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Identification of dehydration tolerance genes in triticale (x *Triticosecale* Wittm.) seedlings

by

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Dedication

To Ana and Andrew - my family.

Abstract

Dehydration stress triggers many changes in plant gene expression. Triticale (x *Triticosecale* Wittm.) sequencing using next generation sequencing allowed identification of 107,000 transcripts of which 4,467 were differentially regulated ≥2-fold. Abscisic acid co-receptors, pyrabactin and protein phosphatases 2C were highly regulated and identified for the first time in dehydration stress. Many ABA-independent genes such as DREB/CBFs family were down-regulated or non-differentially expressed compared to non-stressed plants. This suggested that the solid polyethylene glycol system successfully led to identification of dehydration stress-specific genes from other stresses. Only 5% from the 87% annotated differentially regulated transcripts showed homology to previously identified dehydration responsive genes in wheat, maize, barley or Arabidopsis. Thus, this study revealed a larger number of novel genes potentially involved in dehydration stress, many of which were highly differentially regulated.

Identification of differentially expressed genes and their functions during dehydration stress is essential towards improvement of dehydration tolerance crops.

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List of symbols, nomenclature, and abbreviations

AAO: abscisic aldehyde oxygenase

AAPK: ABA-activated protein kinase

ABA: abscisic acid

ABAld: abscisic aldehyde

ABF: ABA-responsive element binding factor

ABRE: ABA-responsive element

AREB: ABRE binding protein

BLAST: basic local alignment search tool

bZIP: basic-region leucine zipper

CAGE: cap analysis of gene expression

CCD: charge-coupled device

CE: coupling element

CRT: C-repeat

DHN: dehydrin

DRE/CBF: drought responsive element/C-repeat binding factor

ERD: early response to dehydration

FCA: flowering time control protein A

GCR: G protein-coupled receptor

GO: gene ontology

GUN: genome uncoupled

ICE: inducer of CBF expression

LEA: late embryogenesis abundance

LTRE: low-temperature responsive element

MoCo: molybdenum cofactor

MYB: myeloblastosis

MYC: myelocytomatosis

NCED: 9-*cis*-epoxycarotenoid dioxygenase

NGS: next generation sequencing

P5CS: pyrroline-5-carboxylate-synthetase

P5CR: pyrroline-5-carboxylate-reductase

PEG: polyethylene glycol

PP2C: protein phosphatase 2C

PYR/PYL/RCAR: pyrabactine/pyrabactine-like/regulatory component of ABA receptor

RPK: leucine-rich repeat receptor-like kinase

RPKM: Reads Per Kilobase of exon model per Million mapped reads

SAGE: serial analysis of gene expression

SNF: sucrose non-fermenting

SNP: single nucleotide polymorphism

SnRK: SNF-related protein kinase

SR: signal responsive to biotic stimulus

SRD: short-chain dehydrogenase/reductase

START: StAR-related lipid transfer

USDA: United States Department of Agriculture

WUE: water use efficiency

ZEP: zeaxanthin epoxidase

Chapter 1. Introduction

Drought is a devastating event that occurs globally and could cause severe environmental and economic damages. In recent years, lack of water has become a major issue worldwide and this topic has generated heated debates in most countries on how water should be distributed, used and conserved. Moreover, global arable land surface is decreasing due in part to urbanization as well as detrimental agricultural practices to the soil.

For crop development, water is one of the most important component and the rapid increase in global population demands larger amounts of food supplies and puts a high pressure on rapidly increasing yields. Therefore, using more land or water to increase the food supplies are not viable solutions anymore. There is a need for development of crops with high water use efficiency and higher yields that could grow on less fertile soil and more extreme climatic conditions.

All plants have a degree of dehydration tolerance, some are more tolerant such as the desert plants which are resurrection plants (*Selaginella lepidophylla, Myrothamnus flabellifolius, Anastatica hierochuntica, Craterostigma plantagineum*), but crop species are much less tolerant and to produce high yields they have high demands for water. The goal of recent breeding programs is towards developing crops that use less water and maintain or increase the yield. Both rice and wheat varieties, the most cultivated crops on Earth, have high water requirements to maintain their yield potential. Therefore, plant breeders have to find new breeding sources to use as parents in their breeding programs to redesign the crop of the future. Triticale (x *Triticosecale* Wittm.) is a man-made crop obtained from hybridizing wheat and rye that has the potential to be an important source for developing dehydration-tolerant crops. Triticale is tolerant to most abiotic stresses, including salt and dehydration. It can grow on marginal land and has a high biomass and grain yields, highly superior to wheat. It is mostly used as forage crop due to the fact that its grain quality for end-use products is not as good as of wheat.

Our decision to use hexaploid triticale to identify the genes and mechanisms involved in the response to dehydration rather than the diploid rye, its abiotic stress tolerant parent, was based on the fact that not all rye genes are expressed in triticale. The objective was to identify genes contributing to the dehydration tolerance in triticale, a newly designed species for development of a biorefinery industry in Canada based on the complete utilization of the plant.

The objectives of this study were to identify a reproducible system for applying dehydration stress and using RNA-Seq to identify the transcript complement and differentially regulated genes in triticale seedlings exposed to different degrees of dehydration stress. After identification and classification of these genes, an additional objective was to identify which signalling and biochemical pathways might be involved in responding to the dehydration stress in triticale seedlings.

Different concentrations of solid polyethylene glycol (PEG) media were used to induce different levels of dehydration stress. This method was recently developed by Comeau et al. (2010) and evaluated on different cereals such as barley, wheat and triticale.

The identification of the transcripts was achieved using next generation sequencing (NGS) technology through sequencing of cDNA libraries obtained from poly $(A)^+$ RNA from triticale seedlings of control and three dehydration treatments.

RNA-Seq, which is a newly developed method for transcript profiling, was used to identify the transcript complement and to identify and analyze the genes that are statistically significantly regulated between the four libraries. The identification and classification of the transcripts was analyzed by bioinformatics methods, using different platforms and softwares such as DNASTAR and Blast2GO or developed in house.

There were three goals set for this study. The first one was to demonstrate that solid PEG medium is a highly reliable and reproducible system to study different levels of dehydration stress in triticale and cereals. The second was to generate the first transcriptome of triticale seedlings under control and dehydration stress. The last goal was to identify statistically differentially regulated genes involved in the response of triticale to dehydration stress at an early stage of development.

Chapter 2. Literature Review

Crop species have played an important part in shaping the civilized world, helping the human race to settle at specific locations and build new nations by providing the necessary food supply. Today, crop species are getting much attention, as there is increasing demand to feed the growing population of the world. It is estimated that by 2050, the world population will reach 9 billion, which means that the food demand will increase by at least 2.5-fold (Godfray et al., 2010). Efforts to increase yield have been successful for the majority of the staple crops e.g. wheat, rice, corn, soybean, oats, rye (Karaba et al., 2007; Vendruscolo et al., 2007; Cattivelli et al., 2008; Chen et al., 2008; Ergen et al., 2009); however, there are factors negatively affecting plant development and yield such as drought, wind, heat, cold, and freezing that are grouped under abiotic stresses. These factors have detrimental impacts on crops and can lead to significant yield losses (Passioura, 2007; Cattivelli et al., 2008; Jaleel et al., 2009; Sheffield et al., 2009). In this chapter, a review of the economic and social impacts caused by drought will be presented, as well as some of the plant responses to this stress from both physiological and molecular perspectives. The importance of molecular studies and identification of differential regulation of the genes implicated in the response to drought stress will also be discussed in detail.

2.1. Economic and social impacts of drought on plant productivity

Drought is among the most devastating of all abiotic stresses in plants, causing yield losses of up to 80%. There are many definitions of drought, depending on the field of study, but the common element in all these definitions is that drought represents a period of low or no precipitation (Passioura, 2007). Drought is very unique because it affects the only resource that plants cannot live without - water. Plants are made up of approximately 90% water and its scarcity has implications for all tissues and organs of any plant species. Recent research reports have shown that water supplies worldwide have dropped significantly in the past decades, due in part to climate change and to the rapid increase in the human population (Godfray et al., 2010).

According to the Intergovernmental Panel on Climate Change, global warming will cause more drought events that will last for a longer period of time and will extend to greater cultivated areas (Sheffield et al., 2009). Other factors that could impact drought events, such as large-scale atmospheric mechanisms associated with climate variability, and sea surface temperature anomalies, have also been identified (Sheffield et al., 2009). Drought could result, in part, from evapotranspiration that depletes moisture from soils and from a decrease in precipitations in different areas around the globe thus making soil unusable for agricultural purpose and diminishing the already limited and available arable land areas globally. For the past 60 years, hundreds of drought events of different intensities and durations have been recorded worldwide (Sheffield et al., 2009). Some of the most severe droughts occurred in North America in the 1950s and in Asia in the mid 1980s, and these regions have recorded the highest number of drought events in the past 50 years (Table 2.1). The high percentage of arable land affected by drought events (Sheffield et al., 2009) also provides a good indication

Table 2.1 Drought occurrence between 1950 and 2000 on all continents (adapted from Sheffield et al., 2009)

Continent	**Number of droughts**	**Longest duration (months and years)**	**Area affected (km^2)**
Africa	44	19 (82'-84')	11,346,000 (40%*)
Asia	86	49 (84'-88')	8,193,000 (18.5%)
Europe	40	20 (59'-61')	4,264,000 (42.8%)
North America	57	44 (50'-53')	8,231,000 (39.3%)
Oceania	24	12 (51'-52')	6,525,000 (80.2%)
South America	45	16 (58'-59')	9,038,000 (51.2%)

* % of total continental arable land area

of how they can affect agriculture on a larger scale. In all, 446 drought events between years 1968-1992 were blamed for 1.8 million deaths, second only to the 2.5 million lives claimed by civil wars over the same period (Foster, 2010).

Some drought events have only a local impact on the economy, while others could have a global impact as demonstrated by the two following news headlines: a) Recent events in Somalia have shown that “Fighting and drought worsen Somalia’s humanitarian crisis” and that “1.4 million people are in need of food aid due to an ongoing drought” (Wakabi, 2009); b) The latest Russian drought event in the summer of 2010 led to titles such as “USDA to gauge Russia drought impact on world crops” where “the U.S. government is set to forecast a bigger decline in world wheat stock and modest drop in global production as Russia’s worst drought in over a century halts exports and threatens to whither the next crop” (Abbott, 2010).

In Canada, Agriculture and Agri-Food Canada proposed the creation of the National Drought Strategy in 2004, that could “help agriculture policy and decision-makers, and all stakeholders to make timely and informed decisions to prepare for, mitigate, respond to, and adapt to drought” (Hanuta, 2007). Over the past 70 years, seven major droughts have cost Canada $22.2 billion. The 1980 drought on the Canadian Prairies alone cost $5.8 billion, roughly 2% of Canada’s Gross Domestic Product. These costs only reflect direct losses to the agricultural sector, and do not account for downstream losses in retail and goods and services to farmers and the food industry.

Given, drought cannot be easily localized or predicted, and considering its impact on the world's human population, one can see the importance of minimizing its negative impact. The threat of climate change and the current reality of rapid population growth demanding greater food production, has brought together many research groups, national, and international panels, all with the mandate of mitigating the impact of drought on crop productivity and food security.

2.2. Drought stress physiology and plant development

Drought can occur over a few hours or days in the eyes of molecular biologists or plant physiologists, or up to several years or decades from the perspective of meteorologists, farmers or historians (Passioura, 2007). However, when studying drought tolerance in plants, one mostly refers to a brief period (hours to days) of water scarcity. Plants are influenced by drought stress in different ways, depending on several factors such as the severity and the duration of the drought event as well as the developmental stage of the plant at the time of occurrence (Fukai and Cooper, 1995; Morant-Manceau et al., 2004; Pieters and El Souki, 2005; Hura et al., 2009; Jaleel et al., 2009).

Many factors can influence physiological functions and initiate responses impacting plant development and yield (Jaleel et al., 2009). Nevertheless, when drought is perceived, one of the first plant responses is to close their stomata to reduce transpiration, thus indirectly reducing photosynthetic activities (Xue et al., 2006; Yoo et al., 2010). After this initial step, a series of events will occur that affects the whole plant at different levels. Plant growth and development is slowed down while the plant tries to cope with the external stress in order to survive and reproduce. It has already been identified that when plants are affected by drought stress during their initial developmental stage, cell elongation and plant expansion is limited (Specht et al., 2001; Su and Wu, 2004; Wu et al., 2008). Specht et al. (2001) demonstrated that under mild drought stress, the length of soybean stems was reduced significantly, while in citrus seedlings, mild water stress led to up to 25% reduction in plant height and a significant reduction in crop survival to drought (Wu et al., 2008). Su and Wu (2004) showed that under drought stress, rice plants had a slower growth rate and were smaller than transformed plants overexpressing a pyrroline-5-carboxylate synthetase (*p5cs*) gene responsible for the production of proline, a known osmoprotectant.

Even if the stress occurred at very early developmental stages, studies have shown that drought events can still have a negative impact on plant development and subsequent yield (Fukai and Cooper, 1995; Leilah and Al-Khateeb, 2005; Monneveux et al., 2006; Foulkes et al., 2007; Cattivelli et al., 2008; Jaleel et al., 2009; Salekdeh et al., 2009). Most researchers agree that several plant developmental stages can be seriously affected by drought, including seedling, juvenile, tillering, grain filling and even full maturity stages. In regions where crop production relies almost exclusively on irrigation, farmers have to manage water for the whole plant growth cycle and must decide when to allocate more water to support different developmental stages. While there is no clear consensus on the stage of plant development most vulnerable to drought-related yield losses, such an understanding would allow for better management of water resources and thus the development of better water conservation strategies.

There are different approaches to studying drought tolerance from a physiological point of view, and among these, one is to examine existing tolerant plant systems, such as seeds and desiccation-tolerant plants (Ingram and Bartels, 1996). Both seeds and desiccation-tolerant plants such as resurrection plants and ferns can withstand severe dehydration stress (Bartels et al., 1990; Leprince et al., 1993). Initially, it was believed that detailed molecular analyses of these systems would reveal the expressed genes that are responsible for imparting tolerance to drought stress, and provide the answer for drought tolerance in crops. The main achievement from studying seeds was the identification and characterization of late-embryogenesis abundant (LEA) proteins. LEA proteins are small proteins in plants and animals which protect other proteins from aggregation following a desiccation, osmotic, cold and high salinity stresses (Park et al., 2005; Tondelli et al., 2006; Kobayashi et al., 2008). The molecular study of the desiccation-tolerant plants revealed that desiccation-induced genes were very similar to the LEA genes expressed in seeds of most angiosperm species (Bartels et al., 1990).

It was also observed that, upon a drought stress event, the resurrection plant activates a survival mechanism that reduces the plant’s growth and development, an adaptive behaviour that is not desirable in crop plants where the main goal is to maintain or increase yield. These observations led to the conclusion that studies should be shifted away from desiccation-tolerant plants and directed towards existing high yielding crops and wild relatives that have good tolerance to drought (Bartels et al., 1990; Ingram and Bartels, 1996).

Rice was among the first crops to be studied for drought tolerance. It is one of the most important crops worldwide, being the major food ingredient for half of the world's population and one of the most dependent on water at all developmental stages. Even in rain-fed areas, drought is the most important risk factor for rice production. Introduction of irrigation in these areas was a major innovation, but with the increased water scarcity and limited energy resources, this approach has become costly and unfortunately, non-sustainable (Wassmann et al., 2009). Consequently, one of the successful strategies in developing drought tolerant rice was the development of short-duration rice cultivars which combine improved water use efficiency (WUE) and yield potential (Reinke et al., 1994). Plant WUE is a term that originates from the economic concept of crop yield and productivity, representing the volume of water used by a plant to produce a unit of output. Reinke et al. (1994) showed that use of short-duration varieties could reduce water consumption by 10%. While this represents a promising solution on a local scale, on a global scale, it remains only a partial solution considering that short-duration rice varieties still have lower yield potential, and therefore require larger arable land areas to achieve the same yields as long-duration varieties in absence of drought.

For semi-arid crops, like sorghum, pearl millet, rye or barley, the water availability is more critical at the juvenile stage for the development of tillers and root expansion (Ozturk et al., 2002; Sahnoune et al., 2004; Buchanan et al., 2005).

The grain development and filling stages are also extremely important to ensure maximum yield.

