carboxylate oxidase 4 OS=Arabidopsis th	4.38242e-97
C_Locus_19000_T	4.83	AT4g27450/F27G19_50 OS=Arabidopsis thaliana GN=At4g27450	2.49947e-102
C_Locus_27154_T	4.84	Putative uncharacterized protein At1g29860 (Fragment) OS=Arabi	1.2889e-31
C_Locus_8191_Tra	4.84	Endoxyloglucan transferase OS=Arabidopsis thaliana GN=At5g13	0
C_Locus_4192_Tra	4.84	Inositol transporter 1 OS=Arabidopsis thaliana GN=INT1 PE=1 SV	2.75115e-46
C_Locus_22914_T	4.85	AAA-type ATPase like protein OS=Arabidopsis thaliana GN=At2g	2.03358e-79
C_Locus_5412_Tra	4.85	Expansin-like A2 OS=Arabidopsis thaliana GN=EXLA2 PE=2 SV=	6.61489e-130
C_Locus_22735_T	4.85	NA	NA
C_Locus_22864_T	4.86	Putative uncharacterized protein F4P12_390 OS=Arabidopsis thali	3.26298e-77
C_Locus_20516_T	4.86	Putative uncharacterized protein T11I11.11 OS=Arabidopsis thalia	1.02515e-28
C_Locus_1795_Tra	4.87	No apical meristem domain-containing transcriptional regulator OS	6.32512e-89
C_Locus_4564_Tra	4.87	12-oxophytodienoate reductase 1 OS=Arabidopsis thaliana GN=O	6.61525e-171
C_Locus_9043_Tra	4.87	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 P	2.36281e-55
C_Locus_3198_Tra	4.88	At2g39220 OS=Arabidopsis thaliana GN=At2g39220/T16B24.14 P	2.92065e-139
C_Locus_582_Tran	4.88	2-nitropropane dioxygenase-like protein OS=Arabidopsis thaliana	2.65228e-16
C_Locus_12242_T	4.88	Respiratory burst oxidase homolog protein C OS=Arabidopsis thal	1.09269e-164
C_Locus_3590_Tra	4.88	Hevein-like protein OS=Arabidopsis thaliana GN=HEL PE=1 SV=1	7.68859e-49

C_Locus_1534_Tra	4.88	Probable inactive poly [ADP-ribose] polymerase SRO5 OS=Arabid	5.60551e-78
C_Locus_113_Tran	4.89	Lipase class 3 family protein OS=Arabidopsis thaliana GN=At3g14	2.98833e-173
C_Locus_2009_Tra	4.90	At5g01980 OS=Arabidopsis thaliana GN=T7H20_30 PE=2 SV=1	1.14882e-106
C_Locus_4421_Tra	4.90	NA	NA
C_Locus_4110_Tra	4.90	Inorganic phosphate transporter 2-1, chloroplastic OS=Arabidopsis	2.64726e-29
C_Locus_1486_Tra	4.91	Protein WAX2 OS=Arabidopsis thaliana GN=WAX2 PE=1 SV=1	0
C_Locus_5589_Tra	4.91	NAC domain-containing protein 72 OS=Arabidopsis thaliana GN=N	1.88578e-97
C_Locus_13383_T	4.91	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	1.48079e-52
C_Locus_1210_Tra	4.91	Sulfite reductase [ferredoxin], chloroplastic OS=Arabidopsis thalia	0
C_Locus_29552_T	4.91	Putative glucuronosyltransferase PGSIP8 OS=Arabidopsis thaliana	0
C_Locus_8884_Tra	4.91	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 PE	2.90234e-171
C_Locus_2138_Tra	4.91	Mitochondrial substrate carrier family protein OS=Arabidopsis thal	2.40115e-141
C_Locus_3198_Tra	4.91	At2g39220 OS=Arabidopsis thaliana GN=At2g39220/T16B24.14 P	0
C_Locus_4110_Tra	4.92	Inorganic phosphate transporter 2-1, chloroplastic OS=Arabidopsis	6.08445e-29
C_Locus_6614_Tra	4.92	Ethylene-responsive transcription factor ERF095 OS=Arabidopsis	1.44506e-22
C_Locus_5906_Tra	4.92	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	1.12592e-109
C_Locus_5291_Tra	4.93	1-aminocyclopropane-1-carboxylate oxidase 4 OS=Arabidopsis tha	5.61099e-80
C_Locus_322_Tran	4.93	Putative farnesylated protein (Fragment) OS=Arabidopsis thaliana	9.22088e-29
C_Locus_483_Tran	4.93	Protein kinase-like protein OS=Arabidopsis thaliana GN=At5g3596	2.38269e-161
C_Locus_3169_Tra	4.93	Probable boron transporter 2 OS=Arabidopsis thaliana GN=BOR2	2.6373e-129
C_Locus_9631_Tra	4.93	9-cis-epoxycarotenoid dioxygenase NCED3, chloroplastic OS=Ara	0
C_Locus_1057_Tra	4.94	At5g07400 OS=Arabidopsis thaliana GN=At5g07400 PE=2 SV=1	0
C_Locus_1754_Tra	4.95	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase OS=Arabidop	3.39677e-20
C_Locus_2232_Tra	4.95	Protein phosphatase 2C 3 OS=Arabidopsis thaliana GN=AIP1 PE	4.20757e-130
C_Locus_4105_Tra	4.95	Receptor like protein 1 OS=Arabidopsis thaliana GN=RLP1 PE=4	4.55234e-164

C_Locus_440_Tra	4.95	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 S	3.27128e-123
C_Locus_26780_T	4.95	Predicted GPI-anchored protein OS=Arabidopsis thaliana GN=At1	9.37211e-141
C_Locus_19053_T	4.96	Putative Myb family transcription factor At1g14600 OS=Arabidopsi	1.84389e-29
C_Locus_12370_T	4.96	Putative disease resistance protein (TMV N-like) OS=Arabidopsis	8.39915e-140
C_Locus_9043_Tr	4.96	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 P	7.60752e-171
C_Locus_4375_Tr	4.96	Coatomer subunit beta'-2 OS=Arabidopsis thaliana GN=At1g5236	2.00294e-54
C_Locus_22148_T	4.96	Phospholipase D p1 OS=Arabidopsis thaliana GN=PLDP1 PE=2	0
C_Locus_481_Tra	4.97	ABC transporter G family member 40 OS=Arabidopsis thaliana G	3.25295e-22
C_Locus_9978_Tr	4.97	Wall-associated receptor kinase 4 OS=Arabidopsis thaliana GN=	6.65462e-93
C_Locus_22914_T	4.97	AAA-type ATPase like protein OS=Arabidopsis thaliana GN=At2g	8.58602e-137
A_Locus_17434_T	4.97	F-box/kelch-repeat protein At1g57790 OS=Arabidopsis thaliana G	2.78909e-104
C_Locus_440_Tra	4.97	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 S	2.55857e-134
C_Locus_22148_T	4.98	Phospholipase D p1 OS=Arabidopsis thaliana GN=PLDP1 PE=2	0
A_Locus_7255_Tr	4.98	Sulfite reductase [ferredoxin], chloroplastic OS=Arabidopsis thalia	0
C_Locus_17508_T	4.99	Phosphorylase-like protein protein OS=Arabidopsis thaliana GN=A	2.45781e-89
A_Locus_23447_T	4.99	BON1-associated protein 2 OS=Arabidopsis thaliana GN=BAP2 P	3.76579e-19
C_Locus_1842_Tr	4.99	UDP-arabinopyranose mutase 1 OS=Arabidopsis thaliana GN=RG	3.28278e-64
C_Locus_113_Tra	5.00	Lipase class 3 family protein OS=Arabidopsis thaliana GN=At3g1	3.36395e-140
C_Locus_3185_Tr	5.00	Probable calcium-binding protein CML16 OS=Arabidopsis thaliana	1.77161e-73
C_Locus_113_Tra	5.01	Lipase class 3 family protein OS=Arabidopsis thaliana GN=At3g1	6.81063e-137
A_Locus_8491_Tr	5.01	Protein TIFY 9 OS=Arabidopsis thaliana GN=TIFY9 PE=1 SV=1	1.24304e-19
C_Locus_7796_Tr	5.01	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_30198_T	5.02	Alanine acetyl transferase-like protein OS=Arabidopsis thaliana G	1.07664e-62
C_Locus_854_Tra	5.02	Probably inactive leucine-rich repeat receptor-like protein kinase A	3.87358e-58
C_Locus_4320_Tr	5.02	Serine/threonine-protein kinase At3g07070 OS=Arabidopsis thalia	1.55439e-139

C_Locus_338_Tra	5.03	At4g37640 OS=Arabidopsis thaliana PE=2 SV=1	8.68511e-155
C_Locus_1210_Tra	5.03	Sulfite reductase [ferredoxin], chloroplastic OS=Arabidopsis thalia	0
C_Locus_2232_Tra	5.03	Protein phosphatase 2C 3 OS=Arabidopsis thaliana GN=AIP1 PE	5.6169e-81
C_Locus_2781_Tra	5.03	Adenine nucleotide alpha hydrolases-domain containing protein ki	7.38054e-113
C_Locus_15843_T	5.04	RING-H2 finger protein ATL60 OS=Arabidopsis thaliana GN=ATL6	2.79975e-51
C_Locus_29787_T	5.04	Putative uncharacterized protein At1g13340 OS=Arabidopsis thali	9.83259e-85
C_Locus_8884_Tra	5.04	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 P	1.71931e-79
C_Locus_7796_Tra	5.04	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_7149_Tra	5.04	Putative uncharacterized protein At1g30320 OS=Arabidopsis thali	3.35922e-85
C_Locus_9787_Tra	5.05	At3g03280 OS=Arabidopsis thaliana GN=T17B22.3 PE=2 SV=1	4.65916e-08
A_Locus_14300_T	5.05	NA	NA
C_Locus_4320_Tra	5.05	Serine/threonine-protein kinase At3g07070 OS=Arabidopsis thalia	1.62962e-163
C_Locus_2062_Tra	5.05	1-aminocyclopropane-1-carboxylate synthase 6 OS=Arabidopsis t	7.45201e-72
C_Locus_483_Tra	5.06	Protein kinase-like protein OS=Arabidopsis thaliana GN=At5g359	8.03996e-168
C_Locus_572_Tra	5.06	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g05280	1.31739e-159
C_Locus_8102_Tra	5.07	Heavy metal P-type ATPase OS=Arabidopsis thaliana GN=HMA5	0
C_Locus_2781_Tra	5.08	T14N5.13 protein OS=Arabidopsis thaliana GN=T14N5.13 PE=3 S	3.73102e-99
C_Locus_17628_T	5.08	NA	NA
A_Locus_23496_T	5.08	AT4g27450/F27G19_50 OS=Arabidopsis thaliana GN=At4g27450	2.72919e-126
C_Locus_440_Tra	5.09	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 SV	6.23343e-101
C_Locus_811_Tra	5.10	Putative quinone-oxidoreductase homolog, chloroplastic OS=Arabi	0
C_Locus_8102_Tra	5.10	Heavy metal P-type ATPase OS=Arabidopsis thaliana GN=HMA5	3.73919e-156
A_Locus_7631_Tra	5.10	NA	NA
C_Locus_322_Tra	5.10	Putative farnesylated protein (Fragment) OS=Arabidopsis thaliana	1.70783e-28
C_Locus_6212_Tra	5.11	Beta-1,3-glucanase 3 OS=Arabidopsis thaliana GN=BG3 PE=3 S	5.24988e-125

C_Locus_22148_T	5.11	Phospholipase D p1 OS=Arabidopsis thaliana GN=PLDP1 PE=2	0
C_Locus_16839_T	5.11	AT4g32400/F8B4_100 OS=Arabidopsis thaliana GN=F8B4.100 PE	2.29212e-74
C_Locus_3526_Tra	5.11	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	7.67061e-54
C_Locus_2870_Tra	5.12	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	0
C_Locus_1101_Tra	5.13	Ethylene receptor 2 OS=Arabidopsis thaliana GN=ETR2 PE=1 SV	2.86694e-52
C_Locus_19555_T	5.13	Putative methylesterase 11, chloroplastic OS=Arabidopsis thalian	1.0404e-135
C_Locus_5412_Tra	5.13	Expansin-like A2 OS=Arabidopsis thaliana GN=EXLA2 PE=2 SV=	6.20228e-117
C_Locus_6739_Tra	5.14	Similar to latex allergen from Hevea brasiliensis OS=Arabidopsis t	3.51081e-76
C_Locus_4375_Tra	5.14	Coatomer subunit beta'-2 OS=Arabidopsis thaliana GN=At1g5236	3.54388e-126
C_Locus_4195_Tra	5.14	NA	NA
A_Locus_30362_T	5.14	VQ motif-containing protein OS=Arabidopsis thaliana GN=At1g17	8.09801e-08
C_Locus_7796_Tra	5.14	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_34735_T	5.15	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 S	1.30352e-05
C_Locus_7796_Tra	5.15	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_322_Tran	5.15	Heavy-metal-associated domain-containing protein OS=Arabidopsi	1.05343e-21
C_Locus_17010_T	5.15	NA	NA
C_Locus_12242_T	5.15	Respiratory burst oxidase homolog protein D OS=Arabidopsis thal	0
C_Locus_16983_T	5.16	Putative uncharacterized protein At1g21340 (Fragment) OS=Arabi	3.8152e-41
C_Locus_6481_Tra	5.16	At3g50700 OS=Arabidopsis thaliana GN=T3A5.80 PE=2 SV=1	5.74501e-100
C_Locus_8963_Tra	5.16	Toll-Interleukin-Resistance domain-containing protein OS=Arabido	0.000286028
C_Locus_24355_T	5.17	Cytochrome P450 82C2 OS=Arabidopsis thaliana GN=CYP82C2	3.18193e-143
C_Locus_30150_T	5.17	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 S	9.28535e-18
C_Locus_2438_Tra	5.17	Protein kinase APK1A OS=Arabidopsis thaliana GN=APK1A PE=	5.69348e-138
C_Locus_22555_T	5.17	At4g31290 OS=Arabidopsis thaliana GN=At4g31290 PE=2 SV=1	2.39704e-32
C_Locus_18738_T	5.17	AAA-ATPase 1 OS=Arabidopsis thaliana GN=AATP1 PE=3 SV=1	0

C_Locus_1073_Tra	5.18	Auxin efflux carrier family protein OS=Arabidopsis thaliana GN=T7	8.26047e-86
C_Locus_2318_Tra	5.18	Transmembrane amino acid transporter family protein OS=Arabido	3.50269e-167
C_Locus_16619_T	5.18	Wall-associated receptor kinase-like 2 OS=Arabidopsis thaliana G	4.32729e-149
C_Locus_416_Tra	5.18	F-box protein At3g56470 OS=Arabidopsis thaliana GN=At3g5647	3.87356e-40
C_Locus_1073_Tra	5.18	Auxin efflux carrier family protein OS=Arabidopsis thaliana GN=T7	2.4746e-110
C_Locus_26704_T	5.18	At1g52565 OS=Arabidopsis thaliana GN=At1g52565 PE=2 SV=1	3.00009e-11
C_Locus_31962_T	5.19	F10A5.18 OS=Arabidopsis thaliana GN=At1g75620 PE=4 SV=1	0
C_Locus_2781_Tra	5.19	Adenine nucleotide alpha hydrolases-domain containing protein ki	2.35574e-102
A_Locus_22228_T	5.20	NA	NA
C_Locus_7468_Tra	5.20	At1g72510 OS=Arabidopsis thaliana GN=T10D10.2 PE=2 SV=1	7.12454e-37
C_Locus_2787_Tra	5.21	Leucine-rich repeat receptor-like protein kinase OS=Arabidopsis th	5.70424e-33
C_Locus_17570_T	5.21	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	9.84854e-141
C_Locus_17364_T	5.22	Toll-Interleukin-Resistance domain-containing protein OS=Arabido	5.54946e-26
C_Locus_947_Tra	5.22	Leucine-rich repeat receptor-like protein kinase OS=Arabidopsis th	4.30723e-80
C_Locus_2318_Tra	5.23	Transmembrane amino acid transporter family protein OS=Arabido	1.08548e-136
C_Locus_26808_T	5.23	COBRA-like protein 7 OS=Arabidopsis thaliana GN=COBL7 PE=1	0
C_Locus_21870_T	5.23	PP2C-type phosphatase AP2C3 OS=Arabidopsis thaliana GN=AP	2.12576e-06
C_Locus_1842_Tra	5.23	UDP-arabinopyranose mutase 2 OS=Arabidopsis thaliana GN=RG	5.71672e-36
C_Locus_8800_Tra	5.23	Uncharacterized protein OS=Arabidopsis thaliana GN=At5g54240	2.67417e-131
C_Locus_5275_Tra	5.24	Shaggy-related protein kinase epsilon OS=Arabidopsis thaliana G	9.84057e-99
C_Locus_10953_T	5.24	NAC domain containing protein 71 OS=Arabidopsis thaliana GN=A	2.35091e-101
C_Locus_5324_Tra	5.24	At2g28570 OS=Arabidopsis thaliana GN=At2g28570 PE=2 SV=1	0.000118945
C_Locus_7000_Tra	5.24	Indole-3-acetic acid inducible 29 (Fragment) OS=Arabidopsis thali	3.113e-21
C_Locus_322_Tra	5.26	Heavy metal transport/detoxification domain-containing protein OS	1.73757e-14
C_Locus_2975_Tra	5.26	Probable nucleoredoxin 1 OS=Arabidopsis thaliana GN=At1g6042	5.6768e-61

C_Locus_8557_Tra	5.26	Urophorphyrin methylase 1 OS=Arabidopsis thaliana GN=UPM1 P	3.08794e-97
A_Locus_17474_T	5.26	Putative farnesylated protein (Fragment) OS=Arabidopsis thaliana	2.61669e-29
C_Locus_2210_Tra	5.27	Mitogen-activated protein kinase kinase kinase 19 OS=Arabidopsi	2.56682e-118
C_Locus_14242_T	5.27	UDP-glucose 6-dehydrogenase OS=Arabidopsis thaliana GN=T20	0
A_Locus_20219_T	5.27	Cytochrome P450 71B36 OS=Arabidopsis thaliana GN=CYP71B3	5.75699e-49
C_Locus_22555_T	5.27	At5g26220 OS=Arabidopsis thaliana GN=At5g26220/T19G15_70	1.73735e-57
C_Locus_26808_T	5.27	COBRA-like protein 7 OS=Arabidopsis thaliana GN=COBL7 PE=1	0
C_Locus_17086_T	5.28	At2g31180 OS=Arabidopsis thaliana GN=MYB14 PE=2 SV=1	2.29201e-49
C_Locus_440_Tran	5.28	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 SV	1.43595e-136
C_Locus_19206_T	5.28	AT-hook motif nuclear localized protein 17 OS=Arabidopsis thalian	4.26066e-64
C_Locus_14147_T	5.28	Pathogenesis-related thaumatin-like protein OS=Arabidopsis thalia	2.41762e-144
C_Locus_29787_T	5.29	Putative uncharacterized protein At1g13340 OS=Arabidopsis thalia	3.12226e-75
C_Locus_7815_Tra	5.29	Heavy-metal-associated domain-containing protein OS=Arabidopsi	4.36261e-11
C_Locus_8884_Tra	5.30	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 PE	6.93828e-171
C_Locus_947_Tran	5.31	Leucine-rich repeat receptor-like protein kinase OS=Arabidopsis th	0
C_Locus_440_Tran	5.31	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 SV	5.29996e-133
C_Locus_17086_T	5.31	At3g23250 OS=Arabidopsis thaliana GN=MYB15 PE=2 SV=1	3.734e-76
C_Locus_39710_T	5.31	Protein NIM1-INTERACTING 1 OS=Arabidopsis thaliana GN=NIMI	4.22429e-07
C_Locus_16619_T	5.31	Wall-associated receptor kinase-like 2 OS=Arabidopsis thaliana G	2.02314e-180
C_Locus_77_Trans	5.32	AT5g12010/F14F18_180 OS=Arabidopsis thaliana GN=F14F18_18	4.62236e-95
C_Locus_4105_Tra	5.32	Receptor like protein 1 OS=Arabidopsis thaliana GN=RLP1 PE=4	1.70984e-154
C_Locus_8557_Tra	5.32	Urophorphyrin methylase 1 OS=Arabidopsis thaliana GN=UPM1 P	1.79263e-64
C_Locus_189_Tran	5.33	NA	NA
C_Locus_7120_Tra	5.34	Phytoalexin deficient 4 OS=Arabidopsis thaliana GN=PAD4 PE=4	9.5284e-35
A_Locus_10291_T	5.34	Putative phi-1-like phosphate-induced protein OS=Arabidopsis thal	8.92845e-94

C_Locus_17205_T	5.34	L-type lectin-domain containing receptor kinase VII.1 OS=Arabido	0
C_Locus_8102_Tra	5.35	Heavy metal P-type ATPase OS=Arabidopsis thaliana GN=HMA5	0
C_Locus_7796_Tra	5.35	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_26791_T	5.35	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 S	1.7654e-49
C_Locus_4564_Tra	5.35	12-oxophytodienoate reductase 1 OS=Arabidopsis thaliana GN=O	0
C_Locus_26664_T	5.36	Putative phi-1-like phosphate-induced protein OS=Arabidopsis thal	4.46324e-147
C_Locus_6739_Tra	5.36	Similar to latex allergen from Hevea brasiliensis OS=Arabidopsis t	3.79886e-18
C_Locus_16619_T	5.37	Wall-associated receptor kinase-like 2 OS=Arabidopsis thaliana G	1.84095e-179
C_Locus_7149_Tra	5.37	Putative uncharacterized protein At1g30320 OS=Arabidopsis thali	6.08824e-120
C_Locus_2870_Tra	5.37	G-type lectin S-receptor-like serine/threonine-protein kinase At4g0	1.28081e-130
A_Locus_17474_T	5.37	Putative farnesylated protein (Fragment) OS=Arabidopsis thaliana	2.38645e-29
C_Locus_8633_Tra	5.37	Putative RNA-binding protein OS=Arabidopsis thaliana GN=T1B9.	4.85252e-33
C_Locus_2860_Tra	5.37	Beta-fructofuranosidase OS=Arabidopsis thaliana GN=ATBFRUCT	0
C_Locus_13753_T	5.38	Proline-rich receptor-like protein kinase PERK13 OS=Arabidopsis	1.44719e-60
C_Locus_2870_Tra	5.38	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	0
C_Locus_6095_Tra	5.38	Putative uncharacterized protein F2J7.21 OS=Arabidopsis thaliana	2.46357e-68
C_Locus_2860_Tra	5.39	Beta-fructofuranosidase, insoluble isoenzyme CWINV1 OS=Arabid	0
C_Locus_19000_T	5.39	Putative uncharacterized protein AT4g27450 OS=Arabidopsis thali	4.04935e-95
C_Locus_945_Tran	5.39	NA	NA
C_Locus_1448_Tra	5.39	Hin1 homolog OS=Arabidopsis thaliana GN=YLS9 PE=2 SV=1	6.21045e-57
C_Locus_2870_Tra	5.39	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	0
C_Locus_4157_Tra	5.40	AT1G76160 protein OS=Arabidopsis thaliana GN=sks5 PE=2 SV=	0
C_Locus_3324_Tra	5.40	U-box domain-containing protein 19 OS=Arabidopsis thaliana GN=	1.73771e-158
C_Locus_4375_Tra	5.41	Coatomer subunit beta'-2 OS=Arabidopsis thaliana GN=At1g5236	6.04447e-121
C_Locus_8633_Tra	5.41	NA	NA

C_Locus_2062_Tra	5.41	1-aminocyclopropane-1-carboxylate synthase 6 OS=Arabidopsis t	0
C_Locus_8889_Tra	5.41	Tricyclene synthase, chloroplastic OS=Arabidopsis thaliana GN=T	2.13026e-161
C_Locus_8102_Tra	5.41	Heavy metal P-type ATPase OS=Arabidopsis thaliana GN=HMA5	0
C_Locus_2649_Tra	5.42	NA	NA
C_Locus_7277_Tra	5.42	Putative glucan endo-1-3-beta-glucosidase OS=Arabidopsis thalian	0
C_Locus_1842_Tra	5.42	UDP-arabinopyranose mutase 2 OS=Arabidopsis thaliana GN=RG	6.20951e-88
C_Locus_27107_T	5.43	Probable carboxylesterase 15 OS=Arabidopsis thaliana GN=CXE1	9.48375e-52
C_Locus_3169_Tra	5.44	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 SV	3.08941e-149
C_Locus_22555_T	5.44	At5g26220 OS=Arabidopsis thaliana GN=At5g26220/T19G15_70 F	2.74837e-56
C_Locus_20580_T	5.44	Heavy metal-associated isoprenylated plant protein 26 OS=Arabid	8.07626e-11
C_Locus_1210_Tra	5.44	Sulfite reductase [ferredoxin], chloroplastic OS=Arabidopsis thalia	0
C_Locus_2350_Tra	5.44	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 S	0.000132282
C_Locus_1389_Tra	5.44	NA	NA
C_Locus_6481_Tra	5.45	At3g50700 OS=Arabidopsis thaliana GN=T3A5.80 PE=2 SV=1	1.24628e-91
C_Locus_3398_Tra	5.45	AT-hook motif nuclear localized protein 27 OS=Arabidopsis thalian	3.76931e-47
C_Locus_295_Tran	5.45	S-adenosyl-L-methionine-dependent uroporphyrinogen III methyltra	5.1199e-70
C_Locus_947_Tran	5.46	Leucine-rich repeat receptor-like protein kinase OS=Arabidopsis th	0
C_Locus_22348_T	5.46	NA	NA
C_Locus_23244_T	5.46	AT1G70780 protein OS=Arabidopsis thaliana GN=AT1G70780 PE	3.15533e-34
C_Locus_2870_Tra	5.46	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	0
C_Locus_4208_Tra	5.47	Trihelix transcription factor GT-3b OS=Arabidopsis thaliana GN=G	6.1247e-61
C_Locus_22518_T	5.48	Probable receptor-like protein kinase At1g11050 OS=Arabidopsis	0
C_Locus_5540_Tra	5.48	Leucine-rich repeat receptor-like protein kinase PEPR2 OS=Arabid	0
C_Locus_7120_Tra	5.48	Phytoalexin deficient 4 OS=Arabidopsis thaliana GN=PAD4 PE=4	2.77476e-8
C_Locus_2252_Tra	5.49	At4g13420 OS=Arabidopsis thaliana PE=2 SV=1	0

C_Locus_1198_Tra	5.49	Probable xyloglucan endotransglucosylase/hydrolase protein 21 O	1.57348e-129
C_Locus_2062_Tra	5.49	1-aminocyclopropane-1-carboxylate synthase 6 OS=Arabidopsis t	0
C_Locus_1305_Tra	5.49	Putative uncharacterized protein (Fragment) OS=Arabidopsis thali	0.000210917
C_Locus_20549_T	5.50	Syntaxin-121 OS=Arabidopsis thaliana GN=SYP121 PE=1 SV=1	1.2625e-124
C_Locus_2993_Tra	5.50	AT5g20880/F22D1_50 OS=Arabidopsis thaliana GN=At5g20885/A	3.28894e-59
C_Locus_22157_T	5.50	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g32928	1.00021e-10
C_Locus_1413_Tra	5.50	NA	NA
C_Locus_16619_T	5.51	Wall-associated receptor kinase-like 8 OS=Arabidopsis thaliana G	6.5925e-167
C_Locus_8319_Tra	5.51	ABC transporter B family member 11 OS=Arabidopsis thaliana GN	0
C_Locus_3370_Tra	5.51	ABC transporter G family member 36 OS=Arabidopsis thaliana GN	0
C_Locus_21929_T	5.52	NAC domain containing protein 36 OS=Arabidopsis thaliana GN=N	6.74644e-79
C_Locus_2062_Tra	5.52	1-aminocyclopropane-1-carboxylate synthase 6 OS=Arabidopsis t	5.76688e-175
C_Locus_6481_Tra	5.52	At3g50700 OS=Arabidopsis thaliana GN=T3A5.80 PE=2 SV=1	3.28716e-98
C_Locus_8102_Tra	5.53	Heavy metal P-type ATPase OS=Arabidopsis thaliana GN=HMA5	0
C_Locus_322_Tran	5.54	Putative farnesylated protein (Fragment) OS=Arabidopsis thaliana	1.80605e-12
C_Locus_17508_T	5.54	Phosphorylase-like protein protein OS=Arabidopsis thaliana GN=A	2.27422e-98
C_Locus_7620_Tra	5.54	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	0
C_Locus_9761_Tra	5.54	NAC domain-containing protein 62 OS=Arabidopsis thaliana GN=T	2.21606e-33
C_Locus_38_Trans	5.55	Putative P450 (Fragment) OS=Arabidopsis thaliana GN=F21J6.1 F	4.95985e-61
C_Locus_8884_Tra	5.55	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 PE	8.69418e-132
C_Locus_5906_Tra	5.55	G-type lectin S-receptor-like serine/threonine-protein kinase At1g6	1.22361e-134
C_Locus_1795_Tra	5.55	No apical meristem domain-containing transcriptional regulator OS	5.10433e-60
C_Locus_18213_T	5.56	Glutathione S-transferase F8, chloroplastic OS=Arabidopsis thalia	2.34487e-64
C_Locus_1789_Tra	5.56	G-type lectin S-receptor-like serine/threonine-protein kinase RLK1	1.98622e-140
C_Locus_4967_Tra	5.56	MtN21 nodulin protein-like OS=Arabidopsis thaliana GN=At5g0705	1.82269e-153