Despite what we have learned to date about the influence of drought on crop physiology over the course of plant development, there is still an incomplete understanding of the underlying molecular events taking place in each cell, tissue or organ. Therefore, molecular studies could reveal the intimate processes involved in the response of plants to drought stress, and lead to the identification of genes that are specifically involved in drought tolerance at various developmental stages.

2.3. Dehydration stress and gene expression: ABA-dependent and ABA-independent pathways

At the molecular level, plants adapt to water shortage by differentially regulating a series of genes or gene families with various functions (Shinozaki et al., 2003; Tuberosa and Salvi, 2006; Seki et al., 2007; Nakashima et al., 2009). Numerous studies have demonstrated that abscisic acid (ABA) is a very important signalling molecule in plant responses to drought stress, and its increased accumulation triggers stomatal closure (Swindell, 2006; Raghavendra et al., 2010; Schramm et al., 2010; Weiner et al., 2010). ABA is a plant hormone which has different developmental and tissue-specific functions in seeds, such as promoting embryo maturation, synthesis of storage reserves and late embryogenesis abundant proteins (LEA), and initiation of seed dormancy. After seeding, emergence is delayed by ABA when there is a lack of water in the soil.

ABA-dependent pathway

ABA acts as a signalling molecule, inducing changes at the cellular and whole-plant levels in response to a water stress during all stages of plant development (Grill and Himmelbach, 1998; Leung and Giraudat, 1998). In addition to promoting stomatal closure to minimize water loss from transpiration, ABA also activates many stress-responsive genes that encode LEA proteins and enzymes for the biosynthesis of compatible osmolytes, which collectively increase plant tolerance to this stress (Himmelbach et al., 2003; Wang et al., 2006; Shao et al., 2007; Cattivelli et al., 2008; Mazzucotelli et al., 2008; Nakashima et al., 2009).

ABA also initiates the expression of numerous drought-related genes containing an ABA-responsive element (ABRE) or ABRE-binding protein (AREB) elements in their promoters (Shinozaki et al., 2003; Nakashima et al., 2009). However, other studies have shown that there are additional transcriptional regulatory systems that respond to drought stress independently of ABA, such as genes with drought responsive element/c-repeat binding factor (DRE/CBF) in their promoters (Agarwal et al., 2007; Chen et al., 2008; Gutha and Reddy, 2008; Xu et al., 2008; Wang and Dong, 2009; Wei et al., 2009). Consequently, there are two independent molecular pathways: ABA-dependent and ABA-independent involved in drought stress responses, and these two pathways include genes with different functions. The majority of the drought studies have shown that these differentially regulated genes fall into two categories: genes that have an important function in sensing a reduction in water availability, and genes that transduce these differentially regulated genes into signals and stimulate expression of other genes responsible for inducing the tolerance response and thus protecting plants from the stress (Seki et al., 2003; Kathiresan et al., 2006; Swindell, 2006; Shao et al., 2007; Mazzucotelli et al., 2008; Deng et al., 2009; Rodrigues et al., 2009; Lata et al., 2010; Moriwaki et al., 2010).

ABA is considered the most important component of plant responses to drought. Understanding its biosynthesis and mechanism of action is an important step toward deciphering its role in mediating drought responses (Liotenberg et al., 1999; Qin and Zeevaart, 1999; Seo et al., 2000; McCourt and Creelman, 2008; Kurahashi et al., 2009). Studies have shown that ABA is synthesized *de novo* in different tissues such as leaf, root and seed, primarily in response to drought and salinity stresses (Seki et al., 2003; Shinozaki et al., 2003). Genes encoding the majority of enzymes in ABA biosynthetic pathway in leaf and seed tissue have been identified and cloned in Arabidopsis (Liotenberg et al., 1999; Qin and Zeevaart, 1999; Seo et al., 2000; Raghavendra et al., 2010). The initial steps of the ABA biosynthesis take place in the chloroplast of the green tissue where zeaxanthin epoxidase (known as *ABA1* in Arabidopsis and *ABA2* in tobacco (Liotenberg et al., 1999)) catalyzes the epoxidation of zeaxanthin and antheraxanthin into violaxanthin and subsequently into neoxanthin (Fig. 2.1). Both violaxanthin and neoxanthin are known as 9-*cis*-epoxycarotenoids. In the following step of the ABA biosynthetic pathway, xanthoxin is cleaved from neoxanthin by the catalytic action of 9-*cis*-epoxycarotenoid dioxygenase (NCED)

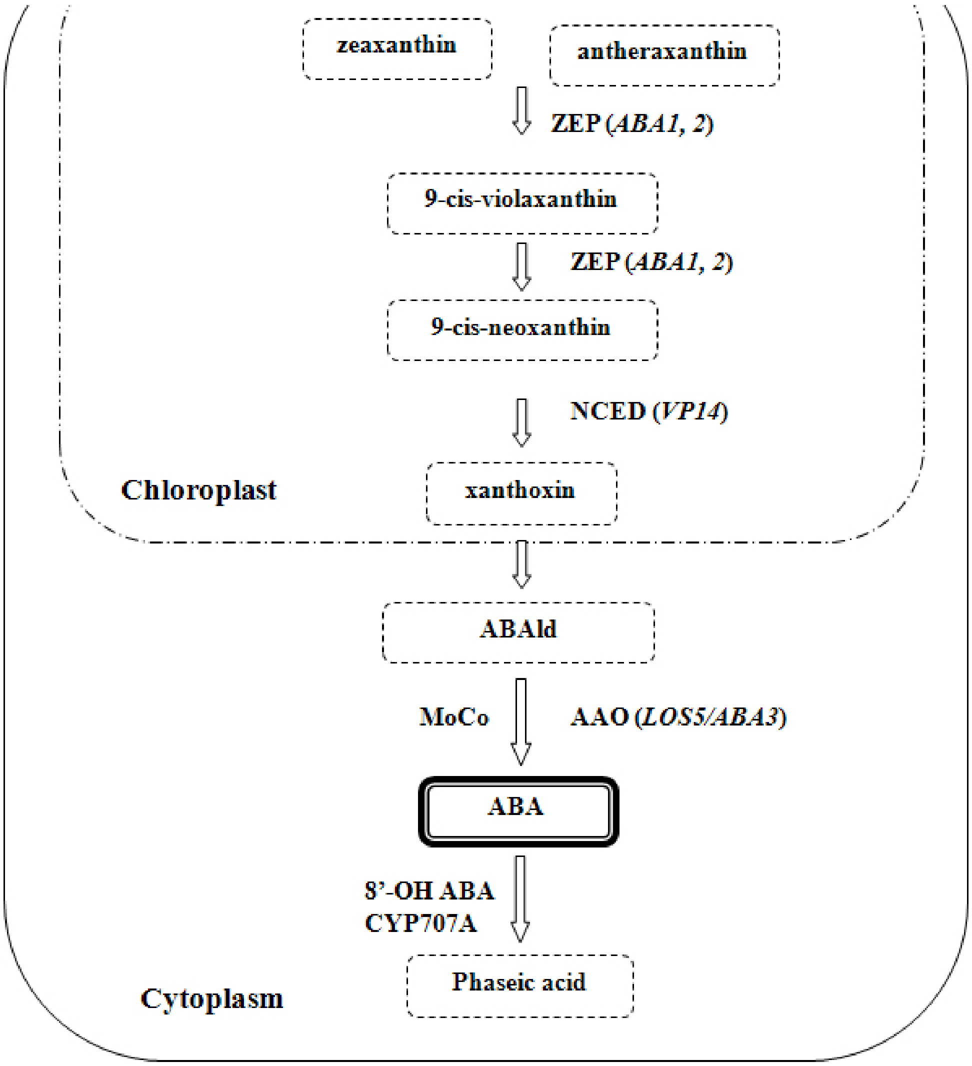

Figure 2.1. Abscisic acid (ABA) biosynthetic and catabolic pathway in plant cells (adapted from Liotenberg et al., 1999).

Zeaxanthin and antheraxanthin are reduced to 9-*cis*-epoxycarotenoids (9-cis-violaxanthin and 9-*cis*-neoxanthin) by zeaxanthin epoxydase (ZEP), known as *ABA1* in Arabidopsis and *ABA2* in tobacco. Xanthoxin is cleaved from 9-*cis*-epoxycarotenoids by the catalytic action of 9-*cis*-epoxycarotenoid dioxygenase (NCED – *VP14*) and then released from chloroplasts into the cytoplasm. Abscisic aldehyde (ABAld) is produced from xanthoxin catalyzed by short-chain dehydrogenaze/reductase (SRD – *ABA2*) and abscisic acid (ABA) is produced through ABAld by the catalytic action of abscisic aldehyde oxygenase (AAO – *LOS5/ABA3*) in presence of a molybdenum cofactor (MoCo) sulfurase). ABA is catabolized to phaseic acid through an oxidative route, with the help of ABA 8'-hydroxylation and catalyzed by the CYP707A enzyme family.

and then released from chloroplasts into the cytoplasm (Fig. 2.1). Abscisic aldehyde (ABAld) is produced from xanthoxin catalized by a short-chain dehydrogenase/reductase (SRD) enzyme encoded by the *ABA2* gene (Liotenberg et al., 1999). ABA is then produced from ABAld by the catalytic action of abscisic aldehyde oxygenase (AAO), also known as *LOS5/ABA3*, which requires the molybdenum cofactor (MoCo) sulfurase (Liotenberg et al., 1999; Weiner et al., 2010). AAO catalyzes the last step in ABA biosynthesis (Fig. 2.1) (Liotenberg et al., 1999; Himmelbach et al., 2003; Raghavendra et al., 2010; Weiner et al., 2010). Other ABA biosynthesis genes that were also upregulated in leaf or seed tissues upon osmotic stress are *ABA1, ABA3* and *AAO3* (Liotenberg et al., 1999; Seo et al., 2000; Xiong et al., 2001). Many studies have suggested that NCED is consistently upregulated in green tissues during drought stress (Ishitani et al., 1997; Qin and Zeevaart, 1999).

Many candidate ABA receptors, both cytoplasmic and plasma membrane-associated, have been identified in past decades, but their ability to bind and to regulate ABA responses has not been unequivocally demonstrated so far (Himmelbach et al., 2003; Hirayama and Shinozaki, 2007; Raghavendra et al., 2010; Weiner et al., 2010). Several groups have identified potential candidate receptor genes for ABA, including the G protein-coupled receptors 1 and 2 (GCR1/2) which are signalling GTP binding proteins (Finkelstein et al., 2002; Eckardt, 2004), or the leucine-rich repeat receptor-like kinase 1 (RPK1), which is also present in the plasma membrane (Osakabe et al., 2005). Subsequent studies (Gao et al., 2009) have challenged the identification of GCR1/2 as ABA receptors; however, the current understanding suggests that GCR2 is in fact an ABA receptor and that two other proteins, the RNA-binding flowering time control protein A (FCA) and the genome uncoupled 5 (GUN5) proteins also function as ABA receptors (McCourt and Creelman, 2008).

Recent crystallographic studies showed that other proteins must be considered as ABA receptors as they form a multi-protein complex capable of sequestering ABA (Fig. 2.2 A) (Fujii et al., 2009; Ma et al., 2009; Melcher et al., 2009; Park et al., 2009). Park at al. (2009) identified the pyrabactin resistance 1 (*PYR1*) and PYR1-like (*PYL*s) genes in a chemical genetic screen using pyrabactin, a selective ABA agonist that inhibits only some of the pathways that are regulated by ABA. *PYR1* encodes one of the 14 members of StAR related lipid transfer (START) proteins which share a conserved hydrophobic ligand-binding pocket in Arabidopsis. The other members of START proteins have different functions unrelated to ABA. Triple and quadruple *pyr1* and *pyl* mutants are insensitive to ABA *in vitro*, but expression of both *PYR1* and one of the PYLs (*PYL4*) revert the phenotype. These results suggest that PYR1 and PYLs are functionally redundant and mediate multiple ABA responses *in vivo* (Park et al., 2009). The group further proposed that *PYR1* and *PYL*s are at the apex of a negative regulatory pathway that controls ABA signaling by inhibiting type 2C protein phosphatases (PP2Cs) activity. Ma et al. (2009) independently identified the same 14 member protein family, and named it regulatory component of ABA receptor (RCAR), in a yeast two-hybrid screen for plant proteins that interact with

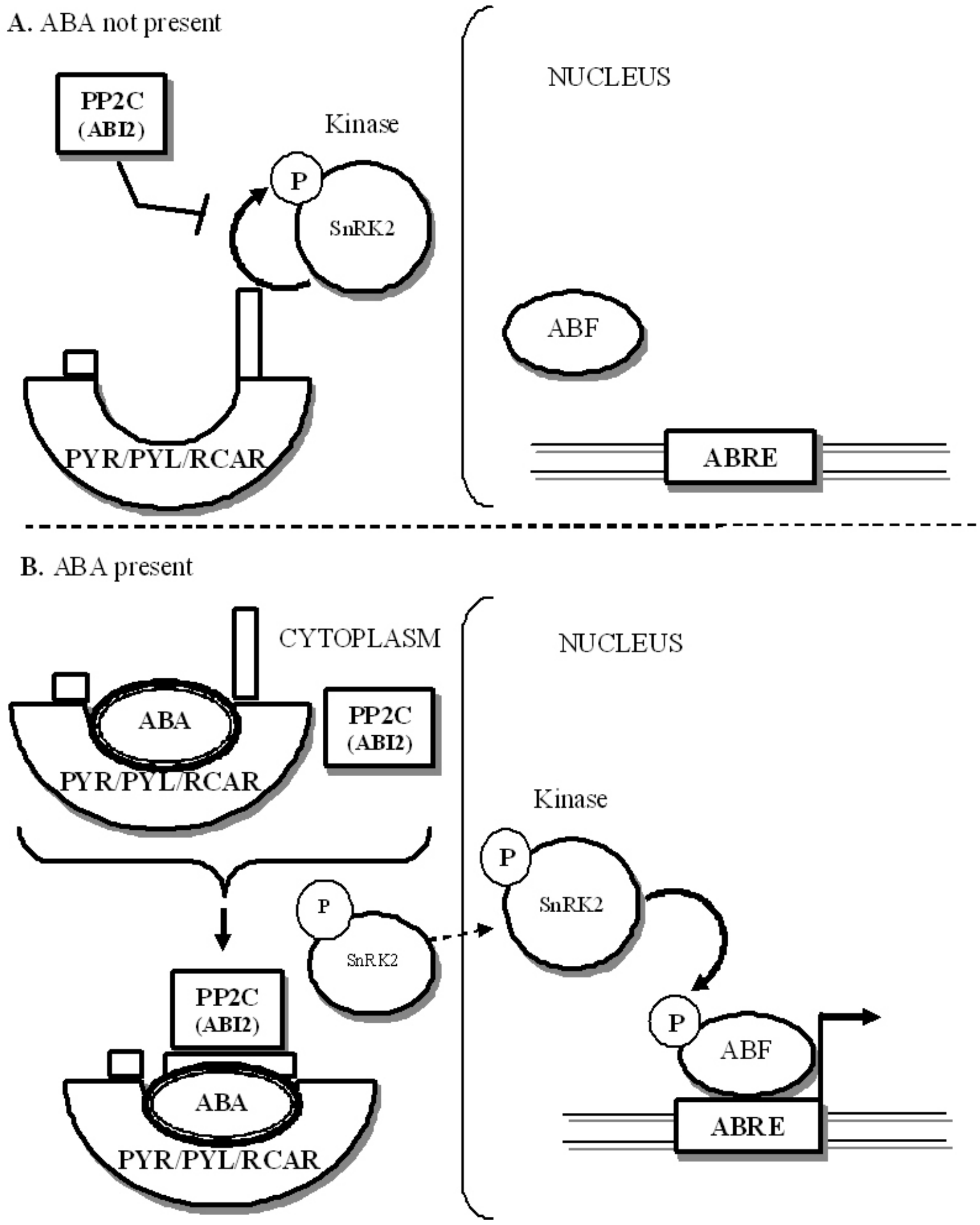

Figure 2.2. Abscisic acid (ABA) signalling pathway (adapted from Sheard and Zheng, 2009).

A) When ABA is absent, the phosphatase PP2C inhibits autophosphorylation of the SnRK2 kinases, thus impairing phosphorylation of the ABF transcription factor. B**)** When ABA is present and binds to the receptor, it enables the PYR/PYL/RCAR receptor protein to bind PP2C. The kinase is not inhibited and is autophosphorylated and subsequently activates ABF to initiate transcription of genes having an ABA-responsive promoter element (ABRE).

ABI2. ABI2 is one of the nine redundant type 2C protein phosphatases (PP2Cs) and negatively regulate ABA signalling. RCAR1 and related proteins bind ABA and block the phosphatase activity of PP2Cs in the presence of ABA in the cytoplasm (Fig. 2.2 B). Detailed crystallographic studies on the composition and function of ABA receptors as well as other studies that confirmed this discovery (Melcher et al., 2009; Peterson et al., 2010; Raghavendra et al., 2010; Weiner et al., 2010; Santiago et al., 2012) validate the identity of the ABA receptor. These results were recognized unanimously by the research community. Due to the requirement to form a heteromeric receptor complex, it was proposed and agreed that PYR/PYL/RCAR and PP2C be considered co-receptors rather than independent receptors (Peterson et al., 2010; Raghavendra et al., 2010; Weiner et al., 2010).

In the cytoplasm, in absence of ABA, a PP2C phosphatase inhibits the autophosphorylation of a sucrose non-fermenting 1 (SNF1)-related protein kinase 2 (SnRK2), which is responsible for the downstream activation of the ABA-responsive element binding factor (ABF) to initiate transcription of ABA-dependent genes. ABFs are transcription factors of the basic-region leucine zipper (bZIP) family (Choi et al., 2000; Uno et al., 2000). In the presence of ABA, the heteromeric PYR/PYL/RCAR co-receptor complex binds ABA and this complex enables binding and sequestering of PP2C phosphatase to form a heteromeric complex which no longer impairs the autophosphorylation of the SnRK2 kinase. Subsequently, the kinase migrates to the nucleus where it phosphorylates and activates ABF to initiate transcription of genes exhibiting the presence of *cis*-acting ABRE domain through the ABA-dependent pathway.

Many of the components of the ABA-dependent gene expression pathway are known (Fig. 2.3). ABRE is a conserved eight nucleotide *cis*-acting element (C/TACGTGGC) present in ABA-dependent transcriptional regulators promoter regions. This *cis*-acting element was first identified in the wheat seed-specific *Em* gene, which is a member of a gene family encoding the Group 1 late embryogenesis abundant (LEA) proteins (Paradkar and Marcotte, 2001), and in the *RAB16* gene from rice, which is expressed in both vegetative tissues and seeds (Mundy et al., 1990). Different studies have shown that a single copy of ABRE is not sufficient for ABA-dependent gene transcription as a minimum of two copies of the motif are needed for the expression of Arabidopsis *RD29B* in seeds and in vegetative tissue (Shen and Ho, 1995; Shen et al., 1996; Uno et al., 2000). ABRE was also identified in association with coupling elements (CE) CE1 and CE3, thus forming ABA-dependent complexes in the regulation of wheat *HVA1* and *HVA22* genes, respectively. Studies have shown that short periods of dehydration or addition of exogenous ABA triggered overexpression of ABA-response element binding (AREB), *AREB1/ABF2, AREB2/ABF4*, and *ABF3* (Seki et al., 2003; Kurahashi et al., 2009; Nakashima et al., 2009; Weiner et al., 2010). Upon sequencing and gene annotation of the Arabidopsis genome, 75 bZIP transcription factors have been identified, of which 13 could be represented by members of the AREB protein family (Bensmihen et al., 2002; Jakoby et al., 2002; Nakashima et al., 2009). Most of the AREB proteins are involved in ABA-responsive signal

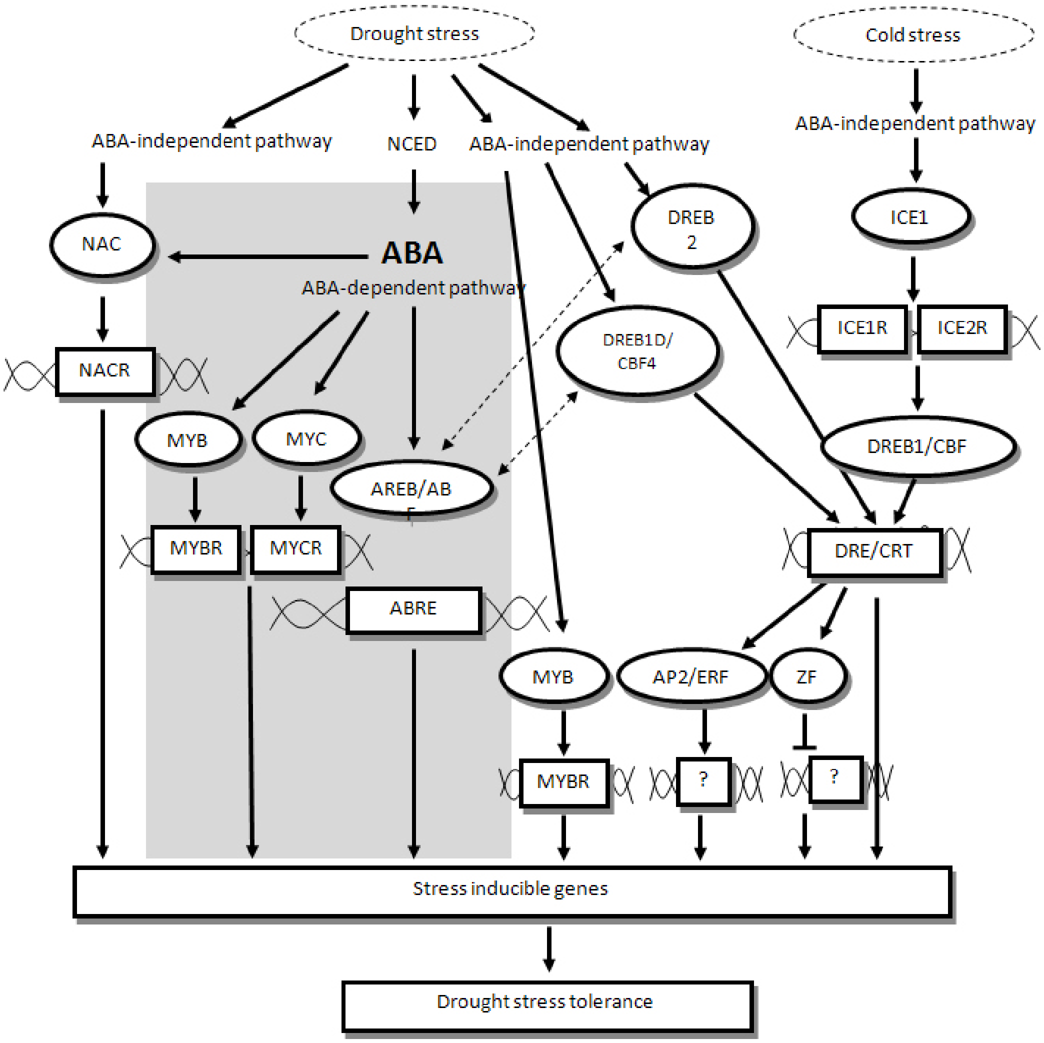

Figure 2.3. ABA-dependent and ABA-independent gene expression pathways (adapted from Seki et al., 2003).
Transcriptional factors involved in controlling stress-inducible genes are presented in oval cases and the *cis*-acting elements are shown in the boxes with double-helix strands. The ABA-dependent pathway is emphasized by the grey box. The interrupted arrows show the cross talk between drought and cold stress genes.

transduction pathways in both vegetative tissue and seed (Choi et al., 2000; Finkelstein and Lynch, 2000; Lopez-Molina and Chua, 2000). However, *AREB1/ABF2, AREB2/ABF4* and *ABF3* from Arabidopsis are family members expressed in vegetative tissues (Choi et al., 2000; Uno et al., 2000), while *ABI5* and *EEL* were mostly expressed in seeds at different developmental stages (e.g. maturation and germination) (Finkelstein and Lynch, 2000; Bensmihen et al., 2002; Nakashima et al., 2009). Homologous factors of AREB/ABFs in rice, *TRAB1*, and barley, *HvABI5*, are also specifically expressed in seed (Hobo et al., 1999; Casaretto and Ho, 2003; Tondelli et al., 2006).

Other transcriptional regulators of the ABA-dependent pathway include some SnRK2-type kinases such as AAPK (ABA activated protein kinase) and OST1/SRK2E, which have been shown to mediate regulation of stomatal aperture (Baena-González and Sheen, 2008; Raghavendra et al., 2010; Weiner et al., 2010). Additional studies have shown that 9 out of the 10 SnRK2s kinases in Arabidopsis are activated by hyperosmolarity, while only 5 are activated by ABA (Boudsocq et al., 2004; Nakashima et al., 2009). This family of kinases is responsible for activating downstream ABFs, initiating transcription at the ABRE *cis*-acting elements.

ABA also mediates the expression of the dehydration inducible *RD22* gene. MYB and MYC *cis*-acting element have also been identified in the promoter of *RD22* gene (Abe et al., 1997). A MYC transcription factor, AtMYC2 (also known as *rd22BP1*), and a MYB transcription factor, AtMYB2, bind these elements in the promoter of *RD22* (Abe et al., 2003). The synthesis of MYBs was observed to take place after the accumulation of ABA, which suggested that they play a role in later stages of the plant's response to drought stress (Abe et al., 2003; Tondelli et al., 2006).

ABA is a very important component of plant response to drought stress and most of the enzymes involved in its biosynthetic pathway are differentially regulated during drought stress (Liotenberg et al., 1999; Qin and Zeevaart, 1999; Seo et al., 2000; Xiong et al., 2001; Welsch et al., 2008). Identification and characterization of the cytoplasmic ABA co-receptors, PYR/PYL/RCAR and PP2C, is considered the major discovery that led to the understanding of the ABA signaling pathway in plants as it revealed some of the missing pieces in the ABA-dependent pathway between ABA and ABRE *cis*-acting element (Fig. 2.2). Complete knowledge and characterization of the transcriptional regulators in the ABA-dependent pathway is essential for the understanding of the plant response mechanism to drought stress and subsequently the development of crops with improved tolerance to drought.

ABA-independent pathway

Plants have other transcriptional regulators that are differentially expressed during drought and act independently of ABA. This pathway commonly known as the ABA-independent pathway is also very important in plants response to drought stress. The majority of these differentially regulated genes are specific to the plant response to other abiotic stresses such as cold, freezing, high salinity, as well as biotic stresses such as insects and pathogens

(Fig. 2.3) (Shinozaki et al., 2003; Agarwal et al., 2007; Seki et al., 2007; Chen et al., 2008; Mazzucotelli et al., 2008; Xu et al., 2008; Gao et al., 2009; Medina et al., 2011).

The most abundant gene family in the ABA-independent pathway is the drought responsive element/c-repeat binding factor (DREB/CBF), which has been studied extensively in different plant species including Arabidopsis, wheat and barley (Ozturk et al., 2002; Seki et al., 2003; Swindell, 2006; Agarwal et al., 2007; Chen et al., 2008; Kobayashi et al., 2008; Xu et al., 2008; Wei et al., 2009; Medina et al., 2011; Tondelli et al., 2011). There are two types of DREB proteins, DREB1 and DREB2, which have different roles (Seki et al., 2003; Wang et al., 2006; Gutha and Reddy, 2008; Xu et al., 2008; Nakashima et al., 2009; Nayak et al., 2009; Wang and Dong, 2009; Medina et al., 2011). Initially, three genes were identified that encoded DREB1/CBFs: *DREB1B/CBF1*, *DREB1A/CBF3* and *DREB1C/CBF2*, as well as two genes encoding DREB2 proteins: *DREB2A* and *DREB2B*, which were found initially in Arabidopsis (Seki et al., 2003; Furihata et al., 2006). Exhaustive studies have shown that the expression of type 1 *DREB, DREB1/CBF* is induced by cold (Fig. 2.3), and not by dehydration or salinity stresses (Shinwari et al., 1998; Lopez-Molina and Chua, 2000). On the other hand, *DREB2* gene expression is driven by dehydration (Fig. 2.3) and salinity and is nonresponsive to cold stress (Liu et al., 1998; Shen et al., 2003). However, two groups, Haake et al. (2002) and Magome et al. (2004) have identified new *DREB1* genes and shown that the expression of one of them, *DREB1D/CBF4* (Fig. 2.3), is induced by osmotic stress, while two other genes, *DREB1F/DDF1* and

DREB1E/DDF2, are induced by a salinity stress (Haake et al., 2002; Magome et al., 2004). These studies suggested that there is crosstalk between the two types of DREBs pathways. It is known that the DRE *cis*-acting element is a 9-bp conserved sequence, TACCGACAT, essential for regulating *RD29A* induction in the ABA-independent response to dehydration and cold (Fig. 2.3). This *cis*-acting element is also found in the promoters of many other drought- and cold-responsive genes (Yamaguchi-Shinozaki and Shinozaki, 1994; Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000).

Other *cis*-acting elements, namely C-repeat (CRT), containing an A/GCCGAC motif that forms the core of DRE, and low temperature responsive element (LTRE), regulate cold-inducible promoters in an ABA-independent manner (Jiang et al., 1996). Studies have shown that DRE/CRT functions in response to abiotic stresses in many plant species, and were reported in tobacco (Yamaguchi-Shinozaki and Shinozaki, 1994; Kobayashi et al., 2008), wheat (Shen et al., 2003; Wang et al., 2006; Xu et al., 2008; Wei et al., 2009), rapeseed (Jiang et al., 1996; Park et al., 2005), rice (Dubouzet et al., 2003; Chen et al., 2008; Gutha and Reddy, 2008; Gao et al., 2009), barley (Ozturk et al., 2002; Tondelli et al., 2006), maize (Qin et al., 2004; Wang and Dong, 2009), and tomato (Hsieh et al., 2002a, 2002b). In rice, five DREBs have been isolated, four DREB1/CBF, *OsDREB1A, B, C, D* and one *DREB2* homologous gene, *OsDREB2A* (Dubouzet et al., 2003). In maize, one homologous DREB1/CBF protein, ZmDREB1A was shown to be produced at relatively high levels during drought and salinity stresses (Qin et al., 2004). These studies demonstrated that a similar regulatory system has been conserved from monocots to dicots when plants respond to external factors, either abiotic or biotic. However, some transcription factors such as the inducer of CBF expression 1 (ICE1), were identified to function upstream of DREB1/CBF in Arabidopsis (Fig. 2.3) and are differentially regulated during a drought stress. ICE1 regulates the expression of *DREB1A/CBF3*, but not *DREB1C/CBF2*, indicating that different mechanisms are regulating the expression of the different types of DREB proteins (Shinwari et al., 1998; Chinnusamy et al., 2003; Zarka et al., 2003).

It has been clearly demonstrated that DREB/CBFs play a major role in the ABA-independent gene expression pathway and they are present and differentially regulated during drought stress in a majority of plants, both monocots and dicots. Some studies have suggested the presence of ABA-independent transcriptional regulators in the dehydration response, which is independent of the DRE/CRT regulon (Nakashima et al., 1997; Mohammadi et al., 2007; Suprunova et al., 2007). One example is the early response to dehydration (ERD1), which encodes a nuclear ATP-dependent chloroplastic protease regulatory subunit. Also, other *cis*-acting elements such as a MYC-like binding protein, containing a conserved CATGTG motif, were also identified in the promoters of the all three overexpressed genes - *ANAC019*, *ANAC055*, and *ANAC072* - in Arabidopsis during dehydration stress (Tran et al., 2004).

Even if there are other genes differentially expressed during drought in an ABA-independent manner, the DREB/CBF gene family appears to be the major regulator of the ABA-independent pathway and has been observed in most plant species (Seki et al., 2003; Furihata et al., 2006). The conservation of this pathway among all plant species demonstrates the broad existence of cross talk between transcriptional regulators and the complexity of the plant response to drought stress.

Many genes are differentially expressed simultaneously during different stresses, and the activity of some gene products is influenced by other genes that act as activators or repressors, creating an interconnected network among gene families, considered gene crosstalk (Yamaguchi-Shinozaki and Shinozaki, 2006; Seki et al., 2007; Shao et al., 2007; Nakashima et al., 2009). Studies have shown that during drought stress, genes from both the ABA-dependent and ABA-independent pathways were differentially regulated at the same time (Seki et al., 2003; Shinozaki et al., 2003; Tondelli et al., 2006; Mazzucotelli et al., 2008; Nakashima et al., 2009; Ashraf, 2010). This could also be due in part to the fact that it is often hard to separate the stresses from each other; cold is often associated with freezing and subsequent dehydration stress. To further complicate the matter, plants exposed to stress become more vulnerable to diseases. Thus, it is difficult to assess the impact of gene crosstalk in plant defense reactions, since upregulation of some gene products with protective action against one stress could be repressors of other genes with protective roles against another stress. For example, ABA represses the production of other hormones such as jasmonic acid, which is responsible for plant response to diseases (Memelink, 2009). More studies are needed to define the balance in which dehydration stress response does not interfere with other stress responses.