C_Locus_780_Tra	5.56	At3g54420 OS=Arabidopsis thaliana GN=T12E18_110 PE=2 SV=	1.00007e-90
C_Locus_8319_Tr	5.56	ABC transporter B family member 11 OS=Arabidopsis thaliana G	0
C_Locus_911_Tra	5.56	Probable WRKY transcription factor 48 OS=Arabidopsis thaliana	9.98374e-55
C_Locus_5691_Tr	5.57	9-cis-epoxycarotenoid dioxygenase NCED3, chloroplastic OS=Ar	0
C_Locus_17223_T	5.57	Protein TIFY 9 OS=Arabidopsis thaliana GN=TIFY9 PE=1 SV=1	2.10337e-49
C_Locus_19053_T	5.57	Putative Myb family transcription factor At1g14600 OS=Arabidopsi	1.66582e-29
C_Locus_7916_Tr	5.58	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At5g575	9.70872e-101
C_Locus_17925_T	5.58	NA	NA
C_Locus_19752_T	5.58	Putative uncharacterized protein At5g61820 OS=Arabidopsis thali	1.38688e-167
C_Locus_16659_T	5.58	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_18719_T	5.58	Probable galacturonosyltransferase-like 1 OS=Arabidopsis thalian	4.12944e-148
C_Locus_582_Tra	5.59	2-nitropropane dioxygenase-like protein OS=Arabidopsis thaliana	3.06429e-29
C_Locus_18213_T	5.59	Glutathione S-transferase F6 OS=Arabidopsis thaliana GN=GSTF	1.94454e-53
C_Locus_30147_T	5.60	NA	NA
C_Locus_4320_Tr	5.60	Serine/threonine-protein kinase At3g07070 OS=Arabidopsis thalia	6.65855e-170
C_Locus_38_Tran	5.60	Putative cytochrome P450 OS=Arabidopsis thaliana GN=At3g263	4.25862e-97
C_Locus_9527_Tr	5.61	NA	NA
C_Locus_34660_T	5.61	At3g57450 OS=Arabidopsis thaliana GN=T8H10.50 PE=4 SV=1	1.53044e-11
C_Locus_11494_T	5.61	Probable L-type lectin-domain containing receptor kinase S.5 OS=	3.48827e-129
C_Locus_7120_Tr	5.62	Phytoalexin deficient 4 OS=Arabidopsis thaliana GN=PAD4 PE=4	1.51355e-67
C_Locus_1458_Tr	5.62	IAA-amino acid hydrolase OS=Arabidopsis thaliana GN=At1g4435	5.96791e-29
C_Locus_7796_Tr	5.62	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_411_Tra	5.62	At4g17350 OS=Arabidopsis thaliana GN=At4g17350 PE=2 SV=1	2.43894e-107
C_Locus_19127_T	5.62	Probable aquaporin PIP2-5 OS=Arabidopsis thaliana GN=PIP2-5	1.0688e-65
C_Locus_1534_Tr	5.63	Probable inactive poly [ADP-ribose] polymerase SRO5 OS=Arabid	5.03871e-72

C_Locus_1662_Tra	5.63	At2g41380 OS=Arabidopsis thaliana GN=At2g41380 PE=2 SV=1	1.16517e-14
C_Locus_26719_T	5.64	Sigma factor binding protein 1, chloroplastic OS=Arabidopsis thali	1.82584e-10
C_Locus_1534_Tra	5.64	Probable inactive poly [ADP-ribose] polymerase SRO5 OS=Arabid	8.38726e-78
C_Locus_18735_T	5.64	Mitogen-activated protein kinase kinase kinase 15 OS=Arabidopsi	8.64564e-104
C_Locus_4971_Tra	5.65	NA	NA
A_Locus_32292_T	5.65	At2g28570 OS=Arabidopsis thaliana GN=At2g28570 PE=2 SV=1	3.98982e-07
C_Locus_3526_Tra	5.65	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	3.03614e-26
C_Locus_4110_Tra	5.65	Inorganic phosphate transporter 2-1, chloroplastic OS=Arabidopsis	9.62555e-30
C_Locus_25317_T	5.66	GDSL esterase/lipase At5g45670 OS=Arabidopsis thaliana GN=A	6.68389e-80
C_Locus_2483_Tra	5.66	Acid beta-fructofuranosidase 4, vacuolar OS=Arabidopsis thaliana	0
C_Locus_2860_Tra	5.66	Beta-fructofuranosidase OS=Arabidopsis thaliana GN=ATBFRUCT	1.10338e-70
C_Locus_411_Tran	5.67	At4g17350 OS=Arabidopsis thaliana GN=At4g17350 PE=2 SV=1	4.20898e-105
C_Locus_10380_T	5.67	Putative uncharacterized protein At2g47440 OS=Arabidopsis thalia	0
C_Locus_6095_Tra	5.67	Putative uncharacterized protein F2J7.21 OS=Arabidopsis thaliana	4.26103e-86
C_Locus_13629_T	5.68	Probable protein phosphatase 2C 75 OS=Arabidopsis thaliana GN	8.25442e-107
C_Locus_2318_Tra	5.69	Transmembrane amino acid transporter family protein OS=Arabido	1.1012e-174
C_Locus_22173_T	5.69	Putative phi-1-like phosphate-induced protein OS=Arabidopsis thal	5.56295e-148
C_Locus_26800_T	5.69	Putative uncharacterized protein At1g68470/T26J14_4 OS=Arabido	7.31923e-173
C_Locus_1842_Tra	5.69	UDP-arabinopyranose mutase 1 OS=Arabidopsis thaliana GN=RG	1.01773e-49
C_Locus_16674_T	5.69	Alanine aminotransferase 1, mitochondrial OS=Arabidopsis thalian	1.17793e-133
C_Locus_17650_T	5.70	AT1G32050 protein (Fragment) OS=Arabidopsis thaliana GN=AT1	4.31911e-37
C_Locus_23244_T	5.70	At1g70780 OS=Arabidopsis thaliana GN=F5A18.4 PE=2 SV=1	2.79151e-58
C_Locus_9909_Tra	5.70	Acetyltranferase-like protein OS=Arabidopsis thaliana GN=At3g26	1.76629e-76
C_Locus_4375_Tra	5.71	Coatomer subunit beta'-3 OS=Arabidopsis thaliana GN=At3g1598	3.18519e-149
C_Locus_3823_Tra	5.71	Putative uncharacterized protein F7A10.19 OS=Arabidopsis thalia	1.05833e-119

C_Locus_2860_Tra	5.71	Beta-fructofuranosidase, insoluble isoenzyme CWINV1 OS=Arabic	0
C_Locus_911_Tran	5.72	Probable WRKY transcription factor 48 OS=Arabidopsis thaliana G	4.33792e-50
C_Locus_16674_T	5.72	Alanine aminotransferase 1, mitochondrial OS=Arabidopsis thalian	8.34153e-39
A_Locus_7459_Tra	5.72	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0
C_Locus_12692_T	5.72	Leucine-rich repeat receptor-like protein kinase (Fragment) OS=Ar	1.19833e-179
C_Locus_23663_T	5.72	Putative uncharacterized protein AT4g36500 OS=Arabidopsis thali	1.57066e-23
A_Locus_28715_T	5.72	Auxin efflux carrier family protein OS=Arabidopsis thaliana GN=T7	3.27694e-155
C_Locus_4234_Tra	5.73	Putative serine/threonine-protein kinase-like protein CCR3 OS=Ara	0
C_Locus_7403_Tra	5.73	Dehydration-responsive element-binding protein 2C OS=Arabidops	1.29819e-26
C_Locus_18373_T	5.73	UDP-glycosyltransferase 88A1 OS=Arabidopsis thaliana GN=UGT	3.51868e-52
C_Locus_1754_Tra	5.74	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase OS=Arabidop	3.55275e-48
C_Locus_23346_T	5.74	Probable caffeoyl-CoA O-methyltransferase At4g34050 OS=Arabic	7.85164e-153
C_Locus_1906_Tra	5.75	Aquaporin TIP1-3 OS=Arabidopsis thaliana GN=TIP1-3 PE=1 SV=	1.04528e-92
C_Locus_8017_Tra	5.75	Non-specific lipid-transfer protein 1 OS=Arabidopsis thaliana GN=L	5.26944e-36
C_Locus_2210_Tra	5.75	Mitogen-activated protein kinase kinase kinase 19 OS=Arabidopsis	2.30054e-119
C_Locus_760_Tran	5.75	At4g24380 OS=Arabidopsis thaliana GN=At4g24380/T22A6_210 P	5.27312e-90
C_Locus_2801_Tra	5.76	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	0
C_Locus_411_Tran	5.76	Putative uncharacterized protein At4g17350 OS=Arabidopsis thalia	1.0152e-68
C_Locus_10722_T	5.77	Putative uncharacterized protein not annotated OS=Arabidopsis th	3.5639e-09
C_Locus_1458_Tra	5.77	IAA-amino acid hydrolase ILR1-like 4 OS=Arabidopsis thaliana GN	3.76054e-160
A_Locus_13458_T	5.77	Probable WRKY transcription factor 48 OS=Arabidopsis thaliana G	9.78659e-53
C_Locus_5842_Tra	5.78	NA	NA
C_Locus_2830_Tra	5.78	MLP-like protein 423 OS=Arabidopsis thaliana GN=MLP423 PE=1	0.000542458
C_Locus_322_Tran	5.79	Putative farnesylated protein (Fragment) OS=Arabidopsis thaliana	7.88805e-30
C_Locus_16912_T	5.79	At5g26330 OS=Arabidopsis thaliana GN=F9D12.16 PE=2 SV=1	6.74978e-12

C_Locus_7120_Tra	5.79	Phytoalexin deficient 4 OS=Arabidopsis thaliana GN=PAD4 PE=4	2.37953e-81
C_Locus_24908_T	5.79	SPX domain-containing protein 3 OS=Arabidopsis thaliana GN=SP	1.07195e-52
C_Locus_3169_Tra	5.80	Boron transporter 4 OS=Arabidopsis thaliana GN=BOR4 PE=2 SV	0
C_Locus_2860_Tra	5.80	Beta-fructofuranosidase OS=Arabidopsis thaliana GN=ATBFRUCT	1.16017e-92
C_Locus_18330_T	5.80	Aquaporin TIP1-3 OS=Arabidopsis thaliana GN=TIP1-3 PE=1 SV=	2.96514e-19
C_Locus_12880_T	5.80	Laccase-17 OS=Arabidopsis thaliana GN=LAC17 PE=2 SV=1	4.17611e-103
C_Locus_3238_Tra	5.81	Yip1 integral membrane domain-containing protein OS=Arabidopsi	9.46551e-116
C_Locus_33337_T	5.81	Ethylene-responsive transcription factor ERF017 OS=Arabidopsis	1.46103e-57
C_Locus_5615_Tra	5.81	Ammonium transporter 2 OS=Arabidopsis thaliana GN=AMT2 PE=	8.15428e-150
C_Locus_1198_Tra	5.81	Probable xyloglucan endotransglucosylase/hydrolase protein 21 O	2.12417e-137
C_Locus_21994_T	5.81	NA	NA
C_Locus_2826_Tra	5.82	At2g38470 OS=Arabidopsis thaliana PE=2 SV=1	2.64468e-89
C_Locus_9906_Tra	5.83	Nudix hydrolase 12, mitochondrial OS=Arabidopsis thaliana GN=N	2.33782e-53
C_Locus_9761_Tra	5.83	CDS OS=Arabidopsis thaliana GN=F9L11.21 PE=4 SV=1	1.04909e-33
C_Locus_18330_T	5.83	Aquaporin TIP1-3 OS=Arabidopsis thaliana GN=TIP1-3 PE=1 SV=	6.06748e-61
A_Locus_23744_T	5.84	Pathogen-induced CaM-binding protein OS=Arabidopsis thaliana G	7.99169e-26
C_Locus_917_Tran	5.85	NADH dehydrogenase OS=Arabidopsis thaliana GN=NDA2 PE=2	9.60379e-99
C_Locus_2781_Tra	5.86	Adenine nucleotide alpha hydrolases-domain containing protein kir	1.18353e-112
C_Locus_3034_Tra	5.86	Y19 protein OS=Arabidopsis thaliana GN=Y19 PE=4 SV=1	6.39642e-49
C_Locus_10884_T	5.87	At2g46620/F13A10.15 OS=Arabidopsis thaliana GN=At2g46620 P	1.49947e-05
C_Locus_12242_T	5.87	Respiratory burst oxidase homolog protein C OS=Arabidopsis thal	4.25362e-112
C_Locus_18700_T	5.87	NA	NA
C_Locus_26756_T	5.87	Putative uncharacterized protein At1g08630 OS=Arabidopsis thalia	0
C_Locus_411_Tran	5.87	At5g47440 OS=Arabidopsis thaliana GN=At5g47440 PE=2 SV=1	2.3354e-91
C_Locus_7796_Tra	5.87	Calmodulin-binding protein OS=Arabidopsis thaliana GN=At2g243	0

C_Locus_17359_T	5.88	Dof zinc finger protein DOF1.6 OS=Arabidopsis thaliana GN=DOF	5.89991e-37
C_Locus_7796_Tra	5.88	At2g24300 OS=Arabidopsis thaliana GN=At2g24300 PE=2 SV=1	5.72453e-130
C_Locus_2801_Tra	5.88	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	0
C_Locus_27466_T	5.89	Putative uncharacterized protein OS=Arabidopsis thaliana PE=4 S	3.54625e-07
C_Locus_14147_T	5.89	Pathogenesis-related thaumatin-like protein OS=Arabidopsis thalia	2.78141e-143
C_Locus_19879_T	5.89	Glutamate receptor 2.8 OS=Arabidopsis thaliana GN=GLR2.8 PE=	6.01206e-65
C_Locus_891_Tran	5.89	NA	NA
C_Locus_7857_Tra	5.89	NA	NA
C_Locus_16659_T	5.89	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_152_Tran	5.89	Phosphoglycerate dehydrogenase-like protein OS=Arabidopsis tha	8.46045e-44
C_Locus_809_Tran	5.89	Leucine-rich receptor-like protein kinase OS=Arabidopsis thaliana	0
C_Locus_2830_Tra	5.89	MLP-like protein 423 OS=Arabidopsis thaliana GN=MLP423 PE=1	4.25076e-08
C_Locus_911_Tran	5.90	Probable WRKY transcription factor 48 OS=Arabidopsis thaliana G	2.20024e-44
C_Locus_10617_T	5.90	AT5g35570/K2K18_1 OS=Arabidopsis thaliana GN=At5g35570 PE	1.02286e-53
C_Locus_21901_T	5.91	Probable E3 ubiquitin-protein ligase ARI7 OS=Arabidopsis thaliana	6.46174e-22
C_Locus_1534_Tra	5.91	Probable inactive poly [ADP-ribose] polymerase SRO5 OS=Arabid	1.22558e-77
C_Locus_665_Tran	5.91	Heme oxygenase-like, multi-helical protein OS=Arabidopsis thalian	0
C_Locus_22555_T	5.91	At5g26220 OS=Arabidopsis thaliana GN=At5g26220/T19G15_70 P	2.51015e-57
C_Locus_2866_Tra	5.91	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	9.06292e-104
C_Locus_1906_Tra	5.91	Aquaporin TIP1-3 OS=Arabidopsis thaliana GN=TIP1-3 PE=1 SV=	4.55045e-98
C_Locus_27335_T	5.91	U-box domain-containing protein 21 OS=Arabidopsis thaliana GN=	4.2652e-125
C_Locus_9906_Tra	5.91	Nudix hydrolase 12, mitochondrial OS=Arabidopsis thaliana GN=N	3.14384e-55
C_Locus_16738_T	5.91	Inorganic phosphate transporter 1-4 OS=Arabidopsis thaliana GN=	0
A_Locus_25570_T	5.92	IAA-amino acid hydrolase OS=Arabidopsis thaliana GN=At1g4435	6.15475e-07
C_Locus_3238_Tra	5.92	Yip1 integral membrane domain-containing protein OS=Arabidopsi	7.84254e-115

C_Locus_8884_Tra	5.92	AT3g19970/MZE19_2 OS=Arabidopsis thaliana GN=At3g19970 P	1.72658e-65
C_Locus_9527_Tra	5.92	NA	NA
C_Locus_3238_Tra	5.92	Yip1 integral membrane domain-containing protein OS=Arabidopsi	9.6888e-115
C_Locus_22555_T	5.92	Putative uncharacterized protein OS=Arabidopsis thaliana PE=2 S	2.47835e-33
C_Locus_2704_Tra	5.93	NA	NA
C_Locus_12689_T	5.93	At2g27690 OS=Arabidopsis thaliana GN=At2g27690/F15K20.21 P	0
C_Locus_16737_T	5.94	Heavy-metal-associated domain-containing protein OS=Arabidopsi	1.51108e-19
C_Locus_24659_T	5.94	Respiratory burst oxidase homolog protein D OS=Arabidopsis thal	0
C_Locus_5412_Tra	5.94	Expansin-like A2 OS=Arabidopsis thaliana GN=EXLA2 PE=2 SV=	1.01516e-123
C_Locus_17086_T	5.96	At3g23250 OS=Arabidopsis thaliana GN=MYB15 PE=2 SV=1	3.48899e-68
C_Locus_16737_T	5.96	Heavy-metal-associated domain-containing protein OS=Arabidopsi	2.30121e-12
C_Locus_4375_Tra	5.96	Coatomer subunit beta'-2 OS=Arabidopsis thaliana GN=At1g5236	5.03936e-149
A_Locus_11070_T	5.96	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_9291_Tra	5.96	NA	NA
C_Locus_2693_Tra	5.97	Putative uncharacterized protein T32M21_100 OS=Arabidopsis tha	8.80946e-26
C_Locus_760_Tran	5.97	Uncharacterized protein OS=Arabidopsis thaliana GN=At4g24380	1.00362e-74
C_Locus_2232_Tra	5.97	Probable protein phosphatase 2C 78 OS=Arabidopsis thaliana GN	3.5949e-61
C_Locus_14060_T	5.97	Proline-rich receptor-like protein kinase PERK1 OS=Arabidopsis t	3.43518e-54
C_Locus_1534_Tra	5.97	Probable inactive poly [ADP-ribose] polymerase SRO5 OS=Arabid	1.9943e-72
C_Locus_780_Tran	5.97	Class IV chitinase OS=Arabidopsis thaliana GN=CHIV PE=4 SV=	3.93638e-76
C_Locus_17902_T	5.98	Protein PHLOEM PROTEIN 2-LIKE A1 OS=Arabidopsis thaliana G	1.45663e-53
C_Locus_8319_Tra	5.98	ABC transporter B family member 11 OS=Arabidopsis thaliana GN	0
C_Locus_5615_Tra	5.98	Ammonium transporter 2 OS=Arabidopsis thaliana GN=AMT2 PE=	1.04864e-122
C_Locus_7619_Tra	5.98	GATA transcription factor 5 OS=Arabidopsis thaliana GN=GATA5	1.18085e-46
C_Locus_13875_T	5.98	AAA-type ATPase family protein OS=Arabidopsis thaliana GN=At	1.93442e-55

C_Locus_11847_T	5.98	U-box domain-containing protein 15 OS=Arabidopsis thaliana GN=	1.95724e-15
C_Locus_8319_Tra	5.98	ABC transporter B family member 11 OS=Arabidopsis thaliana GN	0
C_Locus_5194_Tra	5.98	Peroxidase 43 OS=Arabidopsis thaliana GN=PER43 PE=2 SV=2	2.11939e-73
C_Locus_1534_Tra	5.98	Probable inactive poly [ADP-ribose] polymerase SRO5 OS=Arabid	3.01722e-70
C_Locus_411_Tra	5.99	At4g17350 OS=Arabidopsis thaliana GN=At4g17350 PE=2 SV=1	4.72081e-105
C_Locus_2252_Tra	6.00	At4g13420 OS=Arabidopsis thaliana PE=2 SV=1	0
C_Locus_10987_T	6.00	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase famil	6.0614e-158
C_Locus_2975_Tra	6.00	Probable nucleoredoxin 1 OS=Arabidopsis thaliana GN=At1g6042	9.20203e-50
C_Locus_16659_T	6.01	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_7120_Tra	6.01	Phytoalexin deficient 4 OS=Arabidopsis thaliana GN=PAD4 PE=4	3.99837e-98
C_Locus_6148_Tra	6.01	At5g08480 OS=Arabidopsis thaliana GN=At5g08480 PE=2 SV=1	2.23336e-18
C_Locus_26520_T	6.01	NA	NA
C_Locus_15758_T	6.01	NA	NA
C_Locus_917_Tra	6.02	Putative NADH dehydrogenase (Ubiquinone oxidoreductase) OS=	0
C_Locus_1111_Tra	6.02	WRKY like transcription factor OS=Arabidopsis thaliana GN=At4g	3.57376e-25
C_Locus_5194_Tra	6.02	Peroxidase 43 OS=Arabidopsis thaliana GN=PER43 PE=2 SV=2	1.50041e-58
C_Locus_21901_T	6.03	Probable E3 ubiquitin-protein ligase ARI7 OS=Arabidopsis thalian	6.96568e-22
C_Locus_2860_Tra	6.03	Beta-fructofuranosidase, insoluble isoenzyme CWINV1 OS=Arabid	0
C_Locus_10987_T	6.03	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase famil	8.85078e-97
C_Locus_16726_T	6.03	Ethylene-responsive transcription factor ABR1 OS=Arabidopsis th	2.11043e-25
C_Locus_31766_T	6.03	Putative diacylglycerol kinase OS=Arabidopsis thaliana PE=2 SV=	6.56171e-07
C_Locus_8127_Tra	6.03	Probable F-box protein At1g44080 OS=Arabidopsis thaliana GN=	3.1928e-05
C_Locus_9024_Tra	6.03	At1g02660/T14P4_9 OS=Arabidopsis thaliana GN=At1g02660 PE	0
C_Locus_5194_Tra	6.04	Peroxidase 43 OS=Arabidopsis thaliana GN=PER43 PE=2 SV=2	2.79326e-118
C_Locus_917_Tra	6.04	Putative NADH dehydrogenase (Ubiquinone oxidoreductase) OS=	1.89865e-143

C_Locus_18280_T	6.04	MJM20.4 protein OS=Arabidopsis thaliana GN=MJM20.4 PE=2 S	3.42126e-167
C_Locus_13753_T	6.04	Proline-rich receptor-like protein kinase PERK13 OS=Arabidopsis	3.73278e-62
C_Locus_21901_T	6.05	AT4g19390/T5K18_170 OS=Arabidopsis thaliana GN=At4g19390	1.57568e-19
C_Locus_18648_T	6.05	Receptor-like protein kinase FERONIA OS=Arabidopsis thaliana G	4.12226e-135
C_Locus_2870_Tra	6.05	G-type lectin S-receptor-like serine/threonine-protein kinase At1g1	0
C_Locus_10502_T	6.05	Probable LRR receptor-like serine/threonine-protein kinase At1g53	0
C_Locus_2866_Tra	6.06	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	4.53161e-76
C_Locus_3292_Tra	6.06	Ethylene-responsive transcription factor 1A OS=Arabidopsis thalia	6.60836e-72
C_Locus_4192_Tra	6.06	Inositol transporter 1 OS=Arabidopsis thaliana GN=INT1 PE=1 SV	1.48493e-65
C_Locus_22555_T	6.07	F9D12.14 protein OS=Arabidopsis thaliana GN=F9D12.14 PE=4 S	7.4084e-46
C_Locus_26600_T	6.07	Dehydrin ERD14 OS=Arabidopsis thaliana GN=ERD14 PE=1 SV=	0.000235884
C_Locus_4234_Tra	6.07	Putative serine/threonine-protein kinase-like protein CCR3 OS=Ara	0
C_Locus_38_Trans	6.07	Cytochrome P450 71B34 OS=Arabidopsis thaliana GN=CYP71B3	9.117e-78
C_Locus_7620_Tra	6.07	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	0
C_Locus_10318_T	6.08	Probable LRR receptor-like serine/threonine-protein kinase At3g47	0
C_Locus_9024_Tra	6.08	At1g02660/T14P4_9 OS=Arabidopsis thaliana GN=At1g02660 PE	1.62592e-112
C_Locus_11134_T	6.08	NA	NA
C_Locus_2459_Tra	6.08	NA	NA
C_Locus_648_Tran	6.08	NA	NA
C_Locus_4375_Tra	6.09	Coatomer subunit beta'-2 OS=Arabidopsis thaliana GN=At1g5236	2.67407e-126
C_Locus_2860_Tra	6.09	Beta-fructofuranosidase, insoluble isoenzyme CWINV1 OS=Arabid	0
C_Locus_801_Tran	6.09	Putative transcription factor bHLH041 OS=Arabidopsis thaliana GN	4.83165e-61
C_Locus_1911_Tra	6.10	Probable galacturonosyltransferase-like 10 OS=Arabidopsis thalian	1.70907e-167
C_Locus_27618_T	6.10	Cytochrome P450 82C4 OS=Arabidopsis thaliana GN=CYP82C4	4.26283e-75
C_Locus_7852_Tra	6.10	NA	NA

C_Locus_5291_Tra	6.11	1-aminocyclopropane-1-carboxylate oxidase 4 OS=Arabidopsis th	5.07405e-65
C_Locus_31815_T	6.11	Monothiol glutaredoxin-S2 OS=Arabidopsis thaliana GN=GRXS2 P	3.16533e-31
C_Locus_6956_Tra	6.11	Putative uncharacterized protein At1g29860 (Fragment) OS=Arabi	2.02421e-50
C_Locus_2888_Tra	6.11	PRA1 family protein F2 OS=Arabidopsis thaliana GN=PRA1F2 PE	3.39189e-05
C_Locus_11847_T	6.11	U-box domain-containing protein 15 OS=Arabidopsis thaliana GN=	1.11108e-18
C_Locus_19451_T	6.11	Protein translation factor SUI1 homolog 2 OS=Arabidopsis thalian	1.55758e-31
A_Locus_25698_T	6.12	Mitogen-activated protein kinase kinase kinase 19 OS=Arabidopsi	3.21417e-114
C_Locus_917_Tran	6.12	Putative NADH dehydrogenase (Ubiquinone oxidoreductase) OS=A	0
C_Locus_19879_T	6.12	Glutamate receptor 2.8 OS=Arabidopsis thaliana GN=GLR2.8 PE=	4.74787e-72
C_Locus_7205_Tra	6.13	Glucan endo-1,3-beta-glucosidase 14 OS=Arabidopsis thaliana G	0
A_Locus_11997_T	6.13	Putative uncharacterized protein At4g22530 (Fragment) OS=Arabi	1.63433e-81
C_Locus_5839_Tra	6.13	NA	NA
C_Locus_37128_T	6.13	Gb|AAD20160.1 OS=Arabidopsis thaliana GN=At5g35110 PE=4 S	2.71368e-16
C_Locus_2801_Tra	6.13	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	0
C_Locus_3158_Tra	6.13	Aquaporin TIP1-1 OS=Arabidopsis thaliana GN=TIP1-1 PE=1 SV=	6.62915e-131
C_Locus_13383_T	6.13	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	1.34383e-25
C_Locus_24006_T	6.14	Putative uncharacterized protein F8M21_80 OS=Arabidopsis thali	1.5731e-05
C_Locus_665_Tran	6.15	Heme oxygenase-like, multi-helical protein OS=Arabidopsis thalia	3.25095e-141
C_Locus_27618_T	6.15	Cytochrome P450 82C4 OS=Arabidopsis thaliana GN=CYP82C4	4.31627e-169
C_Locus_665_Tran	6.15	Heme oxygenase-like, multi-helical protein OS=Arabidopsis thalia	2.69747e-140
C_Locus_8800_Tra	6.15	Uncharacterized protein OS=Arabidopsis thaliana GN=At5g54240	6.24382e-123
C_Locus_911_Tran	6.16	Probable WRKY transcription factor 48 OS=Arabidopsis thaliana	1.83539e-48
C_Locus_295_Tran	6.17	Urophorphyrin methylase 1 OS=Arabidopsis thaliana GN=UPM1 P	4.1553e-73
C_Locus_26930_T	6.17	BON1-associated protein 2 OS=Arabidopsis thaliana GN=BAP2 P	1.23778e-22
C_Locus_5615_Tra	6.17	Ammonium transporter 2 OS=Arabidopsis thaliana GN=AMT2 PE=	5.55319e-49