An important characteristic of genes from the two pathways is the presence of common motifs, such as the ABRE and the DRE/CRT *cis*-acting elements, in the promoters of differentially expressed genes in the majority of tissues and plant species (Fig. 2.3). Each of these two elements have different functions, as one is directly dependent on ABA accumulation during a dehydration stress and the other one works independently of ABA as an important element in cold-inducible gene expression as well as in response to dehydration and salinity stresses. Studies have shown that the level of expression of the same genes in response to stress vary significantly based on the plants developmental stage. For example, a higher number of ABA-independent genes were differentially expressed during stress at seedling and tillering stages compared to later stages of development (Shinozaki and Yamaguchi-Shinozaki, 2000; Yamaguchi-Shinozaki and Shinozaki, 2006; Chen et al., 2008; Gutha and Reddy, 2008; Xu et al., 2008; Nakashima et al., 2009; Wang and Dong, 2009) while studies on tissues from later developmental stages such as flowering and grain filling, included more ABA-dependent genes differentially expressed in the plant response to drought stress (Finkelstein and Lynch, 2000; Abe et al., 2003; Furihata et al., 2006; Gao et al., 2009; Weiner et al., 2010). These studies could really help in shaping a general understanding of the mode of action in plant responses to drought at the molecular level, which could be very important in developing crop species with better tolerance to drought. However, efforts are needed to identify and understand other factors that could be influenced positively or negatively by these two major factors presented above, ABRE and

DREB/CBF, to better understand the plant response to drought stress during the entire life cycle.

2.4. Conclusions

Drought stress is well recognized as one of the most destructive abiotic stresses in plants, with a very negative economic impact depending on the severity, location, and duration of the drought event. Thus, efforts to predict and mitigate the impact of drought on agronomical important crops are in place, based on ongoing research in both crop and model plant systems.

All plants respond to drought stress, but only few species have effective mechanisms that will allow them to be somewhat tolerant to such stress. Most of the plants that have a very effective mechanism against drought stress, such as resurrection plants or cacti, respond to drought stress using a survival mechanism in which their most common response is a reduction in growth and development processes. Therefore, studies on their response mechanism, although interesting, are not valuable for crop improvement. Research groups are now focussing more on crop plants with a good tolerance to drought. This may lead to an understanding of the mechanism of response, which will allow for the improvement of crop species to maintain yield under drought conditions. Current studies have shown strong sequence homologies and similar functions of many transcriptional regulators among different plant species from Arabidopsis to wheat. However, the fact that most crop species have large genomes and thus a high number of genes makes this task very difficult. Moreover, information from model plants may insufficiently represent the genes or gene families involved in the response mechanism of a more complex plant such as wheat or triticale (Akhunov et al., 2007). Therefore, there is a need for more studies at the molecular level involving crop species such as triticale or rye, which have good tolerance to drought and could reveal novel genes involved in the response mechanism.

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Chapter 3. A system to study water stress in triticale seedlings

3.1. Introduction

Drought is among the most devastating abiotic stresses in plants, and studies on drought go back a century. Early empirical studies mainly focused on comparing crop yields between drought and wet years, or dry and wet areas. However, a study at University of Wyoming in the early 1920s, in a semi-arid area, proposed several solutions that farmers could adopt to maintain sustainable operations in areas with low precipitations (Luebke, 1980). These included deep-ploughing and utilizing crops such as barley, rye, and sorghum, which exhibited better resistance to drought. These studies were deceiving because during these experiments, the region was experiencing a cycle of several wet years. Thus, the plants were not exposed to severe drought conditions. Severe droughts occurred several years after the farmers adopted these solutions, and were catastrophic to these farmers and their communities (Luebke, 1980).

Until the late 1970s, the majority of drought studies were field experiments, which were weather-dependent and very costly due to the high number of trained personnel and specialized infrastructure (e.g. rain shelters, soil probes) needed for the experiments. Moreover, this type of experiment required a high number of precise observations at variable time intervals (reviewed in Liu et al., 2006; Xue et al., 2006; Kamoshita et al., 2008). A typical project generated several data sets on yield, morphological characteristics, growth, and development of different genotypes (reviewed in Cattivelli et al., 2008; Ergen et al., 2009). The evaluation and classification of the drought resistance of different crops (wheat, corn, sorghum, rice, and potato) was based on a global drought index derived from several measurements, including drought coefficient and drought index of yield. Unlike field conditions where the trials were grown, the measurements were very accurate and provided a wide range of results that could be compared among experiments (reviewed in Cattivelli et al., 2008; Ergen et al., 2009). These studies were highly important in characterizing the physiology of plants in response to drought. However, they introduced errors, leading to misidentification of genes apparently critical in explaining plant drought tolerance traits in plants (reviewed in Cattivelli et al., 2008; Ergen et al., 2009).

Later on, drought studies were moved into greenhouses and growth cabinets, which provided a more defined and controlled growth environment for physiological studies, required far less manpower, and accelerated experiment timelines (Ramalingam et al., 2006; Swindell, 2006; Chen et al., 2008; Nakashima et al., 2009).

Recently, new transcriptome profiling methods were developed to identify any changes in gene expression (Ansorge, 2009; Hurd and Nelson, 2009; Mane et al., 2009; Morozova et al., 2009). These methods can be applied to study plant responses against abiotic stress (Papdi et al., 2009; Varshney et al., 2009; Franssen et al., 2011). Molecular methods require accurate techniques to induce and isolate each stress individually for a more precise identification of the specific genes involved in the plant response to stress (Shinozaki et al., 2003; Seki et al., 2007).

A variety of methods to study drought stress have been used, most of which are based on reducing or withholding the plant's water supply. Xue et al. (2006) used silica gel-based artificial soil to identify genes associated with the plant response to a drought stress in wheat. Tardif et al. (2007) grew wheat using vermiculite and ProMix to identify genes involved in the response to a severe drought. In drought studies by both Subramanyam et al. (2006) and Mohammadi et al. (2007) TurfaceMVP, a porous ceramic soil conditioner used in horticulture was used as physical support. All these substrates can be sterilized and reused without the fear of carrying contaminants such as diseases or pests. Mixtures of soil, sand, and peat do not have this advantage, but have been used by many groups (De Leonardis et al., 2007; Karaba et al., 2007; Vendruscolo et al., 2007), with the major advantage being that it more accurately reflects the composition of natural soil environments. These methods in which the water supply is interrupted have advantages such as control over the amount of water supplied or available, time of application, and avoidance of the plant removal from the soil. A major disadvantage is the cost of the experiment because these types of experiments require a greenhouse with controlled conditions and a controllable water system. Furthermore, the issue of uneven evaporation among pots is not accounted for. Other limitations are interference with other abiotic and biotic stresses that could lead to misidentification of genes related to dehydration stress. Braam and Davis (1990), showed that Arabidopsis plants activated a set of genes named Touch genes (*TCH 1 - 4*) that were overexpressed in response to wind, handling or wounding. Later Johnson et al. (1998) and Lee et al. (2005) confirmed these results as well as reporting that additional genes were also differentially regulated during plant handling or following their exposure to wind.

Osmotic agents such as polyethylene glycol (PEG) or mannitol are also used in drought stress studies (Liu et al., 2007; Mohammadi et al., 2007; Qiu and Yu, 2009; Comeau et al., 2010; Lata et al., 2010). They act by binding water molecules, thus reducing the available water to the plants. PEG has a number of properties that make it particularly useful for biological experiments: (a) it is non-toxic and does not interfere with cellular function, (b) it is highly hydrophilic, which decreases aggregation of proteins and other biomolecules and increases their solubility, and (c) it is available in molecular weights too large to be assimilated by plant cells. Early studies with osmotic agents were based on liquid media that contained different concentrations of osmoticum, and plants initially grown in solid substrate were transferred into this media for a pre-determined period of time (h to days). One of the advantages of using osmotic agents is that they can be measured very accurately by determining their osmotic potentials (ψ) and experimental conditions are easily reproducible.

Michel and Kaufmann (1973) first observed that when using PEG as an osmotic agent, it is important to identify its ψ. They reported a distinctly curvilinear relationship between ψ and concentration of PEG, where an increase in concentration resulted in more negative values of ψ, regardless of the measuring technique used (Steuter et al., 1981; Michel, 1983). Steuter et al. (1981) also observed that the relationship between ψ and PEG was almost identical for PEG 6,000 and PEG 20,000 and came to the conclusion that long and short chains have a similar effect on ψ at equal solution concentration.

Using solidified PEG media could offer several advantages such as accurate measurements and removal of handling plants from one substrate to another. These solidified PEG media were considered very difficult to prepare and previous studies showed that it was not possible to use PEG in solid media above 14% concentration. Furthermore, this was only achieved with low molecular PEG which can create phytotoxicity (Attree, 2003). Comeau et al. (2010) published a new medium formula that allowed utilization of high molecular weight PEG in solid media up to 34% concentration. They have found the best mineral composition that kept the media solidified was based on reduced cations and calcium salts (Comeau et al., 2010). They used an isopiestic method to calculate ψ for the most concentrated solution (34% PEG) of PEG 35,000 and obtained a value of -1.5 MPa at 22ºC. This concentration greatly reduced development of different cereals and other crops (Comeau et al., 2010).

Previous studies showed a curvilinear relationship between ψ and PEG concentration in which weaker concentrations have a smaller ψ (Fig. 3.1) (Michel and Kaufmann, 1973; Steuter et al., 1981; Michel, 1983).

Another important aspect of studying drought is the plant developmental stage at which the stress is occurring, since not all phases of growth are equally affected by water deficit. There are different arguments about which stage of development a plant is the most susceptible to drought. Droughts during seedling,

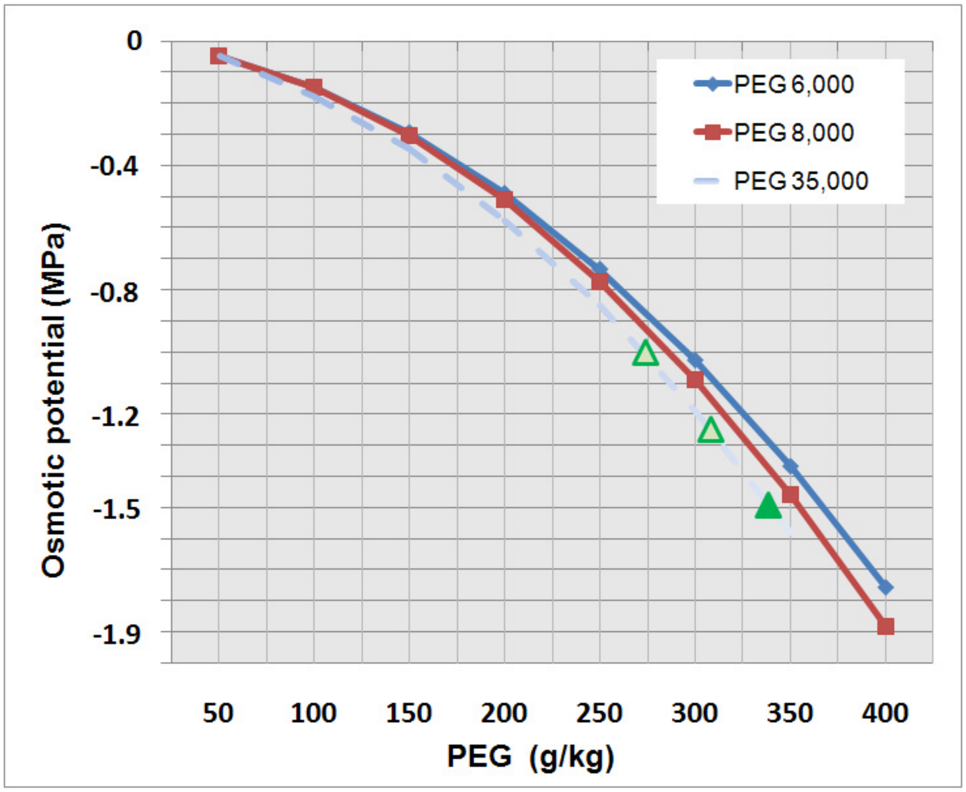


Figure 3.1. Osmotic potential (ψ) as a function of increasing concentrations of three different molecular weight PEG (modified from Michel and Kaufmann, 1973 and Michel, 1983)**.**

The solid green triangle represents the ψ value of PEG 35,000 at 34% as reported by Comeau et al. (2010). The empty green triangles are predicted values for 27%, 31% based on the predicted curve for PEG 35,000.

milking or grain filling stages have all been shown to severely affect crop yields (Mohammadi et al., 2007). Even a small reduction in ψ of the cell has marked effects in some cell components, such as the cell wall. The development and enlargement of cells terminates earlier and structural differentiation processes are completed earlier (Jones, 2007; Mohammadi et al., 2007; Deng et al., 2009; Hura et al., 2009a and 2009b; Moriwaki et al., 2010). When internal water deficiency occurs in a plant, water often moves from different organs towards the meristematic tissue, thus having an indirect effect on tissues that are not directly affected by lack of water. Moreover, the reallocation of plant resources to respond to drought weakens the plant and makes it more vulnerable to stresses such as diseases and pests (Swindell, 2006; Liu et al., 2007; Memelink, 2009). Knowledge of the response mechanism to water shortage in a plant at the seedling stage is critical and should be considered the initial step in drought studies for any crop species.

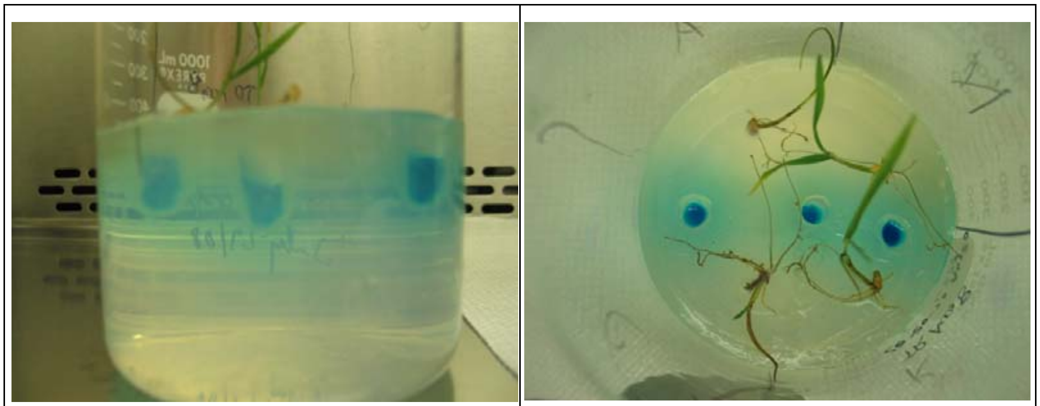
The hypothesis of this study is that using solid PEG medium as an osmotic agent will help develop a highly reliable and reproducible system to study different levels of dehydration stress in triticale and cereals in general. The method takes advantage of the solid PEG media developed by Comeau et al. (2010), and plant culture in vessels under sterile environment in growth chambers with controllable conditions. This system offers the ability to isolate dehydration stress from other stresses, allowing identification of genes specific to the stress.

3.2. Materials and methods

Triticale (x *Triticosecale* Wittm.) is a man-made plant resulting from the hybridization of wheat (*Triticum* ssp.) and rye (*Secale cereale*) and was selected as the plant of choice for this experiment. There are several reasons triticale was chosen as the candidate in this dehydration study over its diploid parent rye, which is known for its good tolerance to abiotic stresses, including dehydration. The first factor was the fact that not all rye genes are expressed in the triticale. Moreover, triticale has not only good tolerance to dehydration stress, but also has superior grain and biomass yields when compared to wheat. Despite its large genome, these facts make triticale the perfect candidate for a biorefinery crop and justify the current effort to identify candidate genes that will help in improving cereals with dehydration tolerance while maintaining or increasing grain and biomass yields.

The cultivar used for this experiment was AC Certa, which is a spring hexaploid variety registered in 1995, exhibiting good yield, good drought tolerance and excellent disease resistance. The experiment consisted of four treatments: a control 0% PEG, and three PEG concentrations at 27%, 31% and 34% PEG imposing a decreasing exponential ψ. The PEG medium was prepared using two separate solutions that were mixed upon preparation. One solution contained the very high molecular weight PEG 35,000 (Sigma, St-Louis, Mo, USA, P-2263), $MgSO_4$, KCl, and iota carrageenan (FMC, GP379). The other solution contained Gelrite gellan gum (Sigma, St-Louis, Mo, USA, G1910). Both solutions were autoclaved prior to mixing (under sterile conditions) at high temperature to avoid clogs. A complete description of the method is presented in Comeau et al. (2010).