C_Locus_35229_T	6.17	F21J9.19 OS=Arabidopsis thaliana GN=At1g24530 PE=2 SV=1	1.76774e-160
C_Locus_25186_T	6.18	NA	NA
C_Locus_21811_T	6.18	At1g68440/T2E12_1 OS=Arabidopsis thaliana GN=T2E12.1 PE=2	3.43052e-37
C_Locus_7620_Tra	6.18	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	0
C_Locus_354_Tran	6.19	Ent-kaur-16-ene synthase, chloroplastic OS=Arabidopsis thaliana	6.77655e-87
C_Locus_37757_T	6.19	1-aminocyclopropane-1-carboxylate oxidase 4 OS=Arabidopsis tha	9.497e-06
C_Locus_10884_T	6.20	At2g46620/F13A10.15 OS=Arabidopsis thaliana GN=At2g46620 P	1.84628e-157
C_Locus_7788_Tra	6.20	Probable trehalose-phosphate phosphatase G OS=Arabidopsis tha	4.67283e-75
C_Locus_8800_Tra	6.20	Uncharacterized protein OS=Arabidopsis thaliana GN=At5g54240	8.2945e-132
C_Locus_10666_T	6.20	Protein MOTHER of FT and TF 1 OS=Arabidopsis thaliana GN=MF	3.18472e-42
C_Locus_2826_Tra	6.21	Truncated WRKY33 protein OS=Arabidopsis thaliana PE=2 SV=1	1.37235e-32
C_Locus_1198_Tra	6.21	Probable xyloglucan endotransglucosylase/hydrolase protein 21 O	1.81774e-104
C_Locus_3327_Tra	6.21	Probable inactive poly [ADP-ribose] polymerase SRO2 OS=Arabid	1.88407e-45
C_Locus_3034_Tra	6.22	At3g23250 OS=Arabidopsis thaliana GN=MYB15 PE=2 SV=1	1.24735e-79
C_Locus_422_Tran	6.23	ABC transporter C family member 9 OS=Arabidopsis thaliana GN=	9.1941e-86
C_Locus_4564_Tra	6.23	12-oxophytodienoate reductase 1 OS=Arabidopsis thaliana GN=O	6.44451e-137
C_Locus_665_Tran	6.23	Putative uncharacterized protein At5g32470 (Fragment) OS=Arabi	1.20959e-116
C_Locus_20148_T	6.24	Rossmann-fold NAD(P)-binding domain-containing protein OS=Ara	1.23249e-80
C_Locus_17792_T	6.25	Basic endochitinase B OS=Arabidopsis thaliana GN=CHI-B PE=1	2.79508e-56
C_Locus_12361_T	6.25	Trehalose-phosphate phosphatase A OS=Arabidopsis thaliana GN	3.77264e-42
C_Locus_27403_T	6.26	R2R3-MYB transcription factor OS=Arabidopsis thaliana GN=AtM	1.64219e-53
C_Locus_38_Trans	6.26	Putative cytochrome P450 OS=Arabidopsis thaliana GN=At3g263	5.02151e-86
C_Locus_2801_Tra	6.26	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	3.79312e-169
C_Locus_14651_T	6.27	Aspartyl protease family protein OS=Arabidopsis thaliana GN=At3	1.17113e-22
C_Locus_1754_Tra	6.27	Alpha-humulene/(-)-(E)-beta-caryophyllene synthase OS=Arabidop	1.55104e-17

C_Locus_921_Tra	6.28	Transcription factor BEE 2 OS=Arabidopsis thaliana GN=BEE2 P	1.49083e-13
C_Locus_5615_Tra	6.28	Ammonium transporter 2 OS=Arabidopsis thaliana GN=AMT2 PE=	0
C_Locus_3534_Tra	6.28	Putative BCS1 protein OS=Arabidopsis thaliana GN=At3g50940/F	7.20622e-105
C_Locus_7620_Tra	6.29	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	0
C_Locus_411_Tra	6.29	At4g17350 OS=Arabidopsis thaliana GN=At4g17350 PE=2 SV=1	5.93964e-87
C_Locus_2801_Tra	6.29	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	0
C_Locus_16659_T	6.30	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_5615_Tra	6.31	Ammonium transporter 2 OS=Arabidopsis thaliana GN=AMT2 PE=	3.15543e-117
C_Locus_780_Tra	6.31	Class IV chitinase OS=Arabidopsis thaliana GN=CHIV PE=4 SV=	1.25115e-85
C_Locus_21901_T	6.32	AT4g19390/T5K18_170 OS=Arabidopsis thaliana GN=At4g19390	4.40255e-35
C_Locus_17240_T	6.32	NA	NA
C_Locus_809_Tra	6.32	Leucine-rich receptor-like protein kinase OS=Arabidopsis thaliana	0
C_Locus_9421_Tra	6.33	Probable LRR receptor-like serine/threonine-protein kinase At3g47	0
C_Locus_801_Tra	6.33	Putative transcription factor bHLH041 OS=Arabidopsis thaliana G	3.5046e-48
C_Locus_33305_T	6.34	Chlorophyllase-1 OS=Arabidopsis thaliana GN=CLH1 PE=1 SV=1	6.70211e-85
C_Locus_801_Tra	6.34	Putative transcription factor bHLH041 OS=Arabidopsis thaliana G	7.28673e-74
C_Locus_1458_Tra	6.34	IAA-amino acid hydrolase ILR1-like 4 OS=Arabidopsis thaliana G	3.27086e-126
C_Locus_5658_Tra	6.35	Putative phi-1-like phosphate-induced protein OS=Arabidopsis thal	1.54818e-160
C_Locus_13753_T	6.35	Proline-rich receptor-like protein kinase PERK1 OS=Arabidopsis t	1.16595e-62
C_Locus_2826_Tra	6.35	At2g38470 OS=Arabidopsis thaliana PE=2 SV=1	2.21092e-83
C_Locus_18839_T	6.35	NA	NA
C_Locus_783_Tra	6.35	UDP-D-glucuronate 4-epimerase 1 (Fragment) OS=Arabidopsis th	0
C_Locus_665_Tra	6.36	Heme oxygenase-like, multi-helical protein OS=Arabidopsis thalia	6.53747e-140
C_Locus_917_Tra	6.37	Putative NADH dehydrogenase (Ubiquinone oxidoreductase) OS=	1.93645e-143
A_Locus_31039_T	6.37	Peroxidase ATP29a OS=Arabidopsis thaliana PE=3 SV=1	1.38815e-27

C_Locus_19115_T	6.38	At5g61890 OS=Arabidopsis thaliana PE=2 SV=1	1.23445e-33
C_Locus_18285_T	6.39	NA	NA
C_Locus_7205_Tr	6.39	Glucan endo-1,3-beta-glucosidase 14 OS=Arabidopsis thaliana G	0
C_Locus_7619_Tr	6.39	GATA transcription factor 5 OS=Arabidopsis thaliana GN=GATA5	2.0288e-47
C_Locus_21901_T	6.39	Probable E3 ubiquitin-protein ligase ARI7 OS=Arabidopsis thalian	6.71494e-22
C_Locus_760_Tra	6.40	AT4G24380 protein OS=Arabidopsis thaliana GN=AT4G24380 PE	6.02503e-06
C_Locus_28852_T	6.40	Ethylene-responsive transcription factor 12 OS=Arabidopsis thalia	3.51093e-38
C_Locus_21901_T	6.41	Probable E3 ubiquitin-protein ligase ARI7 OS=Arabidopsis thalian	7.30792e-22
C_Locus_13875_T	6.42	AAA-ATPase 1 OS=Arabidopsis thaliana GN=AATP1 PE=3 SV=1	6.45555e-144
C_Locus_17798_T	6.42	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP	5.94702e-106
C_Locus_5994_Tr	6.42	NA	NA
C_Locus_5485_Tr	6.43	Cys2/His2-type zinc finger protein 2 OS=Arabidopsis thaliana GN=	1.89037e-21
C_Locus_1223_Tr	6.44	NA	NA
A_Locus_33145_T	6.44	At2g32030 OS=Arabidopsis thaliana GN=At2g32030 PE=2 SV=1	1.20453e-68
C_Locus_16906_T	6.44	VQ motif-containing protein OS=Arabidopsis thaliana GN=At1g17	1.91864e-06
C_Locus_22226_T	6.44	Peroxidase 4 OS=Arabidopsis thaliana GN=PER4 PE=2 SV=1	8.56287e-139
C_Locus_1586_Tr	6.44	AT5g14040/MUA22_4 OS=Arabidopsis thaliana GN=PHT3;1 PE=	0
C_Locus_1661_Tr	6.45	Probable alpha,alpha-trehalose-phosphate synthase [UDP-forming	0
C_Locus_17427_T	6.45	Putative phi-1-like phosphate-induced protein OS=Arabidopsis thal	7.30003e-153
C_Locus_1911_Tr	6.45	Probable galacturonosyltransferase-like 10 OS=Arabidopsis thalia	2.22955e-167
C_Locus_801_Tra	6.45	Putative transcription factor bHLH041 OS=Arabidopsis thaliana G	2.3318e-48
C_Locus_16674_T	6.45	Alanine aminotransferase 1, mitochondrial OS=Arabidopsis thalia	3.03488e-143
C_Locus_16659_T	6.46	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_23379_T	6.47	Putative uncharacterized protein At5g12340 OS=Arabidopsis thali	1.09803e-55
C_Locus_3238_Tr	6.48	Yip1 integral membrane domain-containing protein OS=Arabidopsi	6.26615e-116

C_Locus_5947_Tr	6.48	Exocyst subunit EXO70 family protein OS=Arabidopsis thaliana G	0
C_Locus_7205_Tr	6.49	Glucan endo-1,3-beta-glucosidase 14 OS=Arabidopsis thaliana G	1.40431e-180
C_Locus_22555_T	6.49	At5g26220 OS=Arabidopsis thaliana GN=At5g26220/T19G15_70	6.95334e-50
C_Locus_17798_T	6.49	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP	8.01494e-31
C_Locus_18904_T	6.49	Carbonic anhydrase 2, chloroplastic OS=Arabidopsis thaliana GN	1.10154e-92
C_Locus_6878_Tr	6.50	NA	NA
C_Locus_855_Tra	6.50	At5g10830 OS=Arabidopsis thaliana GN=T30N20_100 PE=2 SV=	2.44435e-60
C_Locus_8702_Tr	6.50	Ethylene-responsive transcription factor 1B OS=Arabidopsis thalia	1.92903e-40
C_Locus_23975_T	6.50	At2g01340 OS=Arabidopsis thaliana GN=At17.1 PE=2 SV=1	3.40777e-49
C_Locus_13753_T	6.51	Proline-rich receptor-like protein kinase PERK13 OS=Arabidopsis	8.75681e-61
C_Locus_12907_T	6.51	Putative uncharacterized protein OS=Arabidopsis thaliana PE=4 S	5.40245e-11
C_Locus_16650_T	6.51	S-adenosylmethionine synthase 2 OS=Arabidopsis thaliana GN=S	4.43986e-26
C_Locus_5213_Tr	6.51	Protein TIFY 5A OS=Arabidopsis thaliana GN=TIFY5A PE=1 SV=	1.32279e-23
C_Locus_8574_Tr	6.52	NA	NA
C_Locus_855_Tra	6.52	At5g10830 OS=Arabidopsis thaliana GN=T30N20_100 PE=2 SV=	2.50729e-73
C_Locus_13383_T	6.53	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	7.1588e-26
C_Locus_13753_T	6.54	Proline-rich receptor-like protein kinase PERK1 OS=Arabidopsis t	8.47043e-39
C_Locus_18903_T	6.54	Calcium-dependent protein kinase 1 OS=Arabidopsis thaliana GN	0
C_Locus_16977_T	6.54	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	7.77409e-15
C_Locus_4381_Tr	6.55	Probable WRKY transcription factor 28 OS=Arabidopsis thaliana	2.69564e-64
C_Locus_16965_T	6.55	U-box domain-containing protein 21 OS=Arabidopsis thaliana GN=	7.16887e-118
C_Locus_22226_T	6.55	Peroxidase 5 OS=Arabidopsis thaliana GN=PER5 PE=2 SV=2	3.1528e-126
C_Locus_354_Tra	6.55	Ent-kaur-16-ene synthase, chloroplastic OS=Arabidopsis thaliana	3.27488e-109
C_Locus_2826_Tr	6.56	At2g38470 OS=Arabidopsis thaliana PE=2 SV=1	1.09072e-82
C_Locus_2210_Tr	6.56	Mitogen-activated protein kinase kinase kinase 19 OS=Arabidopsi	1.182e-118

C_Locus_1789_Tra	6.56	G-type lectin S-receptor-like serine/threonine-protein kinase RLK1	7.15663e-107
A_Locus_30001_T	6.56	Gb|AAD20160.1 OS=Arabidopsis thaliana GN=At5g35110 PE=4 S	1.30482e-22
C_Locus_16659_T	6.56	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_1661_Tra	6.56	Probable alpha,alpha-trehalose-phosphate synthase [UDP-forming]	0
C_Locus_801_Tran	6.56	Putative transcription factor bHLH041 OS=Arabidopsis thaliana GN	8.87032e-57
C_Locus_21901_T	6.56	Probable E3 ubiquitin-protein ligase ARI7 OS=Arabidopsis thaliana	1.63068e-19
C_Locus_19258_T	6.57	AR781, similar to yeast pheromone receptor OS=Arabidopsis thali	2.17033e-52
C_Locus_6456_Tra	6.58	Branched-chain-amino-acid aminotransferase OS=Arabidopsis tha	0
C_Locus_8127_Tra	6.58	Probable F-box protein At1g44080 OS=Arabidopsis thaliana GN=A	0.000135857
C_Locus_5361_Tra	6.58	Probable LRR receptor-like serine/threonine-protein kinase At1g74	0
C_Locus_15429_T	6.59	Blue copper protein OS=Arabidopsis thaliana GN=BCB PE=1 SV=	1.01884e-20
C_Locus_4808_Tra	6.59	Beta-fructofuranosidase, insoluble isoenzyme CWINV5 OS=Arabid	5.20479e-16
C_Locus_3327_Tra	6.59	Probable inactive poly [ADP-ribose] polymerase SRO2 OS=Arabid	4.27373e-90
C_Locus_17798_T	6.60	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP	7.72896e-80
C_Locus_5955_Tra	6.60	Calmodulin-like protein 8 OS=Arabidopsis thaliana GN=CML8 PE=	1.27691e-54
C_Locus_2801_Tra	6.61	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	0
A_Locus_28623_T	6.62	NA	NA
C_Locus_38005_T	6.62	Extensin-3 OS=Arabidopsis thaliana GN=EXT3 PE=2 SV=3	0.00033303
C_Locus_2703_Tra	6.62	Myb-like HTH transcriptional regulator-like protein OS=Arabidopsis	9.61021e-41
C_Locus_16674_T	6.62	Alanine aminotransferase 1, mitochondrial OS=Arabidopsis thalian	1.83625e-56
C_Locus_9521_Tra	6.63	Probable xyloglucan endotransglucosylase/hydrolase protein 25 O	1.88606e-118
C_Locus_37800_T	6.63	NA	NA
C_Locus_665_Tran	6.64	Heme oxygenase-like, multi-helical protein OS=Arabidopsis thalian	6.53747e-140
C_Locus_353_Tran	6.64	UDP-glycosyltransferase 85A3 OS=Arabidopsis thaliana GN=UGT	2.45024e-110
C_Locus_38_Trans	6.64	Cytochrome P450 71B34 OS=Arabidopsis thaliana GN=CYP71B3	9.65345e-67

C_Locus_12215_T	6.64	Cysteine-rich receptor-like protein kinase 3 OS=Arabidopsis thalia	1.27203e-151
C_Locus_24508_T	6.65	Glutathione S-transferase U8 OS=Arabidopsis thaliana GN=GSTU	1.42499e-74
C_Locus_5310_Tra	6.66	Calcium-transporting ATPase 12, plasma membrane-type OS=Ara	0
C_Locus_353_Tran	6.67	UDP-glucosyl transferase 85A2 OS=Arabidopsis thaliana GN=UG	7.31438e-156
C_Locus_17792_T	6.67	Basic endochitinase B OS=Arabidopsis thaliana GN=CHI-B PE=1	8.49222e-124
C_Locus_7619_Tra	6.67	GATA transcription factor 5 OS=Arabidopsis thaliana GN=GATA5	4.27395e-54
C_Locus_864_Tran	6.67	Putative DEF (CLA1) protein (Fragment) OS=Arabidopsis thaliana	0
C_Locus_12810_T	6.67	At2g15730 OS=Arabidopsis thaliana GN=At2g15730 PE=2 SV=1	5.10414e-127
C_Locus_2680_Tra	6.67	AT5g35570/K2K18_1 OS=Arabidopsis thaliana GN=At5g35570 PE	0
C_Locus_216_Tran	6.67	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	1.26173e-60
C_Locus_22553_T	6.69	Calmodulin-like protein 8 OS=Arabidopsis thaliana GN=CML8 PE=	3.77972e-62
C_Locus_2826_Tra	6.70	NA	NA
C_Locus_16050_T	6.70	NA	NA
C_Locus_41158_T	6.70	NA	NA
C_Locus_33612_T	6.70	Probable 6-phosphogluconolactonase 1 OS=Arabidopsis thaliana	1.399e-48
C_Locus_1661_Tra	6.70	NA	NA
C_Locus_2703_Tra	6.70	Myb-like HTH transcriptional regulator-like protein OS=Arabidopsis	2.35778e-32
C_Locus_17798_T	6.71	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP	4.24996e-105
C_Locus_12361_T	6.71	Trehalose-phosphate phosphatase A OS=Arabidopsis thaliana GN	2.57521e-40
C_Locus_26703_T	6.74	MLP-like protein 423 OS=Arabidopsis thaliana GN=MLP423 PE=1	2.22404e-08
C_Locus_1073_Tra	6.74	Auxin efflux carrier family protein OS=Arabidopsis thaliana GN=T7	6.54498e-60
C_Locus_1586_Tra	6.74	Mitochondrial phosphate transporter (Fragment) OS=Arabidopsis t	4.18043e-147
C_Locus_12810_T	6.74	At2g15730 OS=Arabidopsis thaliana GN=At2g15730 PE=2 SV=1	1.56874e-161
C_Locus_665_Tran	6.75	Heme oxygenase-like, multi-helical protein OS=Arabidopsis thalia	5.73062e-78
C_Locus_17246_T	6.76	At3g57830 OS=Arabidopsis thaliana GN=At3g57830/T10K17_40 P	7.76884e-112

C_Locus_1592_Tra	6.76	At1g36580/F28J9_6 OS=Arabidopsis thaliana GN=At5g35735 PE=	3.47762e-88
C_Locus_13629_T	6.77	Probable protein phosphatase 2C 75 OS=Arabidopsis thaliana GN	1.63898e-72
C_Locus_4790_Tra	6.77	F-box/LRR-repeat protein 17 OS=Arabidopsis thaliana GN=FBL17	0
C_Locus_6834_Tra	6.77	G-type lectin S-receptor-like serine/threonine-protein kinase At1g6	0
C_Locus_18091_T	6.78	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	1.55945e-32
C_Locus_21991_T	6.78	Mitochondrial dicarboxylate carrier OS=Arabidopsis thaliana GN=	9.7682e-148
C_Locus_8488_Tra	6.78	Putative transcription factor OS=Arabidopsis thaliana GN=MYB20	4.25663e-53
C_Locus_383_Tra	6.78	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 S	4.69333e-11
C_Locus_760_Tra	6.78	Uncharacterized protein OS=Arabidopsis thaliana GN=At4g24380	2.10034e-73
C_Locus_3122_Tra	6.79	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	5.90377e-57
C_Locus_2826_Tra	6.81	At2g38470 OS=Arabidopsis thaliana PE=2 SV=1	4.31761e-88
C_Locus_23407_T	6.81	NA	NA
C_Locus_4590_Tra	6.81	Heat stress transcription factor A-4a OS=Arabidopsis thaliana GN	1.58934e-88
C_Locus_17126_T	6.82	At4g39830 OS=Arabidopsis thaliana GN=AT4g39830 PE=2 SV=1	0
C_Locus_16726_T	6.82	Ethylene-responsive transcription factor ABR1 OS=Arabidopsis th	3.61394e-33
C_Locus_353_Tra	6.83	UDP-glycosyltransferase 85A2 OS=Arabidopsis thaliana GN=UGT	2.35652e-126
C_Locus_4475_Tra	6.83	At3g23250 OS=Arabidopsis thaliana GN=MYB15 PE=2 SV=1	7.2322e-46
C_Locus_5324_Tra	6.84	NA	NA
C_Locus_4381_Tra	6.85	Probable WRKY transcription factor 28 OS=Arabidopsis thaliana	7.53466e-55
C_Locus_9125_Tra	6.86	NA	NA
C_Locus_12215_T	6.86	Cysteine-rich receptor-like protein kinase 3 OS=Arabidopsis thalia	0
C_Locus_1663_Tra	6.87	Peroxidase 34 OS=Arabidopsis thaliana GN=PER34 PE=1 SV=1	2.74953e-91
C_Locus_7025_Tra	6.88	Putative uncharacterized protein AT4g28460 OS=Arabidopsis thali	1.57624e-05
C_Locus_3280_Tra	6.90	1-aminocyclopropane-1-carboxylate oxidase homolog 4 OS=Arabi	4.81639e-105
C_Locus_4900_Tra	6.90	ADP-ribosylation factor GTPase-activating protein AGD1 OS=Arab	1.47614e-42

C_Locus_5194_Tra	6.91	Peroxidase 43 OS=Arabidopsis thaliana GN=PER43 PE=2 SV=2	4.70227e-73
C_Locus_7072_Tra	6.91	Acidic endochitinase OS=Arabidopsis thaliana GN=CHIB1 PE=2	2.3507e-115
C_Locus_9761_Tra	6.91	At1g33060 OS=Arabidopsis thaliana GN=NAC014 PE=2 SV=1	1.0196e-27
C_Locus_4381_Tra	6.91	Putative uncharacterized protein At1g29860 (Fragment) OS=Arabi	5.55195e-56
C_Locus_22559_T	6.91	At5g47530 OS=Arabidopsis thaliana GN=At5g47530 PE=2 SV=1	7.77747e-131
C_Locus_26770_T	6.92	SRPBCC ligand-binding domain-containing protein OS=Arabidopsi	0.000438629
C_Locus_38947_T	6.92	At2g01340 OS=Arabidopsis thaliana GN=At17.1 PE=2 SV=1	5.49208e-53
C_Locus_33305_T	6.92	Chlorophyllase-1 OS=Arabidopsis thaliana GN=CLH1 PE=1 SV=1	7.51732e-92
C_Locus_22555_T	6.93	At4g31290 OS=Arabidopsis thaliana GN=At4g31290 PE=2 SV=1	1.05895e-35
C_Locus_8488_Tra	6.94	MYB transcription factor OS=Arabidopsis thaliana GN=MYB43 PE	3.26112e-97
C_Locus_643_Tra	6.94	ABC transporter G family member 40 OS=Arabidopsis thaliana G	2.02586e-18
C_Locus_18700_T	6.94	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 S	2.89395e-25
A_Locus_11452_T	6.95	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	5.30708e-57
C_Locus_383_Tra	6.95	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 S	2.54706e-29
C_Locus_17223_T	6.95	NA	NA
C_Locus_3370_Tra	6.95	ABC transporter G family member 36 OS=Arabidopsis thaliana G	3.62077e-28
C_Locus_6684_Tra	6.95	Glutamate receptor 2.9 OS=Arabidopsis thaliana GN=GLR2.9 PE=	2.17784e-140
C_Locus_18539_T	6.95	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
C_Locus_36586_T	6.96	Xyloglucan endotransglucosylase/hydrolase protein 22 OS=Arabid	1.03229e-20
C_Locus_18182_T	6.96	Inorganic pyrophosphatase 2 OS=Arabidopsis thaliana GN=At1g1	3.06361e-104
C_Locus_4375_Tra	6.96	Coatomer subunit beta'-2 OS=Arabidopsis thaliana GN=At1g5236	5.94456e-33
C_Locus_30103_T	6.97	Peroxidase 5 OS=Arabidopsis thaliana GN=PER5 PE=2 SV=2	6.22464e-143
C_Locus_344_Tra	6.97	Uncharacterized protein OS=Arabidopsis thaliana GN=At1g32928	4.46763e-11
C_Locus_1305_Tra	6.98	Putative uncharacterized protein (Fragment) OS=Arabidopsis thali	0.000274162
C_Locus_3327_Tra	6.98	Probable inactive poly [ADP-ribose] polymerase SRO2 OS=Arabid	5.31665e-87

C_Locus_23404_T	6.98	NA	NA
A_Locus_13670_T	6.99	NA	NA
C_Locus_9761_Tra	7.00	At1g33060 OS=Arabidopsis thaliana GN=NAC014 PE=2 SV=1	1.04511e-27
C_Locus_6119_Tra	7.00	Cation/H(+) antiporter 18 OS=Arabidopsis thaliana GN=CHX18 PE	0
C_Locus_18700_T	7.00	Protein TIFY 10B OS=Arabidopsis thaliana GN=TIFY10B PE=1 S	8.83858e-31
C_Locus_17570_T	7.01	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	9.63284e-132
C_Locus_7205_Tra	7.02	Glucan endo-1,3-beta-glucosidase 14 OS=Arabidopsis thaliana G	1.56498e-160
C_Locus_354_Tran	7.02	Ent-kaur-16-ene synthase, chloroplastic OS=Arabidopsis thaliana	5.37873e-114
C_Locus_16977_T	7.02	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	4.63518e-07
C_Locus_1311_Tra	7.03	Calmodulin-like protein 3 OS=Arabidopsis thaliana GN=CML3 PE=	2.34729e-79
C_Locus_2950_Tra	7.03	Expressed protein OS=Arabidopsis thaliana GN=At2g18690 PE=2	5.76894e-44
C_Locus_4475_Tra	7.04	R2R3-MYB transcription factor OS=Arabidopsis thaliana GN=AtM	2.42e-61
C_Locus_12215_T	7.04	Cysteine-rich receptor-like protein kinase 3 OS=Arabidopsis thalia	0
C_Locus_5458_Tra	7.05	Peroxidase 62 OS=Arabidopsis thaliana GN=PER62 PE=2 SV=1	9.40547e-106
C_Locus_16628_T	7.05	Cytochrome P450 OS=Arabidopsis thaliana GN=CYP72A15 PE=2	1.7863e-135
C_Locus_8319_Tra	7.05	ABC transporter B family member 11 OS=Arabidopsis thaliana G	0
C_Locus_18572_T	7.06	NA	NA
C_Locus_280_Tran	7.06	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=1	4.07232e-118
C_Locus_2025_Tra	7.06	Putative uncharacterized protein F9G14_60 OS=Arabidopsis thalia	9.50023e-09
C_Locus_12215_T	7.08	Cysteine-rich receptor-like protein kinase 3 OS=Arabidopsis thalia	0
C_Locus_35189_T	7.08	NA	NA
C_Locus_20549_T	7.09	Syntaxin-121 OS=Arabidopsis thaliana GN=SYP121 PE=1 SV=1	1.71811e-158
C_Locus_22018_T	7.09	NA	NA
C_Locus_911_Tran	7.10	AT4g37660/F19F18_150 OS=Arabidopsis thaliana GN=F19F18.15	1.45183e-08
C_Locus_760_Tran	7.10	At4g24380 OS=Arabidopsis thaliana GN=At4g24380/T22A6_210 F	1.13493e-72

C_Locus_19890_T	7.11	UDP-glycosyltransferase 85A7 OS=Arabidopsis thaliana GN=UGT	9.84556e-33
C_Locus_2680_Tra	7.12	AT5g35570/K2K18_1 OS=Arabidopsis thaliana GN=At5g35570 PE	0
C_Locus_1586_Tra	7.12	Mitochondrial phosphate transporter (Fragment) OS=Arabidopsis t	2.72547e-147
C_Locus_2866_Tra	7.13	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	6.58454e-101
C_Locus_3280_Tra	7.13	1-aminocyclopropane-1-carboxylate oxidase homolog 4 OS=Arabi	2.85382e-98
C_Locus_111_Tran	7.14	NA	NA
C_Locus_1590_Tra	7.14	Probable WRKY transcription factor 53 OS=Arabidopsis thaliana G	9.48399e-38
C_Locus_1754_Tra	7.14	Terpenoid synthase 6 OS=Arabidopsis thaliana GN=TPS06 PE=2	1.01819e-47
C_Locus_111_Tran	7.14	NA	NA
C_Locus_17798_T	7.15	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP	9.01821e-134
C_Locus_917_Tran	7.17	Putative NADH dehydrogenase (Ubiquinone oxidoreductase) OS=A	1.61474e-69
C_Locus_13629_T	7.18	Probable protein phosphatase 2C 75 OS=Arabidopsis thaliana GN	1.77171e-49
A_Locus_19016_T	7.19	6-phosphofructokinase 3 OS=Arabidopsis thaliana GN=PFK3 PE=	0
C_Locus_35709_T	7.21	At1g58420 OS=Arabidopsis thaliana GN=F9K23.5 PE=2 SV=1	9.88751e-34
C_Locus_1497_Tra	7.21	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=1	7.41771e-67
C_Locus_4152_Tra	7.21	Lysine histidine transporter-like 8 OS=Arabidopsis thaliana GN=A	0
C_Locus_14312_T	7.21	Protein IDA OS=Arabidopsis thaliana GN=IDA PE=2 SV=1	2.49138e-07
C_Locus_354_Tran	7.22	Ent-kaur-16-ene synthase, chloroplastic OS=Arabidopsis thaliana	1.0263e-115
C_Locus_760_Tran	7.22	At4g24380 OS=Arabidopsis thaliana GN=At4g24380/T22A6_210 F	2.03177e-80
C_Locus_365_Tran	7.22	At1g75800/T4O12_2 OS=Arabidopsis thaliana GN=At1g75800 PE	1.96789e-149
C_Locus_17792_T	7.23	Basic endochitinase B OS=Arabidopsis thaliana GN=CHI-B PE=1	1.59962e-124
C_Locus_3635_Tra	7.23	PR5-like receptor kinase OS=Arabidopsis thaliana GN=PR5K PE=	1.66821e-101
C_Locus_12242_T	7.24	Respiratory burst oxidase homolog protein D OS=Arabidopsis thal	0
C_Locus_12692_T	7.24	Leucine-rich repeat receptor-like protein kinase (Fragment) OS=Ar	0
C_Locus_216_Tran	7.24	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=1	6.27522e-101