Triticale seeds were sterilized for 3 min on a stirring plate in 80% ethanol, followed by a solution of 50% commercial bleach (Javex Professional Bleach, 6% sodium hypochlorite) for 15 min twice, and rinsed five times for 3 min each time with autoclaved deionized water (Martinez and Wang, 2009). The sterilized seeds were pre-germinated on moist sterilized Whatman no. 1 filter paper for three days in the dark at room temperature (approx. 22°C). In the initial experiment, four pre-germinated seeds were placed in sterile 1 L beakers on the surface of 500 ml of solid media supplemented with four PEG concentrations that were kept in the growth chamber approximately 30 cm beneath Sylvania Gro-Lux Wide Spectrum lamps (40 watts) delivering 80 μM m^{-2} s^{-1} of light (16 h light period) at 18°C. The media was supplemented after 15 days with a sterile 2% nutritive solution 20-20-20 (Nitrogen-Phosphorus-Potassium (NPK), 2 ml per beaker. Holes were cut with a sterile 1 ml pipette tip in the media to let the solution slowly diffuse as presented in Figure 3.2. This nutritive solution would compensate for any lack of macro-nutrients from the initial PEG solution. The beakers were covered with a plastic film to maintain a sterile environment. The experiment was repeated four times for each treatment and the results were averaged by pooling the measurements from 16 plants. Four parameters were measured: the number of leaves; the length of the shoot (cm), which consisted in the seedling parts above the crown; the length of the main root (cm); and the total plant length (cm), which was calculated as the sum of the shoot and root lengths. Observations were taken



Side view

Upper view

Figure 3.2. Application of 20-20-20 nutrient solution on artificial media containing PEG 35,000.
This solution was applied in the first trial after 15 days, when the seedlings were kept on the media for 30 days to supplement the nutrients from the media and reduce the likeliness of inducing another stress other than dehydration. The holes were made using sterile 1 ml pipette tips.

at 21 and 30 days, respectively. The measurements for all the studied parameters were carried out without disturbing the plants, which is another important advantage of this method.

Based on the results from the initial experiment, the duration for the second experiment used for the gene expression analysis was reduced to 21 days. The later experiment was carried out in Magenta boxes, using 4 plants in 50 ml of media per box, under the same conditions as the first experiment. Sixteen replicates were used for each treatment.

Statistical analyses were performed using ANOVA in XLSTAT 2011, a data analysis software package from Microsoft Excel. Results were significantly different when the P value was ≤ 0.05.

Based on the equation of the curvilinear relationship between ψ and PEG 6,000 and 8,000 concentrations and the ψ of 34% PEG 35,000 (Comeau et al., 2010), a similar curve was generated for PEG 35,000 and used to predict the ψ values for 27% and 31% PEG 35,000 solid media (Fig. 3.1).

3.3. Results of PEG-mediated water stress experiment

Solid media supplemented with different concentrations of high molecular PEG (MW 35,000) to induce different levels of dehydration stress were used. The ψ value of -1.5 MPa for the 34% PEG 35,000 medium is shown in comparison with values for PEG 6,000 and PEG 8,000 and predicted values of -1.0 and -1.2 MPa for 27% and 31% PEG 35,000 (Fig. 3.1). Two studies were carried out: a 30 day preliminary study to identify plant development as a response to different levels of ψ and second experiment reduced to 21 days based on the preliminary results.

Measurements during seedling development in the preliminary experiment were recorded at two time intervals, 21 and 30 days. The seedlings and the artificial media were disease- and microbe-free and seedling development was only affected by the levels of dehydration stress, caused by the different PEG concentrations in the media.

Statistical analyses of the studied parameters at 21 and 30 days revealed significant differences in plant development during the experiment between control plants and the ones grown on different PEG concentrations.

The number of leaves was negatively influenced by the increasing levels of dehydration stress. At 21 days, the plants grown on 34% PEG had significantly fewer leaves than the control and 27% and 31% PEG (Fig. 3.3). After 30 days on the media, only an increase in the number of leaves for the control was statistically validated. Additionally, the plants from the 34% PEG media showed chlorotic, wilted and dying leaves.

Root length was affected by the dehydration stress and the reduction was PEG concentration-dependent. After 21 days on the media, there was a clear difference between the lengths of the roots of the PEG-treated plants as plants were separated in five different statistical groups (E to J) (Fig. 3.3). The 34% PEG treatment was so severe that the roots only developed 50% as compared to 31% PEG and 33% of the ones grown on 27% PEG. After 30 days on the media, there

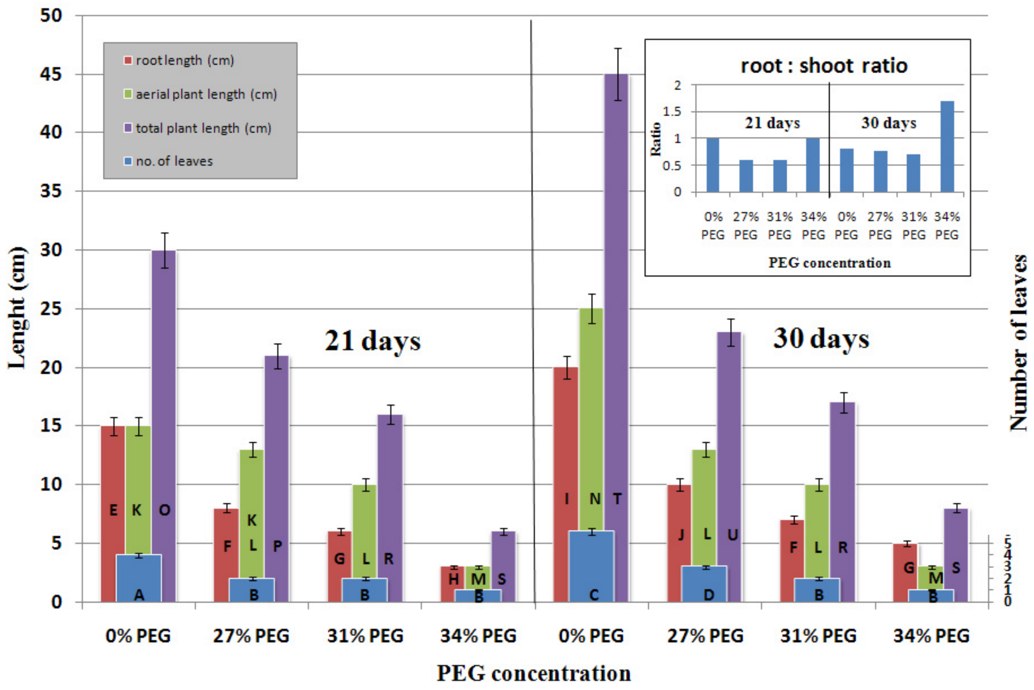


Figure 3.3. Development of triticale seedlings at different time intervals during the drought experiment.
During the experiment measurements were taken for the number of leaves, root length (cm), the aerial plant length (cm) and the total plant length (cm). The standard error (shown as the bars above each column) is very low for all data sets, which demonstrate the high reproducibility of the experiment. In the graph inset (in blue), the root : shoot ratio is presented to show growth differences between two tissues following PEG 35,000 treatments. The capital letters from A to S represent the statistical groups ($P \leq 0.05$) for each parameter at all treatments.

was an increase in root length at all PEG treatments. However, the plants only expanded their roots by no more than 2 mm, in contrast to the ones from the control plants (5 mm) (Fig. 3.3). There were no statistical differences in root lengths between the 31% and 34% PEG treatment.

A decrease in length of the shoot was also observed with the increase of the PEG concentration. The measurements taken after 21 days on the media showed statistical difference between the plants grown at moderate (27%) and high dehydration stresses (31% and 34% PEG). Moreover, the plants grown on 27% PEG were not statistically different than the control plants (Fig. 3.3). After 30 days under dehydration stress, the results showed that the plants exposed to all three PEG treatments ceased to expand their shoot. In contrast, the control plants expanded their shoots by 75%. Statistical analyses grouped the plants in three categories (K to N), where 27% and 31% PEG did not present any significant differences in length (Fig. 3.3).

The last parameter, total plant length, was considered an important parameter to evaluate the impact of dehydration stress. After 21 and 30 days under dehydration stress, statistical differences were observed between all the treatments (Fig. 3.3). Under the highest level of dehydration stress, the plants were only 33% and 17% in size compared to the least severe treatment (27% PEG) and control, respectively (Fig 3.3).

When root : shoot tissue ratios were calculated, the results showed that after 21 days the control had a ratio of 1 and the two intermediate treatments had a lower ratio of 0.6, while the ratio of the most severe treatment (34 % PEG) was also 1. The ratios after 30 days of treatment clearly showed that the growth of the shoot was further impeded than that of the roots (Fig. 3.3).

These results showed that plants reacted differently to the three media exhibiting decreasing ψ. In addition, keeping triticale seedlings under low ψ levels for 30 days generated a very severe dehydration stress, especially at 34% PEG where the plants were chlorotic, wilted and dying. Thus, the experimentation time for transcript profiling was reduced from 30 to 21 days. In addition, to facilitate the production of a larger number of plantlets, the system was moved from 1 L beakers containing 500 ml media into Magenta boxes with 50 ml media. No nutrient deficiency was observed after 21 days (Fig. 3.4). Since adding the NPK supplement could have introduced other stresses, such as manipulation stress and potential pathogens with a potential negative impact for gene expression experiments, no additional supplements were added for the duration of the experiment.

Based on all studied parameters, the results showed that plant development was much reduced under the higher osmotic treatment (34% PEG) compared to the control and the least severe treatment (27% PEG) (Fig. 3.3).

3.4. Discussion

The aim of this study was to identify a reliable method to induce different levels of dehydration stress in plants that can yield easily reproducible results which could be accurately measured. This study also examined the maximum period of time triticale seedlings can withstand when exposed to different levels

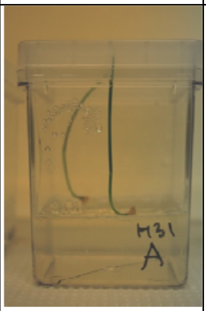
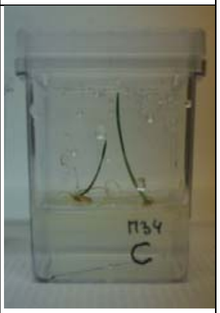
No. of days	0% PEG	27% PEG	31% PEG	34% PEG
10 days				
21 days				

Figure 3.4. Triticale seedlings during development on solid growth media after 10 and 21 days in Magenta boxes under control and increasing PEG concentration, 27%, 31% and 34%.

of ψ. Three concentrations of PEG 35,000 were used to investigate the stress response of triticale seedlings. These experiments were important to determine the appropriate stress period and treatment protocol for a study on drought-induced gene expression.

The three levels of ψ induced by different PEG 35,000 concentrations in solid media showed significant effects on the development of triticale seedlings compared to the control plants. Several aspects of altered morphological developments at this early stage during early drought events were observed at both 21 and 30 days. The number of leaves per plants was substantially reduced from the beginning of the seedling development, possibly a plant response process to reduce transpiration through the leaves or to relocate the resources into other functions such as root growth. The results obtained for this parameter showed that the PEG-treated plants were different from the control due to the lower ψ. However, after 21 days the three levels of dehydration had similar influence in the development of leaves. The low number of leaves reduces the photosynthetic capacity and the plant ability to grow and develop (Shen et al., 2003; Swindell, 2006; Memelink, 2009; Nakashima et al., 2009). Many studies showed that when plants at different developmental stages are affected by drought episodes, they have the tendency to lose their leaves in order to reduce the transpiration and conserve more water (Seki et al., 2003; Magome et al., 2004; Tondelli et al., 2006; Tardif et al., 2007; Memelink, 2009; Nakashima et al., 2009). However, this event can be very detrimental to the plants, especially in their early developmental stages. Although the plants looked healthy after 21 days on the

34% PEG media, after 30 days many plants had etiolated leaves with dying leaf tissues, suggesting that 34% PEG 35,000 concentration was too high a stress to enable continuous triticale development. Comeau et al. (2010) reported that 34% PEG (measured from 40 to 70 days) greatly reduced development of drought tolerant barley seedlings and could represent the maximum concentration barley plants can withstand.

Another aspect observed from the analyses is the root development of the triticale seedlings that was affected by the different ψ during the entire study. This was the only parameter that showed an increase between the 21 and 30 days. This observation strongly suggested that root development was critical when plants are affected by a dehydration stress at this stage of development and under the three levels of stress severity evaluated. Similar observations in other cereals such as barley and wheat were reported by other groups, suggesting that, under dehydration stress, at this early developmental stage the plant's first action is to expand roots and green tissue as much as possible to ensure access to plants main factors for survival: water and light (Tondelli et al., 2006; Tardif et al., 2007; Memelink, 2009).

This was further validated by the observations of shoot development. After 21 days, the triticale seedlings exposed to less severe dehydration stress (27% PEG) did not show signs of dehydration stress in terms of shoot development compared to the control plants. This could be an indication of the ability of triticale to tolerate a relatively intense dehydration stress from the very early stages of development. The two other PEG treatments affected the growth of the shoot. However, after 30 days, the dehydration stress effects were also observed at 27% PEG. The majority of the drought studies showed that during the stress, growth of the shoots ceased, and the plants put their resources into developing roots and accelerating flowering (Seki et al., 2003; Magome et al., 2004; Tondelli et al., 2006; Tardif et al., 2007; Memelink, 2009; Nakashima et al., 2009). The observations that there was no or very little growth at 30 days compared to 21 days on all PEG media, led to two conclusions. A period of 30 days, of which plants were grown on PEG media induced a very severe stress and might be too long a period of time for this type of study. Secondly, this could be an indication of an early response of triticale seedlings to moderate dehydration stress where the shoots expanded faster in the early stage to enhance photosynthetic and survival needs.

All PEG treatments had detrimental effects on plants length, showing a significant reduction compared to the control plants. The results showed that each level of dehydration had significant effects compared with each other, suggesting that different levels of dehydration affected plants development differently. After 21 days under stress, the plants, even though affected by severe dehydration stress, were still able to grow and develop. However, when kept 30 days on solid PEG media, their overall growth and development was dramatically reduced by 80% under the most severe treatment compared with the control.

The results obtained from the root : shoot ratios at all PEG concentrations and a two time intervals suggested that at the seedling stage plants expand the green surface first and later their roots.

The three PEG 35,000 treatments used (27%, 31%, and 34% PEG) offered a unique and reproducible level of different dehydration levels. Similar growth and development patterns to our results shown after 21 days of stress were also observed in seedlings under dehydration stress in maize (Deng et al., 2009), triticale (Hura et al., 2009a and 2009b), wheat (Shen et al., 2003), and Arabidopsis (Swindell, 2006; Moriwaki et al., 2010).

3.5. Conclusions

This experiment demonstrated that the use of different solid PEG media was an excellent reproducible tool to carry out dehydration studies at different levels and under a well-controlled environment. Solid PEG media offer consistency and uniformity of the measured parameters among plants within same repetition and between them for the same treatment. These factors are rather hard to achieve in field trials due to many uncontrollable parameters such as wind, temperature fluctuations and humidity. Thus, this method, using solid PEG media, could become the standard in molecular drought studies for gene expression analyses under different dehydration stress levels due to its ability to isolate dehydration from other abiotic and biotic stresses that could occur simultaneously and thus eliminating any possible confusion in the results of dehydration gene expression studies.

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Chapter 4. Identification of dehydration-responsive genes in triticale seedlings using RNA-Seq

4.1 Introduction

Next-generation sequencing (NGS) technology has dramatically accelerated the sequencing of genomes, regardless of their size or complexity (Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009; Hurd and Nelson, 2009; Morozova et al., 2009; Franssen et al., 2011). The most commonly used NGS platforms are Roche/454 FLX Titanium Sequencer, Illumina/Solexa Genome Analyzer and Applied Biosystems SOLiD System (Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009). A major difference between these platforms is the size of the generated sequenced fragments, which is determined by the chemistry of the system. Initially, the fragment sizes ranged from 30 bp (Illumina) to 350 bp (454 Roche). The size of the generated fragments is significantly reduced from the classical Sanger sequencing (1-2 kb) and has been the most discussed concern of NGS sequencing (Sanger, 1988; Ansorge et al., 1989; Wilson et al., 1990; Ewing et al., 1998; Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009). However, the smaller length of reads is compensated by the larger number (depth) of reads and NGS has been embraced by all fields of genomic research. NGS applications are sequencing and re-sequencing of genomes, transcript profiling, identification of epigenetic modifications and genetic variations, and *de novo* genome/transcriptome assembly (Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009; Hurd and Nelson, 2009; Mane et al., 2009; Morozova et al., 2009; Papdi et al., 2009; Varshney et al., 2009; Franssen et al., 2011).