C_Locus_911_Tra	7.24	Probable WRKY transcription factor 48 OS=Arabidopsis thaliana	2.80711e-24
C_Locus_17792_T	7.24	Basic endochitinase B OS=Arabidopsis thaliana GN=CHI-B PE=1	1.30889e-97
C_Locus_26759_T	7.25	At2g46690 OS=Arabidopsis thaliana GN=At2g46690 PE=2 SV=1	2.47719e-36
C_Locus_12629_T	7.25	VQ motif-containing protein OS=Arabidopsis thaliana GN=At1g17	1.57425e-06
C_Locus_111_Tra	7.25	Putative uncharacterized protein OS=Arabidopsis thaliana PE=4 S	1.86212e-07
C_Locus_36984_T	7.26	At4g11650 OS=Arabidopsis thaliana GN=At4g11650 PE=2 SV=1	1.06121e-09
C_Locus_4157_Tr	7.28	AT1G76160 protein OS=Arabidopsis thaliana GN=sks5 PE=2 SV=	0
C_Locus_18091_T	7.28	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	1.5363e-42
C_Locus_111_Tra	7.29	AT1G25275 protein OS=Arabidopsis thaliana GN=At1g25275 PE=	1.32653e-07
C_Locus_18079_T	7.29	F18B13.24 protein OS=Arabidopsis thaliana GN=F18B13.24 PE=	1.56415e-58
C_Locus_4959_Tr	7.29	Phospholipase D OS=Arabidopsis thaliana GN=PLDDELTA PE=3	4.28885e-134
C_Locus_2583_Tr	7.30	NA	NA
C_Locus_18079_T	7.30	F18B13.24 protein OS=Arabidopsis thaliana GN=F18B13.24 PE=	1.41543e-56
C_Locus_1906_Tr	7.30	Aquaporin TIP1-3 OS=Arabidopsis thaliana GN=TIP1-3 PE=1 SV=	5.0682e-41
C_Locus_2686_Tr	7.31	NA	NA
C_Locus_1586_Tr	7.31	AT5g14040/MUA22_4 OS=Arabidopsis thaliana GN=PHT3;1 PE=	2.67185e-171
C_Locus_25317_T	7.33	GDSL esterase/lipase At5g45670 OS=Arabidopsis thaliana GN=A	1.02894e-63
C_Locus_23728_T	7.34	Calmodulin-binding transcription activator 6 OS=Arabidopsis thalia	3.40145e-10
C_Locus_1887_Tr	7.35	Geranylgeranyl pyrophosphate synthase OS=Arabidopsis thaliana	8.19106e-124
C_Locus_18700_T	7.37	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 S	2.73021e-08
C_Locus_1586_Tr	7.37	AT5g14040/MUA22_4 OS=Arabidopsis thaliana GN=PHT3;1 PE=	7.89889e-178
C_Locus_12215_T	7.38	Cysteine-rich receptor-like protein kinase 3 OS=Arabidopsis thalia	0
C_Locus_21901_T	7.38	Probable E3 ubiquitin-protein ligase ARI7 OS=Arabidopsis thalian	4.5258e-22
C_Locus_9906_Tr	7.39	Nudix hydrolase 13, mitochondrial OS=Arabidopsis thaliana GN=	7.50132e-31
C_Locus_2252_Tr	7.39	At4g13420 OS=Arabidopsis thaliana PE=2 SV=1	0

C_Locus_22829_T	7.39	Putative transcription factor OS=Arabidopsis thaliana GN=MYB36	5.95905e-38
C_Locus_18572_T	7.39	NA	NA
C_Locus_855_Tran	7.39	At5g10830 OS=Arabidopsis thaliana GN=T30N20_100 PE=2 SV=	8.32336e-35
C_Locus_22226_T	7.40	Peroxidase 4 OS=Arabidopsis thaliana GN=PER4 PE=2 SV=1	1.29106e-82
C_Locus_1458_Tra	7.41	At5g56660 OS=Arabidopsis thaliana PE=2 SV=1	3.2147e-55
C_Locus_1592_Tra	7.42	AT4g17280/dl4675c OS=Arabidopsis thaliana GN=At4g17280 PE=	1.83459e-53
C_Locus_16554_T	7.42	Probable carboxylesterase 6 OS=Arabidopsis thaliana GN=CXE6	3.60173e-118
C_Locus_216_Tran	7.43	AT1G80840 *protein OS=Arabidopsis thaliana* GN=AT1G80840 PE	4.05432e-97
C_Locus_7205_Tra	7.43	Glucan endo-1,3-beta-glucosidase 14 OS=Arabidopsis thaliana GN	1.87527e-10
C_Locus_18700_T	7.44	Protein TIFY 10B OS=Arabidopsis thaliana GN=TIFY10B PE=1 SV	1.57138e-29
C_Locus_216_Tran	7.44	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	2.59585e-31
C_Locus_4959_Tra	7.44	Phospholipase D OS=Arabidopsis thaliana GN=PLDDELTA PE=3	9.88757e-110
C_Locus_17798_T	7.44	PP2C-type phosphatase AP2C4 OS=Arabidopsis thaliana GN=AP	9.27813e-80
C_Locus_2866_Tra	7.44	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	2.97337e-102
C_Locus_383_Tran	7.46	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 SV	2.00067e-13
C_Locus_1789_Tra	7.46	G-type lectin S-receptor-like serine/threonine-protein kinase RLK1	2.48256e-27
C_Locus_354_Tran	7.46	Ent-kaur-16-ene synthase, chloroplastic OS=Arabidopsis thaliana	7.41767e-97
A_Locus_27660_T	7.47	Protein TIFY 10A OS=Arabidopsis thaliana GN=JAZ1 PE=4 SV=1	1.05927e-11
C_Locus_24406_T	7.47	At3g13610 OS=Arabidopsis thaliana GN=At3g13610 PE=2 SV=1	1.17082e-169
C_Locus_3280_Tra	7.47	1-aminocyclopropane-1-carboxylate oxidase homolog 1 OS=Arabi	6.31634e-84
C_Locus_27095_T	7.48	Putative uncharacterized protein At1g10880 OS=Arabidopsis thalia	3.98164e-139
C_Locus_365_Tran	7.48	Thaumatin-like protein OS=Arabidopsis thaliana GN=TL1 PE=4 SV	6.31384e-140
C_Locus_471_Tran	7.50	Cupredoxin domain-containing protein OS=Arabidopsis thaliana G	6.99313e-13
C_Locus_1497_Tra	7.50	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	3.02885e-73
C_Locus_1887_Tra	7.50	Geranylgeranyl pyrophosphate synthase 7, chloroplastic OS=Arab	0.000566928

C_Locus_354_Tra	7.50	Ent-kaur-16-ene synthase, chloroplastic OS=Arabidopsis thaliana	6.41635e-114
C_Locus_14810_T	7.51	U-box domain-containing protein 28 OS=Arabidopsis thaliana GN=	8.23156e-95
C_Locus_25855_T	7.51	Class IV chitinase OS=Arabidopsis thaliana GN=CHIV PE=4 SV=	1.4748e-147
C_Locus_26869_T	7.52	E3 ubiquitin-protein ligase PUB22 OS=Arabidopsis thaliana GN=P	0
C_Locus_30201_T	7.53	G-type lectin S-receptor-like serine/threonine-protein kinase At5g2	1.63995e-106
C_Locus_23350_T	7.53	Probable WRKY transcription factor 31 OS=Arabidopsis thaliana	2.79274e-76
C_Locus_9024_Tra	7.53	At1g02660/T14P4_9 OS=Arabidopsis thaliana GN=At1g02660 PE	0
C_Locus_801_Tra	7.54	Putative transcription factor bHLH041 OS=Arabidopsis thaliana G	6.75701e-18
C_Locus_1586_Tra	7.54	AT5g14040/MUA22_4 OS=Arabidopsis thaliana GN=PHT3;1 PE=	2.48937e-171
C_Locus_152_Tra	7.54	D-3-phosphoglycerate dehydrogenase OS=Arabidopsis thaliana G	2.70779e-49
C_Locus_8386_Tra	7.55	Uncharacterized protein OS=Arabidopsis thaliana GN=At5g57510	2.95118e-29
C_Locus_2866_Tra	7.57	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	1.01729e-134
C_Locus_22226_T	7.57	Peroxidase 4 OS=Arabidopsis thaliana GN=PER4 PE=2 SV=1	5.70235e-132
C_Locus_15933_T	7.58	Duplicated SANT DNA-binding domain-containing protein OS=Ara	9.84654e-27
C_Locus_3534_Tra	7.59	Putative mitochondrial protein OS=Arabidopsis thaliana GN=F18B	9.08762e-69
C_Locus_9527_Tra	7.59	NA	NA
C_Locus_16977_T	7.59	Ent-copalyl diphosphate synthase, chloroplastic OS=Arabidopsis	1.47207e-20
C_Locus_2025_Tra	7.60	Putative uncharacterized protein T22E19.19 OS=Arabidopsis thali	9.165e-09
C_Locus_2082_Tra	7.61	Protein kinase-like protein OS=Arabidopsis thaliana GN=T4O24.7	9.06543e-108
C_Locus_18606_T	7.61	NA	NA
C_Locus_26583_T	7.62	Putative uncharacterized protein not annotated OS=Arabidopsis th	1.02189e-07
C_Locus_35775_T	7.62	NA	NA
C_Locus_11847_T	7.62	U-box domain-containing protein 43 OS=Arabidopsis thaliana GN=	7.02783e-17
C_Locus_692_Tra	7.63	F3H9.15 protein OS=Arabidopsis thaliana GN=F3H9.15 PE=4 SV	5.26646e-45
C_Locus_3280_Tra	7.64	1-aminocyclopropane-1-carboxylate oxidase homolog 4 OS=Arabi	4.4754e-62

C_Locus_471_Tra	7.64	At5g26330 OS=Arabidopsis thaliana GN=F9D12.16 PE=2 SV=1	8.41486e-10
C_Locus_760_Tra	7.64	At4g24380 OS=Arabidopsis thaliana GN=At4g24380/T22A6_210	4.24314e-68
C_Locus_1906_Tr	7.64	Aquaporin TIP1-3 OS=Arabidopsis thaliana GN=TIP1-3 PE=1 SV=	2.53486e-42
C_Locus_7530_Tr	7.65	NA	NA
C_Locus_855_Tra	7.65	Putative uncharacterized protein At4g22530 (Fragment) OS=Arabi	2.80233e-58
A_Locus_32468_T	7.65	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 S	6.58757e-31
C_Locus_1242_Tr	7.66	Wall-associated receptor kinase 3 OS=Arabidopsis thaliana GN=	2.52623e-133
C_Locus_4587_Tr	7.66	Calmodulin-binding protein-like protein OS=Arabidopsis thaliana G	0
C_Locus_5039_Tr	7.66	Cytochrome P450 82C4 OS=Arabidopsis thaliana GN=CYP82C4	3.31935e-159
C_Locus_12810_T	7.68	At2g15730 OS=Arabidopsis thaliana GN=At2g15730 PE=2 SV=1	2.36472e-168
C_Locus_17198_T	7.68	Mate efflux domain-containing protein OS=Arabidopsis thaliana G	0
C_Locus_1698_Tr	7.68	At1g36580/F28J9_6 OS=Arabidopsis thaliana GN=At5g35735 PE=	4.66411e-86
C_Locus_9521_Tr	7.69	Probable xyloglucan endotransglucosylase/hydrolase protein 25 O	1.74086e-125
A_Locus_12686_T	7.71	F-box protein SKIP27 OS=Arabidopsis thaliana GN=SKIP27 PE=	5.2627e-08
C_Locus_3283_Tr	7.71	At2g15220/F15A23.4 OS=Arabidopsis thaliana GN=F15A23.4/At2	2.26078e-75
C_Locus_3534_Tr	7.72	Putative mitochondrial protein OS=Arabidopsis thaliana GN=F18B	1.28136e-70
C_Locus_27077_T	7.73	Secretory carrier-associated membrane protein 3 OS=Arabidopsis	6.09076e-08
C_Locus_1242_Tr	7.75	Wall-associated receptor kinase 3 OS=Arabidopsis thaliana GN=	3.84096e-145
C_Locus_134_Tra	7.78	GEM-like protein 5 OS=Arabidopsis thaliana GN=At5g13200 PE=	8.8238e-111
C_Locus_471_Tra	7.80	Blue copper protein OS=Arabidopsis thaliana GN=BCB PE=1 SV=	1.00024e-23
C_Locus_16628_T	7.81	Cytochrome P450 OS=Arabidopsis thaliana GN=CYP72A15 PE=	1.35291e-135
C_Locus_29596_T	7.82	Lactosylceramide 4-alpha-galactosyltransferase OS=Arabidopsis	1.1449e-136
C_Locus_3122_Tr	7.83	Probable WRKY transcription factor 75 OS=Arabidopsis thaliana	3.23733e-18
C_Locus_22442_T	7.84	U-box domain-containing protein 16 OS=Arabidopsis thaliana GN=	0
C_Locus_8574_Tr	7.84	Ethylene-responsive transcription factor ERF110 OS=Arabidopsis	1.65952e-17

C_Locus_3488_Tr	7.84	Uncharacterized protein At1g76070 OS=Arabidopsis thaliana GN=	1.18573e-51
C_Locus_31588_T	7.85	NA	NA
C_Locus_1887_Tr	7.86	Geranylgeranyl pyrophosphate synthase OS=Arabidopsis thaliana	4.49209e-141
C_Locus_2827_Tr	7.86	Globulin-like protein OS=Arabidopsis thaliana PE=2 SV=1	2.34691e-84
C_Locus_10617_T	7.87	AT5g35570/K2K18_1 OS=Arabidopsis thaliana GN=At5g35570 P	5.08094e-28
C_Locus_3129_Tr	7.87	At4g39830 OS=Arabidopsis thaliana GN=AT4g39830 PE=2 SV=1	1.62423e-127
C_Locus_8574_Tr	7.89	Ethylene-responsive transcription factor ERF110 OS=Arabidopsis	2.28461e-17
C_Locus_338_Tra	7.89	Plasma membrane-type calcium ATPase OS=Arabidopsis thalian	1.32489e-69
C_Locus_16952_T	7.90	Protein TIFY 5A OS=Arabidopsis thaliana GN=TIFY5A PE=1 SV=	2.32356e-11
C_Locus_17198_T	7.90	Mate efflux domain-containing protein OS=Arabidopsis thaliana G	8.84288e-160
C_Locus_3635_Tr	7.91	PR5-like receptor kinase OS=Arabidopsis thaliana GN=PR5K PE=	5.33307e-101
C_Locus_16829_T	7.92	Ethylene-responsive transcription factor 12 OS=Arabidopsis thalia	1.09042e-45
C_Locus_3129_Tr	7.93	At4g39830 OS=Arabidopsis thaliana GN=AT4g39830 PE=2 SV=1	0
C_Locus_3158_Tr	7.93	Aquaporin TIP1-1 OS=Arabidopsis thaliana GN=TIP1-1 PE=1 SV=	1.71704e-122
C_Locus_20372_T	7.97	At2g27080/T20P8.13 OS=Arabidopsis thaliana GN=At2g27080/T2	1.18754e-75
C_Locus_16929_T	7.99	NA	NA
C_Locus_917_Tra	8.00	Putative NADH dehydrogenase (Ubiquinone oxidoreductase) OS=	1.61401e-105
C_Locus_10225_T	8.01	AT3g50930/F18B3_210 OS=Arabidopsis thaliana GN=BCS1 PE=	6.20085e-140
C_Locus_1381_Tr	8.02	Uncharacterized protein OS=Arabidopsis thaliana GN=At3g05858	6.30332e-06
C_Locus_610_Tra	8.03	F-box protein At1g61340 OS=Arabidopsis thaliana GN=At1g6134	5.53071e-21
C_Locus_14470_T	8.03	UDP-arabinopyranose mutase 2 OS=Arabidopsis thaliana GN=RG	2.12933e-14
C_Locus_35691_T	8.03	NA	NA
C_Locus_5175_Tr	8.05	Ethylene-responsive transcription factor ERF098 OS=Arabidopsis	2.47332e-21
C_Locus_12215_T	8.06	Cysteine-rich receptor-like protein kinase 3 OS=Arabidopsis thalia	0
C_Locus_23010_T	8.06	Gb|AAF31027.1 OS=Arabidopsis thaliana GN=At5g38200 PE=4 S	0