The usefulness of each of the NGS platforms is based on the size and the number of the sequenced fragments generated and is not restricted to specific applications. However, based on the size of the sequenced fragment generated, platforms that generate smaller fragments (Illumina, Solid) are mostly used for identification of small, rare transcripts and enrichment of sequenced genomes. Platforms that generated larger fragments (454 Roche) are, in general, used for sequencing and re-sequencing of large genomes or transcriptomes of which their sequence is not known (Hurd and Nelson, 2009; Mane et al., 2009; Morozova et al., 2009; Papdi et al., 2009; Varshney et al., 2009; Franssen et al., 2011).

Compared to Sanger sequencing, one advantage of NGS technology is the elimination of transformation of *E. coli* and colony picking that enables the parallelism of sequencing (Kaczorowski and Skybalski, 1996; Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009). Other advantages include array-based sequencing, increased sequencing speed and depth as well as reduced volume of reagents consumed.

Although the specific reagents differ between NGS platforms, they share a common underlying chemistry, which involves sequencing by synthesis methods. In these methods, common adapters are ligated to fragmented DNA, which is subsequently amplified into a million copies and immobilized individually, either on micron-size beads (454 Roche, SOLiD) or directly on the plate surface (Illumina) (Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009). After ligation and amplification, microliter-volumes of reagents are applied for primer hybridization and enzymatic extension reactions. The addition of each nucleotide is captured and recorded instantaneously by image-based detectors (Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009).

In the 454 Roche platform, long DNA fragments (up to 450 bp) are generated using pyrosequencing chemiluminescence (Mardis, 2008; Ansorge, 2009; Balzer et al., 2010). DNA fragments are ligated with specific adapters that bind each fragment to a bead. Fragment amplification is carried out by emulsion PCR using water droplets containing the bead and the PCR reagents immersed in oil. After amplification, each bead is placed into a dedicated picotiter-scale well and covered with smaller beads embedded with reagents (polymerase enzymes and primers) for the next reaction. One unlabelled nucleotide is then introduced into the reaction to start the synthesis. Each time a nucleotide is incorporated, it emits a light generated by a chemical reaction due to the release of a pyrophosphate group. This light is detected by a CCD camera placed under the picotiter-plate and recorded by the software in the computer. Because the identity of the supplied nucleotides is known, the identity of the synthesized DNA fragment is recorded in real time. One full sequencing run is completed in about 10 h and generates approximately up to 1 million reads of 400 nucleotides in length in a full scale reaction (Mardis, 2008; Shendure and Ji, 2008; Ansorge, 2009).

This sequence-based principle has been applied for RNA-sequencing (RNA-Seq), a recently developed method for transcriptome profiling using NGS platforms (Hurd and Nelson, 2009; Mane et al., 2009; Morozova et al., 2009; Papdi et al., 2009; Wang et al., 2009; Franssen et al., 2011). Transcriptome profiling is performed to identify and quantify all transcripts in a cell, usually in relation to a specific experimental condition, such as an environmental stress or developmental stage. Understanding the transcriptome of a plant is very important to identify which genes influence the plant response and to determine how they are regulated. Quantifying changes in expression levels of the transcripts may help in identifying gene role(s) at different developmental stages or under different physiological conditions.

Transcriptome profiling is not new and has been extensively studied using hybridization-based approaches such as microarrays (Ozturk et al., 2002; Denby and Gehring, 2005; Kathiresan et al., 2006; Manavella et al., 2006; Shendure, 2008; Hurd and Nelson, 2009; Mane et al., 2009). There are a few differences between hybridization- and sequence-based methods (Hurd and Nelson, 2009; Morozova et al., 2009). Briefly, hybridization-based methods involve incubation of the fluorescently labeled cDNA with a pre-made microarrays of existing DNA (either genomic or cDNA) of the species studied (Kathiresan et al., 2006; Shendure, 2008). Microarray analysis, although high-throughput and more inexpensive, has several limitations. These includes dependence upon available known sequences, high background signal due to cross-hybridization, limited dynamic range of detection due to the background and the saturation of signals, as well as complicated normalization methods for comparison of expression levels among experiments (Hurd and Nelson, 2009; Morozova et al., 2009; Wang et al., 2009). Even with these limitations, it was preferred over the early sequence-based methods, which were based on Sanger sequencing of cDNA, a low throughput, expensive and non-quantitative approach.

Other methods for transcript profiling have been developed such as serial analysis of gene expression (SAGE) and cap analysis of gene expression (CAGE). They overcame some limitations (Velculescu et al., 1995) but were still expensive and low throughput since they were based on Sanger sequencing technology (Hurd and Nelson, 2009; Wang et al., 2009).

The advantages of sequence-based methods using NGS are numerous, not only compared to microarrays, but also compared to early sequence-based methods. Compared to microarrays, RNA-Seq is used on any transcript template, which is very useful when using non-model organisms without an existing reference genome. RNA-Seq offers the possibility to identify different alternatively spliced gene isoforms as well as single variations (SNPs) in transcribed regions. Moreover, RNA-Seq has very low background and is a very sensitive method to detect very low abundance transcripts. RNA-Seq can also detect very high levels of expression, is very accurate for quantification of expression levels, and is highly reproducible. Transcript abundance is represented by the total number of reads of that transcript, normalized by the length of the exons that are uniquely mapped. This allows direct comparison of the same transcript among treatments or tissues.

Gene ontology (GO) is a standardized system used in genetic and genomic studies to define gene functions. It assigns genes into three categories: biological process, molecular function and cellular component (www.geneontology.org). Blast2Go is a software that uses basic local alignment search tool (BLAST) searches to find similar sequences in ones database and classify the studied sequences based on GO (www.blast2go.org). It offers a complex package that helps not only in mapping and annotating the sequences but also in performing a complete statistical analysis and presentation of the results.

The hypothesis of this study was that utilization of RNA-Seq with an improved method to induce and isolate dehydrations stress based on solid PEG media will allow identification of the transcript complement involved in the response of triticale seedlings to dehydration stress. Identification of these differentially regulated transcripts will enable assignment of genes that are part of the signaling and biochemical pathways responding to the dehydration stress in triticale seedlings.

4.2. Materials and Methods

Plant material and total RNA isolation

Triticale (x *Triticosecale* Wittm., variety AC Certa) pre-germinated seeds were placed on solid PEG media as described in Chapter 3. Above-ground tissue samples were collected from 21-day-old seedlings. At least five biological replicates of each sample were harvested and immediately frozen in liquid nitrogen. These replicates were pooled prior to the total RNA extraction procedure. Frozen tissues (1.0 g) were ground into a fine powder using an RNase-free mortar and pestle and liquid nitrogen to avoid thawing the samples. The powder was transferred into a 2 ml pre-labeled, pre-chilled microcentrifuge tube and immediately submerged in liquid nitrogen. Total RNA was extracted from the samples using *mir*Vana™ miRNA Isolation Kit (Ambion Inc., USA). This method combines both organic (phenol:chloroform) and solid-based extractions, using glass-fiber filters to efficiently isolate total RNA ranging in size from several kilobases down to 10-mers. Each ground sample (1.0 g) was resuspended in 1 ml of lysis/binding buffer and the content was homogenized by pipetting up and down using a cut pipette tip until all visible clumps were dispersed. One hundred microliters of miRNA homogenate additive were added to the lysate and mixed by gently vortexing, and then the tubes were immediately placed on ice for 10 min. The lysate was split into two fresh RNase-free tubes (550 µl each), and 550 µl of acid-phenol:chloroform were added to each tube, followed by vortexting for 30-60 s. The mixture was centrifuged at 9,000 *xg* for 5 min at room temperature. The aqueous phase was removed without disturbing the lower phase and transferred to a fresh 2 ml RNase-free tube. The volume removed was recorded and transferred to a tube containing 1.25 volumes of 100% ethanol. The sample was pipetted onto a filter cartridge previously prepared and centrifuged at 9,000 *xg* for 15 s. The flow-through was discarded and the step was repeated until all the mixture went through the cartridge. Before the wash step, we included an on-column DNase digestion step where 10 µl of DNase 1 stock solution and 70 µl of buffer RDD were added in the center of the filter cartridge and incubated for 15 min at room temperature. After the DNase digestion, the filter was washed by adding 700 µl of miRNA wash solution 1 (provided with the kit) onto the filter cartridge and centrifuged at 9,000 *xg* for 10 s. After discarding the flow-through, the filter was subsequently washed two more times with 500 µl of wash solution 2/3 (provided with the kit) at 9,000 *xg* for 10 s each. Before the elution step, the filter cartridge was centrifuged at 9,000 *xg* one more time for 1 min to remove any residual fluids. The filter cartridge was transferred into a pre-labeled 2 ml tube. One hundred microliters of pre-heated (95ºC) nuclease-free water were pipetted to the center of the filter and centrifuged for 30 s at 13,000 *xg* to recover the total RNA. The eluate containing the total RNA was collected and stored at -80ºC for poly(A)$^+$ RNA isolation. Prior to freezing, a small aliquot was used for quantitative and qualitative analysis of the total RNA. The quantity and quality of each sample were assessed using two methods, spectrometry using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA) and rapid electrophoresis using an Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Nano Kit (Agilent Technologies, Singapore). Agilent 2100 Expert Software (Agilent Technologies, Singapore) was used to visualize the Bioanalyzer results. The electrophoregrams showed the high quality of total RNA for all samples indicated by the distinct 18S and 28S ribosomal peaks, the absence of smaller peaks in between the two peaks, and a relatively flat baseline (Fig. 4.1). Only RNA samples with 260 nm/280 nm ratios from 1.9 to 2.1, and 260 nm/230 nm ratios from 2.0 to 2.5 were used for further analysis. All the biological replicates meeting the above criteria were pooled for mRNA purification.

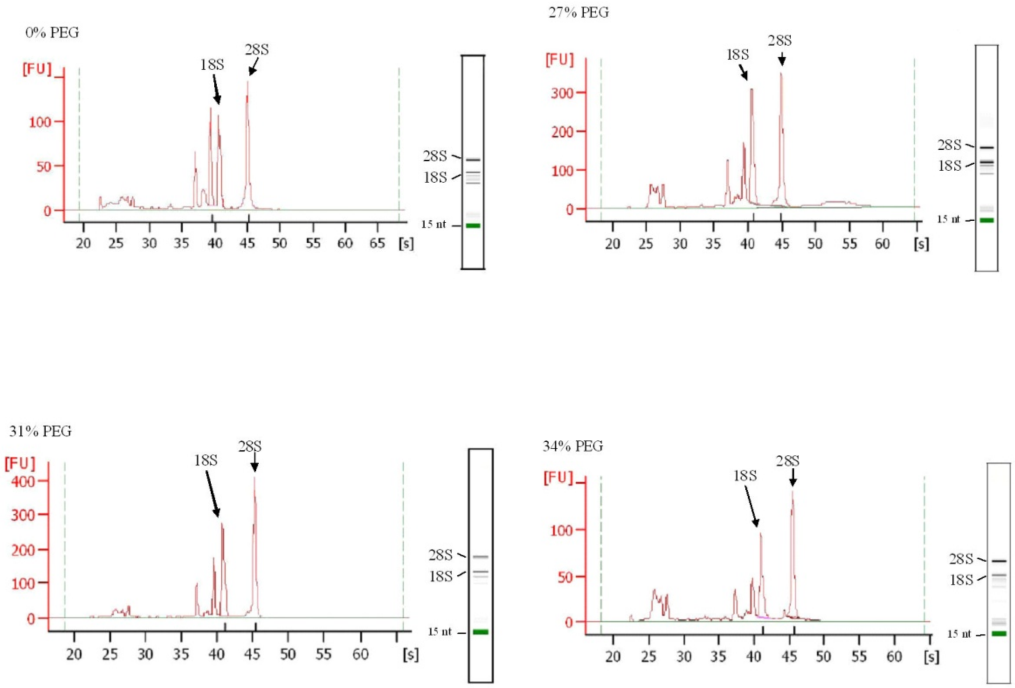


Figure 4.1. Electrophoregrams of isolated total RNA of triticale seedlings from all treatments assessed with an Agilent 2100 Bioanalyzer using the Agilent RNA 6000 kit (FU, Fluorescence Units; s, time for separation in seconds).

The 18S and 28S rRNA peaks are visible. The electronic gel generated on the right side shows total RNA with two more intense bands for 18S and 28S rRNA. The green band represents the lower marker of the sizing ladder at 15 nt.

Poly $(A)^+$ preparation

Poly$(A)^+$ RNA fractions were purified from total RNA samples using a Poly(A)Purist Kit (Ambion Inc., USA). This method was chosen because it uses a high quantity of total RNA (up to 2 mg) that is appropriate for isolating large amount of poly$(A)^+$ RNA needed (up to 5 μg) for the preparation of cDNA libraries for RNA-seq. This method is a solid-based (filter-based) extraction using optimized oligo(dT) cellulose to increase the specificity of poly$(A)^+$ RNA selection. One milligram of total RNA from each sample was combined with nuclease-free water for a total volume of 0.5 ml in a 2 ml pre-labeled RNase-free tube. A 0.5 ml volume of 2x Binding Solution (provided with the kit) was added to the mixture and was mixed by vortexing. This mixture was added to a pre-labeled tube of oligo(dT) cellulose (provided with the kit) and mixed by pipetting up and down with a pipette tip cut to allow a better flow of the mixture until all visible clumps were broken. The resulting mixture was heated for 5 min at 65ºC and then mixed by rocking on a Labnet GyroMini Nutating Mixer for 60 min. The samples were then centrifuged at 4,500 *xg* for 3 min at room temperature. The supernatant was removed with a pipette and saved on ice. Five hundred microliters of wash solution 1 were added to the oligo(dT) cellulose pellet, placed in a column into a pre-labeled 2 ml microfuge tube and vortexed briefly to mix well. The column was incubated for 3 min at room temperature and then centrifuged at 4,500 *xg* for 3 min. The flow-through was discarded. This step was repeated three times. The resulting pellet accumulated in the column was washed three times with 0.5 ml of wash solution 2 with a 3 min incubation at room temperature in between washes. After the last flow-through was discarded, the column was placed into a new 2 ml pre-labeled microfuge tube. Two hundred microliters of the RNA Storage Solution, preheated to 80ºC, were added to the pellet and vortexed until mixed well. The mixture was immediately centrifuged at 6,000 *xg*. The eluate was mixed with 40 µl of ammonium acetate, 1 µl of glycogen and 1.1 ml of 100% ethanol. The mixture was kept overnight at -70ºC and centrifuged the next day at 13,000 *xg* for 30 min at 4ºC. After centrifugation, the supernatant was carefully removed with a fine-tipped pipette without disturbing the pellet. The pellet was air-dried for 10 min at room temperature, then resuspended in 20 µl of 80ºC RNase-free water, from which an aliquot was assessed for quality and quantity with a NanoDrop 1000 and an Agilent 2100 Bioanalyzer using the Agilent RNA 6000 kit. The Bioanalyzer electrophoregram for each sample showed a broad peak, indicating a high-quality poly(A)$^+$ RNA fraction between 25 to 50 s, corresponding to a transcript size range between 1,000 - 4,000 nucleotides long (Fig. 4.2). The higher peaks represent ribosomal contamination, which did not exceed 15% for our samples and did not compromise the poly (A)$^+$ RNA quality. All samples were stored at -80ºC and were subsequently used to generate double-stranded (ds) cDNA libraries.

Library preparation and RNA-Seq

For first strand cDNA synthesis, 1 µg of poly(A)$^+$ RNA was mixed with 2 µl of anchored oligo(dT) primer (0.5 µg/µl) (Invitrogen, Mississauga, ON) and heated for 5 min at 65ºC, then cooled on ice. Six microliters of 5x Superscript III

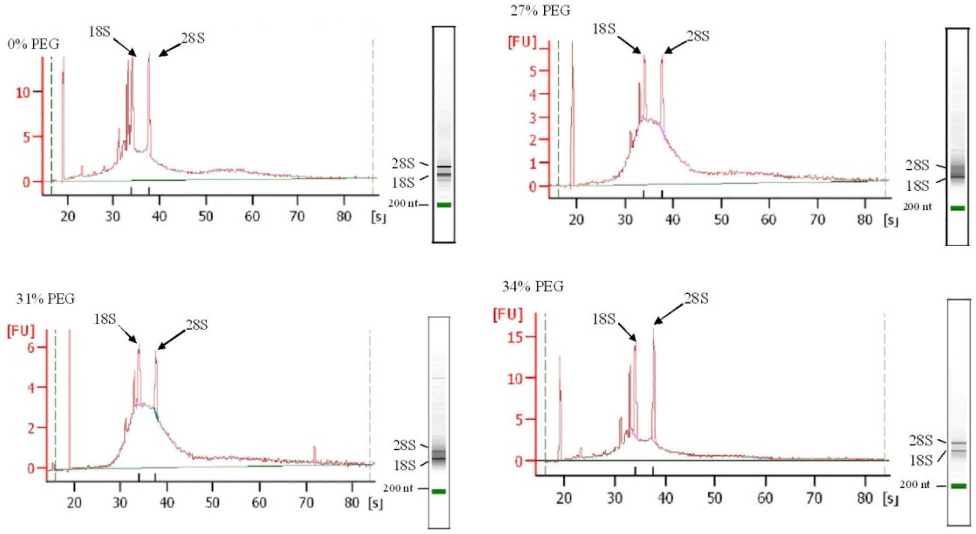


Figure 4.2. Electrophoregrams of poly $(A)^+$ RNA of triticale seedlings from all treatments assessed with an Agilent 2100 Bioanalyzer using the Agilent DNA 7500 kit (FU, Fluorescence Units; s, time for separation in seconds). The poly $(A)^+$ RNA is usually represented by a broad peak. The spikes represent 18S and 28S rRNA that are also visible on the right side on the generated electronic gel image. The green band on the gel represents the lower marker of the ladder at 200 nt.

buffer, 1.5 µl 0.1 M DTT, 1.0 µl RNaseOut (40 U/µl), 1.5 µl dNTP mix (10 mM each dNTP) and 2.0 µl Superscript III (100 U/µl) (Invitrogen, Mississauga, ON) were added to the mixture, and the volume was brought up to 30 µl with RNase-free water. The reactions were placed into an Eppendorf Mastercycler gradient thermocycler and incubated at 35ºC for 10 min and then at 45ºC for 50 min. The reactions were stored overnight at -20ºC. The 30 µl volume of first strand synthesis was added to a mix containing 30 µl 5x second strand buffer, 3 µl 10 mM dNTP mix, 2 µl RNase H (2 U/µl), 4 µl DNA Polymerase I (10 U/µl),1 µl *E.coli* DNA ligase (10 U/µl), and 80 µl RNase-free water. The reactions were placed into the thermocycler at 16º for 2.5 h. The resulting ds cDNA was stored at -20ºC. For each sample, a quantity of 5 µg ds cDNA was obtained for 454 sequencing by pooling several ds cDNA reactions for each experimental condition. The quality of the four cDNA libraries was assessed using an Agilent DNA 7500 kit with an Agilent 2100 Bioanalyzer. The electrophoregrams and electronic gels showed the high-quality DNA indicated by a similar to poly(A)$^+$ RNA broad peak from 500 to 6,000 bp in between the lower and higher markers of the ladder, as well as the relatively flat baseline between 50 and 500 bp (Fig. 4.3).

After the quality assessment, the samples were submitted for 454 whole transcriptome sequencing (RNA-Seq) with GS FLX Titanium 454 Sequencer at the NRC Plant Biotechnology Institute (PBI, Saskatoon).

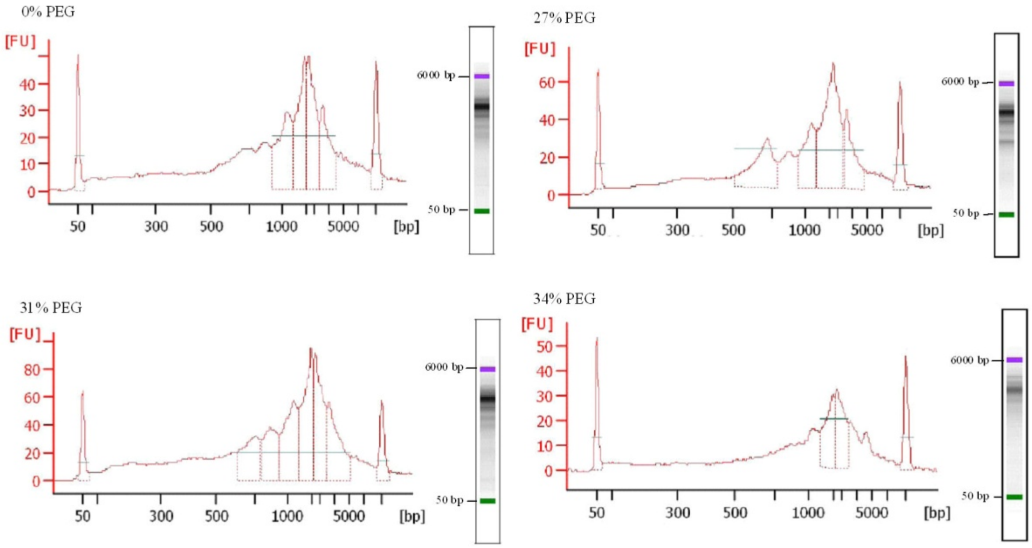


Figure 4.3. Electrophoregrams of the double stranded cDNA of triticale seedlings for all treatments assessed with Agilent 2100 Bioanalyzer using Agilent DNA 7500 kit (FU, Fluorescence Units; bp, base pairs).
The ds cDNA is represented by the broad peak between 500 and 6,000 bp. The peaks at the extremities of the electrophoregrams represent the lower and upper markers at 50 and 6000 bp that are also visible on the generated electronic gel image (the purple and green bands). The horizontal lines in the electropherogram indicate the width of the baseline used to calculate the area under the curves.

Bioinformatic analysis of sequenced cDNA

The GS FLX Titanium raw reads were cleaned with DNASTAR SeqMan NGen 3.0 prior to the sequence assembly step by removing the vectors/adapters and possible contaminants such as ribosomal RNA and mitochondrial RNA sequences. Parameters used for DNASTAR SeqMan NGen 3.0 for assembly of cleaned reads were the following: match size: 19 nucleotides (nt); match spacing: 75 nt; match score: 10; expected coverage: 20 nt; and greater than 92% identity. In the initial assembly, the transcriptome from *Brachypodium distachyon* was selected as reference since it was the most closely related monocot with a fully sequenced and annotated genome. Once the triticale transcriptome reference became available (Xu et al., 2011, and unpublished data), the set of statistically differentially regulated (≥2-fold) genes was run against it to enrich our gene annotation pool.

Identification of differentially regulated genes

RNA-Seq datasets were analyzed with DNASTAR QSeq, part of ArrayStar 3.0 software (www.dnastar.com). The RPKM (Reads Per Kilobase of exon model per Million mapped reads) algorithm was used as the normalization method, with 20 bases matching at least 80% identity within each read. All the samples were compared to each other and to the control to generate differential gene regulation measured in fold differences. The reference coding sequences (cds) file of *B. distachyon* was obtained from www.brachypodium.org. Several software packages were used for the annotation steps such as MegaBlast (http://blast.ncbi.nlm.nih.gov/), Amigo (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi), and Blast2GO (http://www.blast2go.org/), against different databases including *B. dystachion, A. thaliana* (http://www.arabidopsis.org/), and all cereal crops (rice, wheat, maize, oats, rye, sorghum (http://www.plantgdb.org/), and triticale (in-house database).

4.3. Results

Transcriptome of triticale seedlings grown on solid media of different PEG concentrations

To identify the gene complement of triticale seedlings and how dehydration stress affects the regulation of gene expression, cDNA libraries of seedlings grown on 0% (control), 27%, 31% and 34% PEG 35,000 in solid media under axenic conditions were prepared. Four libraries sequenced with the GS FLX Titanium technology yielded from 323,536 to 430,281 raw reads for an average of 386,000 reads per library, and a total of 1,546,210 reads (Table 4.1).

The reads were assembled in two different ways, within the individual and the pooled libraries. The rationale of this approach was to identify similarities between sequences of individual samples. The sum of all libraries yielded 1,133,618 assembled sequences into contigs, where the control had the highest number, 346,763 assembled sequences (Table 4.1). When reads were analyzed together, the number of assembled sequenced increased to 1,228,194. Each transcriptome database was assembled, yielding between 36,876 and 43,038

Table 4.1. Proprieties of the four triticale seedlings libraries sequenced with the 454 GS FLX Titanium technology

Library	Number of raw reads[†]	Number of assembled sequences	Number of contigs	Average contig length (nt)	Number of singletons
0% PEG (control)	430,281	346,763	43,038	605	83,518
27% PEG	422,330	290,997	40,603	535	131,333
31% PEG	323,536	222,551	36,876	646	100,985
34% PEG	370,063	273,307	40,726	597	96,756
Sum of 4 libraries	1,546,210	1,133,618	161,243	595	412,592
Pooled libraries	1,546,210	1,228,194	107,831	668	318,016

[†] **Raw read** – DNA fragments generated by NGS sequencing methods
Contig – a contiguous, overlapping sequence read resulting from the reassembly of the DNA fragments generated by NGS sequencing
Singleton – a sequence that did not show any significant overlap (by default, a 40 bp window of at least 90% similarity) with any other reads

contigs for the individual assemblies, for a total of 161,243 contigs (Table 4.1).

When the reads were pooled together, a significant smaller number of contigs, 107,831, was generated, indirectly suggesting formation of longer contigs. The mean contig length for all databases was 595 nt and ranged from 535 nt at 27% PEG to 646 nt at 31% PEG compared to 668 nt when all reads were pooled together prior to assembly (Table 4.1). The number of singletons was concomitantly decreased by 23% in the pooled libraries since more reads were assembled in contigs. The largest identified contig was 5,496 bp long, and was obtained from reads from the control treatment. A distribution of all contigs and singletons based on their frequency and length was generated using R-based software (developed in-house) (Figs. 4.4 and 4.5). The majority of the contigs for the combined individual assemblies (Fig. 4.4A) were between 316 and 1,000 nt in length with a peak between 400-500 nt of about 40,000 contigs, while a similar pattern with a peak between 400 and 500 nt but with a much smaller frequency of about 23,000 contigs was observed for the pooled assembly (Fig. 4.4B). These numbers suggested that when the libraries were pooled, a large number of reads from all libraries were similar and assembled into single contigs, reducing the number of overall contigs by 33% (Table 4.1). The distribution of the singletons (Fig. 4.5) was spread up to 560 bases with a peak around 350 to 450 bases with frequencies up to 80,000 for the combined individual libraries (Fig. 4.5A) and 60,000 for the pooled libraries (Fig. 4.5B). The smaller number of singletons resulted from the assembly of a large number of reads (Table 4.1).

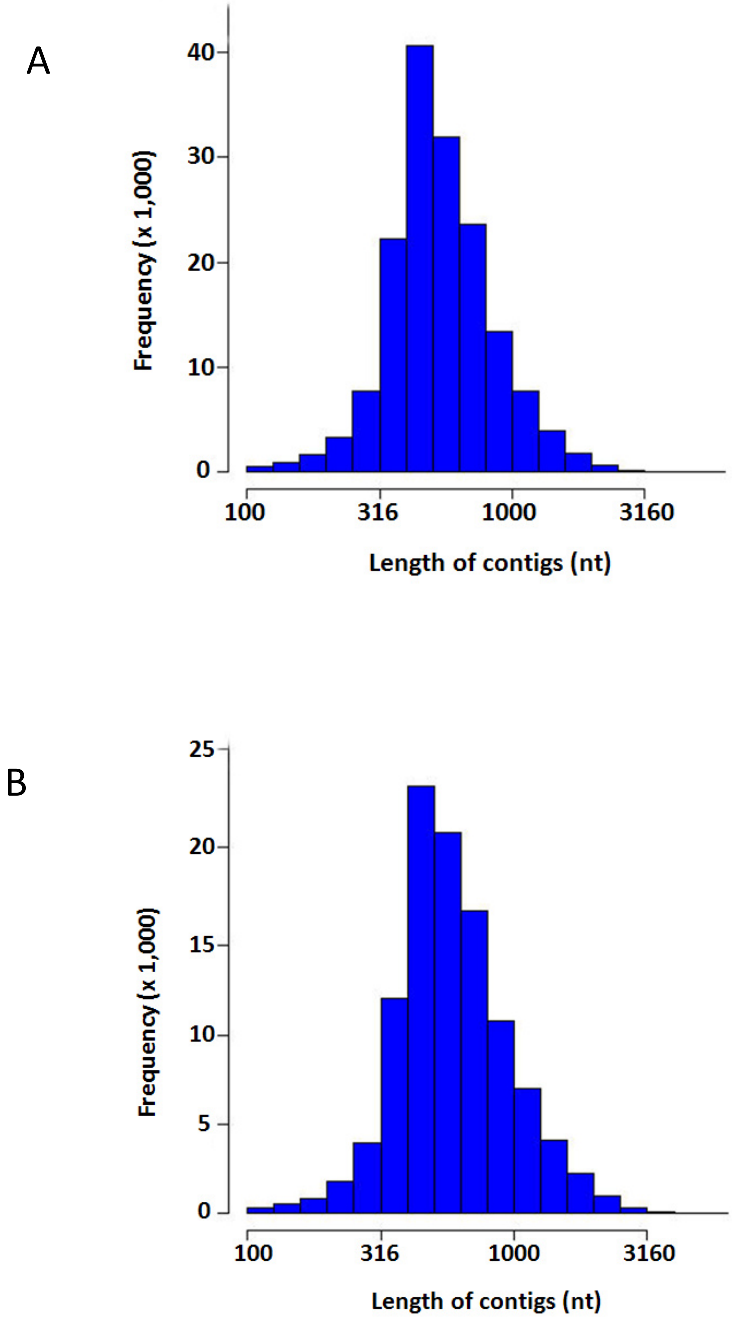


Figure 4.4. Distribution of contig length for combined individual assemblies (A) and pooled assembly (B).
Length distribution of all contigs obtained from assembly with NGen software. There are 161,243 (A) and 107,831 (B) contigs in total.

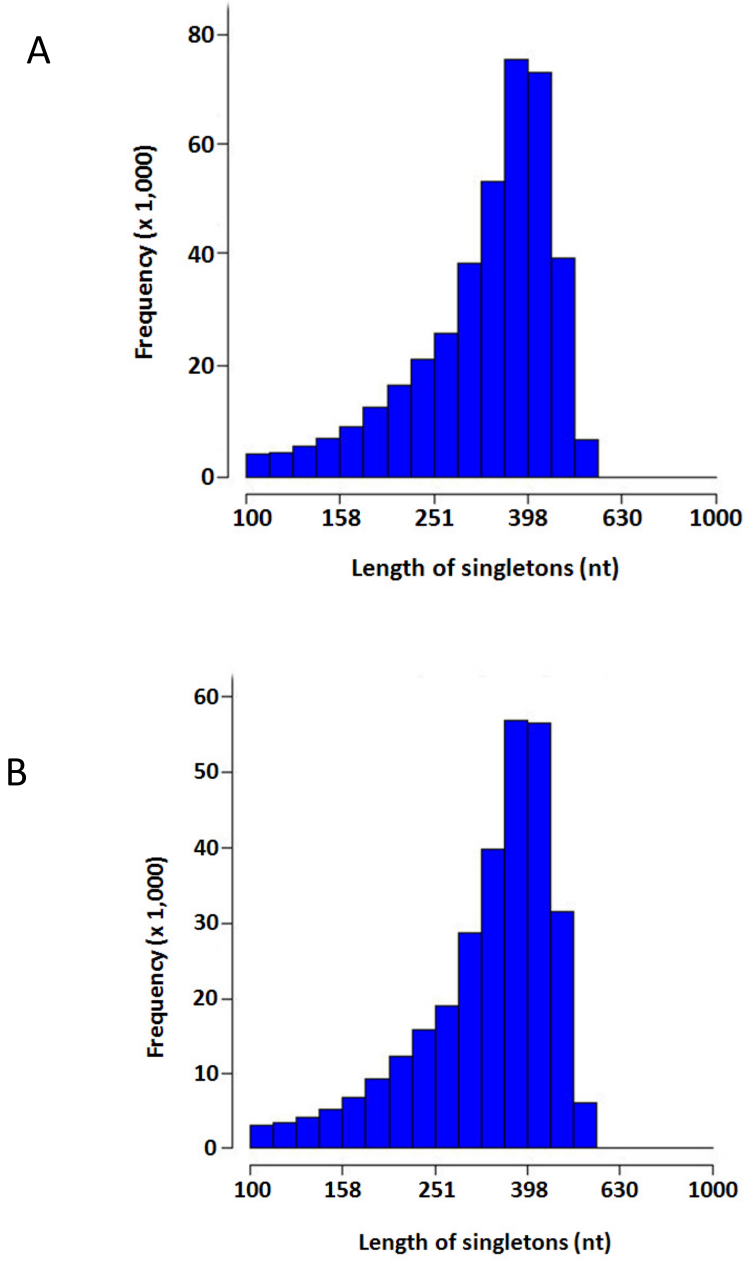


Figure 4.5. Distribution of singleton length for combined individual assemblies (A) and pooled assembly (B).
Length distribution of all singletons resulted from contigs assembly. There are 412,592 (A) and 318,016 (B) singletons in total.