C_Locus_1985_Tra	8.06	Wall-associated receptor kinase-like 22 OS=Arabidopsis thaliana	6.37409e-133
C_Locus_4475_Tra	8.08	MYB-related protein OS=Arabidopsis thaliana GN=myb PE=4 SV=	7.36387e-41
C_Locus_134_Tran	8.13	GEM-like protein 5 OS=Arabidopsis thaliana GN=At5g13200 PE=	1.86775e-75
C_Locus_2827_Tra	8.14	Globulin-like protein OS=Arabidopsis thaliana PE=2 SV=1	1.25803e-90
C_Locus_4828_Tra	8.17	E3 ubiquitin-protein ligase ATL6 OS=Arabidopsis thaliana GN=ATL	1.52214e-84
C_Locus_216_Tran	8.18	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	2.775e-100
C_Locus_25855_T	8.18	At3g54420 OS=Arabidopsis thaliana GN=T12E18_110 PE=2 SV=	6.25432e-155
C_Locus_2082_Tra	8.20	Protein kinase-like protein OS=Arabidopsis thaliana GN=At1g669	1.30164e-110
C_Locus_6631_Tra	8.20	Predicted by genscan and genefinder OS=Arabidopsis thaliana GN	2.86741e-37
C_Locus_2827_Tra	8.21	Globulin-like protein OS=Arabidopsis thaliana PE=2 SV=1	7.7722e-86
C_Locus_6834_Tra	8.21	G-type lectin S-receptor-like serine/threonine-protein kinase At1g6	0
C_Locus_1698_Tra	8.21	At5g47530 OS=Arabidopsis thaliana GN=At5g47530 PE=2 SV=1	1.57161e-137
C_Locus_2750_Tra	8.22	Probable calcium-binding protein CML40 OS=Arabidopsis thaliana	1.67108e-37
C_Locus_15206_T	8.23	Transcription factor bHLH92 OS=Arabidopsis thaliana GN=BHLH9	4.9551e-27
C_Locus_18091_T	8.23	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	5.24502e-43
C_Locus_54_Trans	8.24	Putative metal tolerance protein C3 OS=Arabidopsis thaliana GN=	1.45943e-153
C_Locus_22917_T	8.24	Putative uncharacterized protein At5g43420 OS=Arabidopsis thali	1.06275e-89
C_Locus_13224_T	8.26	Ethylene-responsive transcription factor 1A OS=Arabidopsis thalia	3.19496e-66
C_Locus_17599_T	8.29	Ethylene-responsive transcription factor ERF110 OS=Arabidopsis	7.97234e-17
C_Locus_18539_T	8.32	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0
A_Locus_14526_T	8.33	F-box protein At1g61340 OS=Arabidopsis thaliana GN=At1g6134	2.09349e-09
C_Locus_14875_T	8.33	Hin1 homolog OS=Arabidopsis thaliana GN=YLS9 PE=2 SV=1	1.2799e-47
C_Locus_2826_Tra	8.34	At2g38470 OS=Arabidopsis thaliana PE=2 SV=1	1.16014e-89
C_Locus_20557_T	8.35	NA	NA
C_Locus_1675_Tra	8.35	BON1-associated protein 2 OS=Arabidopsis thaliana GN=BAP2 P	8.15015e-26

C_Locus_12547_T	8.36	GDSL esterase/lipase At3g26430 OS=Arabidopsis thaliana GN=A	1.60326e-143
C_Locus_1073_Tra	8.37	Auxin efflux carrier family protein OS=Arabidopsis thaliana GN=T7	4.67759e-72
C_Locus_2183_Tra	8.38	F3H9.15 protein OS=Arabidopsis thaliana GN=F3H9.15 PE=4 SV	9.89845e-48
C_Locus_15933_T	8.40	Duplicated SANT DNA-binding domain-containing protein OS=Ara	8.22148e-31
C_Locus_1839_Tra	8.42	At4g11650 OS=Arabidopsis thaliana GN=At4g11650 PE=2 SV=1	1.11731e-26
C_Locus_23541_T	8.44	E3 ubiquitin-protein ligase ATL41 OS=Arabidopsis thaliana GN=A	1.04212e-38
C_Locus_17570_T	8.45	Probable xyloglucan endotransglucosylase/hydrolase protein 23 O	5.85911e-105
C_Locus_26558_T	8.48	U-box domain-containing protein 21 OS=Arabidopsis thaliana GN=	7.47378e-113
C_Locus_1532_Tra	8.49	O-fucosyltransferase-like protein OS=Arabidopsis thaliana GN=At	0
C_Locus_11448_T	8.51	60S ribosomal protein L19-2 OS=Arabidopsis thaliana GN=RPL19	1.82372e-76
C_Locus_383_Tra	8.51	Protein TIFY 10A OS=Arabidopsis thaliana GN=TIFY10A PE=1 S	1.14227e-15
C_Locus_1586_Tra	8.51	AT5g14040/MUA22_4 OS=Arabidopsis thaliana GN=PHT3;1 PE=	4.82374e-89
C_Locus_365_Tra	8.52	Pathogenesis-related thaumatin-like protein OS=Arabidopsis thali	4.56397e-144
C_Locus_17126_T	8.52	At4g39830 OS=Arabidopsis thaliana GN=AT4g39830 PE=2 SV=1	0
C_Locus_18091_T	8.52	NA	NA
C_Locus_232_Tra	8.53	NA	NA
C_Locus_6912_Tra	8.57	Putative receptor-like protein kinase At3g47110 OS=Arabidopsis t	0
C_Locus_15206_T	8.58	Transcription factor bHLH92 OS=Arabidopsis thaliana GN=BHLH9	2.27926e-31
C_Locus_1497_Tra	8.58	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	1.40614e-73
C_Locus_9235_Tra	8.63	E3 ubiquitin-protein ligase PUB22 OS=Arabidopsis thaliana GN=P	2.29764e-170
C_Locus_216_Tra	8.65	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	1.63666e-23
C_Locus_7264_Tra	8.66	Uncharacterized protein OS=Arabidopsis thaliana GN=At5g14230	0
C_Locus_4498_Tra	8.69	Putative uncharacterized protein At1g62300 (Fragment) OS=Arabi	8.54316e-104
C_Locus_1497_Tra	8.71	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	4.71891e-72
C_Locus_18539_T	8.73	Abscisic acid 8'-hydroxylase 1 OS=Arabidopsis thaliana GN=CYP	0

C_Locus_2682_Tr	8.79	At2g26190/T1D16.17 OS=Arabidopsis thaliana GN=At2g26190 PE	0
C_Locus_1497_Tr	8.87	Transcription factor, putative OS=Arabidopsis thaliana PE=2 SV=	2.41522e-48
C_Locus_610_Tra	8.92	F-box protein OS=Arabidopsis thaliana GN=At1g61340 PE=4 SV=	1.25433e-17
C_Locus_14771_T	8.94	Wall-associated receptor kinase 1 OS=Arabidopsis thaliana GN=	2.20528e-130
C_Locus_54_Trans	8.94	Putative metal tolerance protein C3 OS=Arabidopsis thaliana GN=	1.57539e-155
C_Locus_20557_T	9.01	NA	NA
C_Locus_1839_Tr	9.01	Osmotin OS=Arabidopsis thaliana PE=2 SV=1	1.96068e-52
C_Locus_472_Tra	9.04	Glutamate decarboxylase 4 OS=Arabidopsis thaliana GN=GAD4	0
C_Locus_17949_T	9.07	Transcription factor bHLH92 OS=Arabidopsis thaliana GN=BHLH9	2.34209e-17
C_Locus_472_Tra	9.07	Glutamate decarboxylase 4 OS=Arabidopsis thaliana GN=GAD4	0
C_Locus_8788_Tr	9.08	NA	NA
C_Locus_14379_T	9.14	NA	NA
C_Locus_280_Tra	9.16	Beta-glucosidase 46 OS=Arabidopsis thaliana GN=BGLU46 PE=	7.70363e-163
C_Locus_54_Trans	9.19	Putative metal tolerance protein C3 OS=Arabidopsis thaliana GN=	1.32105e-114
C_Locus_2613_Tr	9.19	MYB transcription factor OS=Arabidopsis thaliana GN=T22E19.5	4.79456e-71
C_Locus_7245_Tr	9.20	Probable polyol transporter 3 OS=Arabidopsis thaliana GN=PLT3	0
C_Locus_54_Trans	9.22	Putative metal tolerance protein C3 OS=Arabidopsis thaliana GN=	0
C_Locus_591_Tra	9.23	Ethylene-responsive transcription factor 5 OS=Arabidopsis thalian	2.75129e-43
C_Locus_21925_T	9.27	Putative uncharacterized protein OS=Arabidopsis thaliana PE=4 S	1.45172e-10
C_Locus_22811_T	9.31	EDGP OS=Arabidopsis thaliana PE=2 SV=1	2.659e-171
C_Locus_3337_Tr	9.35	At2g27690 OS=Arabidopsis thaliana GN=At2g27690/F15K20.21 P	0
C_Locus_2935_Tr	9.41	Putative non-LTR retroelement reverse transcriptase OS=Arabidop	1.93423e-19
C_Locus_17949_T	9.46	Transcription factor bHLH92 OS=Arabidopsis thaliana GN=BHLH9	6.35449e-39
C_Locus_16967_T	9.47	C-repeat binding factor 4 OS=Arabidopsis thaliana GN=CBF4 PE=	1.95181e-62
C_Locus_1591_Tr	9.49	Zinc finger protein ZAT11 OS=Arabidopsis thaliana GN=ZAT11 PE	1.04506e-49

C_Locus_1698_Tra	9.50	AT4g17280/dl4675c OS=Arabidopsis thaliana GN=At4g17280 PE=	1.53012e-91
C_Locus_2613_Tra	9.54	MYB transcription factor OS=Arabidopsis thaliana GN=T22E19.5	7.71486e-60
C_Locus_2067_Tra	9.56	Sugar transport protein 13 OS=Arabidopsis thaliana GN=STP13 P	0
C_Locus_16967_T	9.61	C-repeat binding factor 4 OS=Arabidopsis thaliana GN=CBF4 PE=	3.59242e-62
C_Locus_2082_Tra	9.63	Protein kinase-like protein OS=Arabidopsis thaliana GN=At1g6692	1.53353e-107
C_Locus_14379_T	9.81	NA	NA
C_Locus_2613_Tra	9.82	MYB transcription factor OS=Arabidopsis thaliana GN=T22E19.5	6.83195e-66
C_Locus_1397_Tra	10.03	Germin-like protein subfamily 1 member 16 OS=Arabidopsis thalia	2.24737e-77
C_Locus_1985_Tra	10.14	Wall-associated receptor kinase-like 22 OS=Arabidopsis thaliana	7.66734e-133
C_Locus_6010_Tra	10.33	Zinc finger protein ZAT12 OS=Arabidopsis thaliana GN=ZAT12 PE	1.00926e-31
C_Locus_134_Tran	10.44	GEM-like protein 5 OS=Arabidopsis thaliana GN=At5g13200 PE=	1.45258e-79
C_Locus_472_Tran	10.74	Glutamate decarboxylase 4 OS=Arabidopsis thaliana GN=GAD4	0

List of Publications

Journals

Luke, L.P., Sathik, M.B.M., Thomas, M., Kuruvilla, L., Sumesh, K.V., Annamalainathan, K. (2015). Quantitative expression analysis of drought responsive genes in clones of *Hevea* with varying levels of drought tolerance. *Physiology and Molecular Biology of Plants*, 21:179-186.

Kuruvilla, L., Sathik, M.B.M., Thomas, M., **Luke, L.P.**, Sumesh, K.V. and Annamalainathan, K. *(2016)*. Expression of miRNAs of *Hevea brasiliensis* under drought stress is altered in clones with varying levels of drought tolerance. *Indian Journal of Biotechnology,* 15: 153-160.

Sathik, M.B.M., Kuruvilla, L., Thomas, M., **Luke, L.P.**, Satheesh, P.R., Annamalainathan, K. and Jacob, J. (2012). Quantitative expression analysis of stress responsive genes under cold stress in *Hevea brasiliensis*. *Rubber Science*, 25(2): 199-213.

Thomas, M., Sathik, M.B.M., **Luke, L.P.**, Sumesh, K.V., Satheesh, P.R., Annamalainathan, K. and Jacob, J. (2012). Stress responsive transcripts and their association with drought tolerance in *Hevea brasiliensis*. *Journal of Plantation Crops*, 40:180–187.

Thomas, M., Sathik, M.B.M., Saha, T., Jacob, J., Schaffner, A.R., **Luke, L.P.**, Kuruvilla, L., Annamalainathan, K. and Krishnakumar, R. (2011). Screening of drought responsive transcripts of *Hevea brasiliensis* and identification of candidate genes for drought tolerance. *Journal of Plant Biology*, 38 & 39:111–118.

Luke, L.P., Sathik, M.B.M., Thomas, M., Sumesh, K.V. and Kuruvilla, L. (2017). Expression of NAC transcription factor is altered under intermittent drought stress and re-watered conditions in *Hevea brasiliensis*. Communicated to *Acta Physiologiae Plantarum.*

Kuruvilla, L., Sathik, M.B.M., Thomas, M., **Luke, L.P.** and Sumesh, K.V. (2016). Identification and validation of cold responsive microRNAs of *Hevea brasiliensis* using high-throughput sequencing. Communicated to *Physiology and Molecular Biology of plants.*

Seminars

Luke, L.P., Sathik, M.B.M., Thomas, M. and Kuruvilla, L. (2015). Quantitative expression analysis of drought responsive genes in clones of *Hevea* with varying levels of drought tolerance. *National Seminar on New frontiers in Plant Sciences and Biotechnology, Department of Botany, Goa University, Abstracts* pp. 138.

Luke, L.P., Sathik, M.B.M., and Thomas, M. (2012). Promising drought tolerance associated genes of *Hevea brasiliensis* In: *Proceedings of 24th Kerala Science Congress.* pp. 223-225.