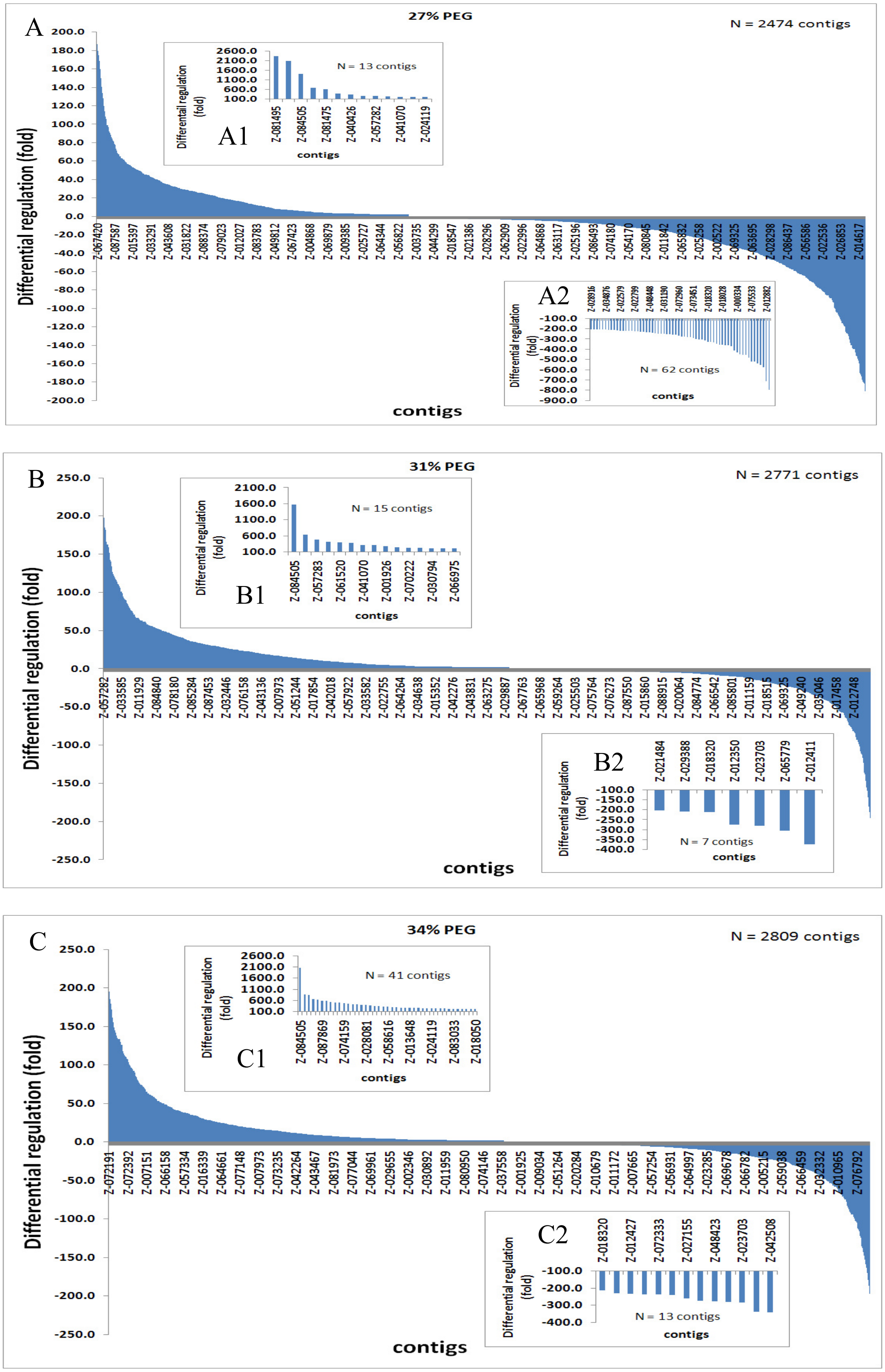
The triticale seedling transcriptome is based on >107,000 contigs representing full and partial length genes that differ from each other by identity differences greater than 8%. This transcriptome was analyzed to identify the transcripts and their regulation using *B. distachyon* as reference genome.

From the >107,000 contigs generated by RNA-Seq, 4,467 were statistically differentially regulated 2-fold or greater for at least one of the PEG treatments among the four libraries (See Table S4.1, supplemental data). The level of gene expression varied up to 2,341-fold for upregulation and 796-fold for downregulation (Fig. 4.6). The highest number of differentially regulated genes was observed at 27% PEG. However, 34% PEG presented the highest number (116) of ≥100-fold overexpressed genes (Figs. 4.6C and C1), suggesting that the higher stress level led to greater overexpression of numerous transcripts.

To further dissect the positively and negatively regulated transcripts, they were divided into four subgroups, each based on the levels of regulation: ≥ 100-, 50- to <100-, 10- to <50-, and 2- to <10-fold. The distribution and the number of genes for each group were quite different from one treatment to the other. This indicated that some genes or gene families were differentially regulated based on the severity of the dehydration treatment.

In the first subgroup of ≥100-fold gene overexpression, there were 19 sequences common between all treatments, 41 common sequences between 34% and 31% PEG treatments, 23 common sequences between 34% and 27% PEG treatments, and 27 common sequences between 31% and 27% PEG treatments.

Figure 4.6. Distribution of all statistically differential regulated genes (≥2-fold) for 27%, 31% and 34% PEG treatments compared to the control treatment (0% PEG).
Differentially regulated genes ranging from 200- to -200-folds for 27%, 31% and 34% PEG treatments are shown in panels A, B and C, respectively. Inset figures represent sets of genes extracted from 27%, 31%, 34% PEG treatments that are upregulated ≥200-folds (A1, B1, and C1, respectively) and downregulated ≥200-folds (A2, B2, and C2, respectively). Partial contigs lists are shown in each panel.


A
27% PEG
N = 2474 contigs
Differential regulation (fold)
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
-40.0
-60.0
-80.0
-100.0
-120.0
-140.0
-160.0
-180.0
-200.0
Z-067420
Z-087587
Z-015397
Z-033291
Z-043608
Z-031822
Z-088374
Z-079023
Z-012027
Z-083783
Z-049812
Z-067423
Z-004868
Z-069879
Z-009385
Z-025727
Z-064344
Z-056822
Z-003735
Z-044299
Z-018547
Z-021386
Z-028296
Z-062909
Z-022996
Z-064868
Z-063117
Z-025196
Z-086493
Z-074180
Z-054170
Z-080845
Z-011842
Z-065832
Z-025258
Z-000522
Z-069325
Z-063695
Z-028298
Z-086437
Z-056586
Z-022536
Z-026853
Z-014617
contigs
A1
Differentail regulation (fold)
2600.0
2100.0
1600.0
1100.0
600.0
100.0
N = 13 contigs
Z-081495
Z-084505
Z-081475
Z-040426
Z-057282
Z-041070
Z-024119
contigs
A2
Differential regulation (fold)
-100.0
-200.0
-300.0
-400.0
-500.0
-600.0
-700.0
-800.0
-900.0
N = 62 contigs
contigs
B
31% PEG
N = 2771 contigs
Differential regulation (fold)
250.0
200.0
150.0
100.0
50.0
0.0
-50.0
-100.0
-150.0
-200.0
-250.0
Z-057282
Z-033585
Z-011929
Z-084840
Z-078180
Z-085284
Z-087453
Z-032446
Z-076158
Z-043136
Z-007973
Z-051244
Z-017854
Z-042018
Z-057922
Z-033582
Z-022755
Z-064264
Z-034638
Z-015352
Z-042276
Z-043831
Z-063275
Z-029887
Z-067763
Z-065968
Z-059264
Z-025503
Z-075764
Z-076273
Z-087550
Z-015860
Z-088915
Z-020064
Z-084774
Z-066542
Z-085801
Z-011159
Z-018515
Z-069325
Z-049240
Z-035046
Z-017458
Z-012748
contigs
B1
Differential regulation (fold)
2100.0
1600.0
1100.0
600.0
100.0
N = 15 contigs
Z-084505
Z-057283
Z-061520
Z-041070
Z-001926
Z-070222
Z-030794
Z-066975
contigs
B2
Differential regulation (fold)
Z-021484
Z-029388
Z-018320
Z-012350
Z-023703
Z-065779
Z-012411
-100.0
-150.0
-200.0
-250.0
-300.0
-350.0
-400.0
N = 7 contigs
contigs
C
34% PEG
N = 2809 contigs
Differential regulation (fold)
250.0
200.0
150.0
100.0
50.0
0.0
-50.0
-100.0
-150.0
-200.0
-250.0
Z-072191
Z-072392
Z-007151
Z-066158
Z-057334
Z-016339
Z-064661
Z-077148
Z-007973
Z-073235
Z-042264
Z-043467
Z-081973
Z-077044
Z-069961
Z-029655
Z-002346
Z-030892
Z-011959
Z-080950
Z-074146
Z-037558
Z-001925
Z-009034
Z-051264
Z-020284
Z-010679
Z-011172
Z-007665
Z-057254
Z-056931
Z-064997
Z-023285
Z-069678
Z-066782
Z-005215
Z-059038
Z-066459
Z-032332
Z-010965
Z-076792
contigs
C1
Differential regulation (fold)
2600.0
2100.0
1600.0
1100.0
600.0
100.0
N = 41 contigs
Z-084505
Z-087869
Z-074159
Z-028081
Z-058616
Z-013648
Z-024119
Z-083033
Z-018050
contigs
C2
Differential regulation (fold)
Z-018320
Z-012427
Z-072333
Z-027155
Z-048423
Z-023703
Z-042508
-100.0
-200.0
-300.0
-400.0
N = 13 contigs
contigs

Seventy-one, 30 and 13 genes were overexpressed only in one treatment, for 34%, 31% and 27% PEG, respectively (Fig. 4.7).

For the subgroup of genes overexpressed between 50- and <100-fold, the number of differentially regulated genes for all treatments increased compared to the previous group (Fig. 4.7). However, only 4 genes were common for all treatments in this subgroup. Ninety genes were unique for 34% PEG, while for 31% and 27% PEG had 99 and 71 specific genes, respectively (Fig. 4.7).

In the next subgroup, 10- to <50-fold upregulation, the number of genes exceeded 400 for each treatment (Fig. 4.7). Fifty seven genes were common for all treatments in this group. The number of genes unique to one treatment exceeded 200, the highest number observed at 31% PEG (339 genes), followed by 34% and 27% PEG showing 301 and 224 genes, respectively (Fig. 4.7).

The last subgroup, 2- to <10-fold contained the highest number of overexpressed genes (Fig. 4.7). One hundred and nine genes were shared by all treatments, which was also the highest number among all groups of upregulated genes (Fig. 4.7).

A total of 189 genes were upregulated ≥2-fold in all three treatments regardless of the dehydration severity. This observation suggested that these genes might be part of the plants general response to dehydration stress. Except for one group (10- to <50-fold) the number of genes was increasing as the intensity of the regulation was decreasing (Fig 4.7).

Conversely, some genes were only upregulated under specific treatments. Of all the upregulated unique genes among all subgroups, some were highly

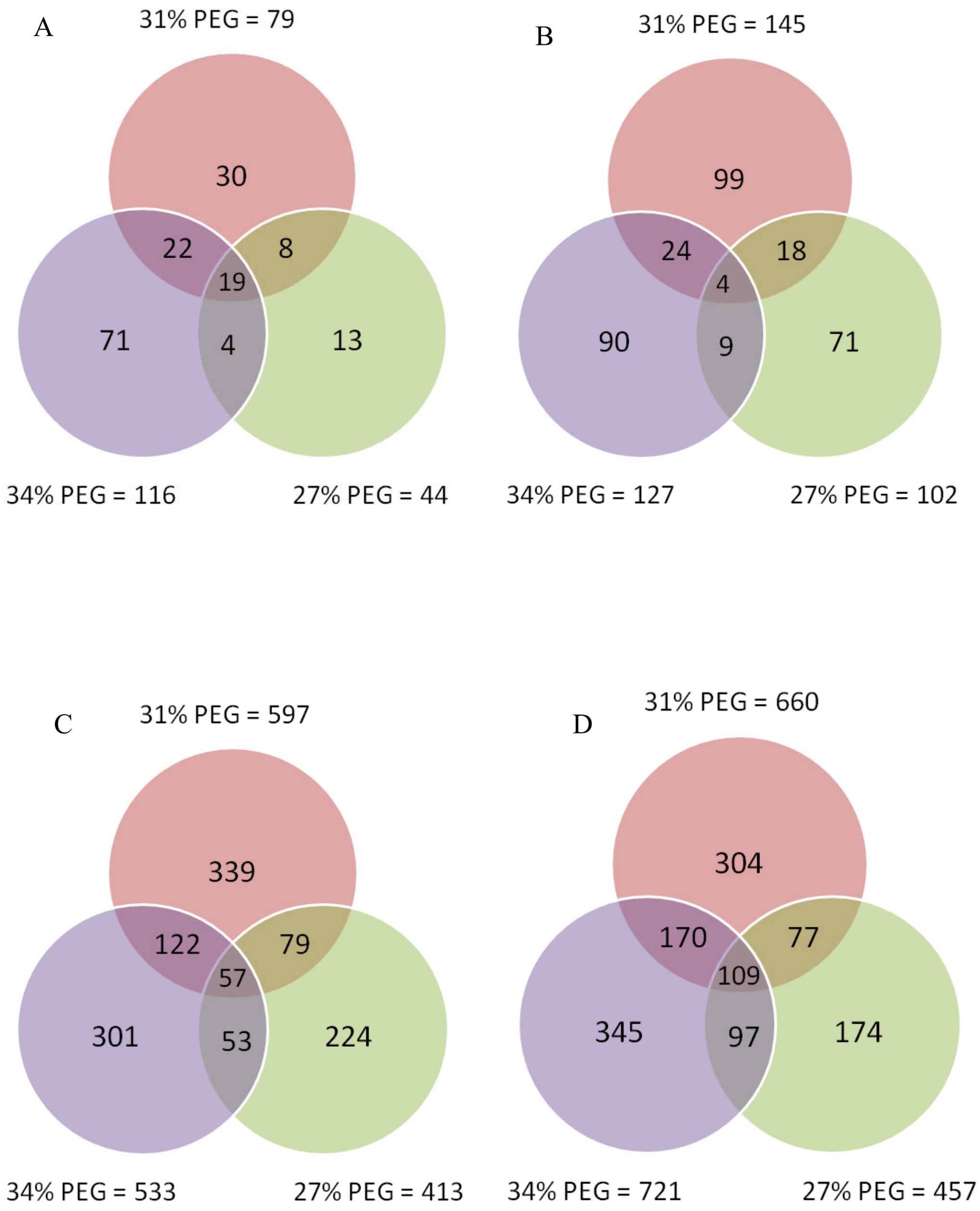


Figure 4.7. Distribution of upregulated genes 2- to >100-fold (A) ≥100-fold; B) 50- to <100-fold; C) 10- to <50-fold; and D) 2- to <10-fold) among the three PEG treatments (27%, 31% and 34% PEG) when compared to control (0% PEG).

upregulated ≥50-fold in two or only one of the treatment(s), without any differential regulation (≥2-fold) in the other treatment(s). Up to 68 (52 + 16) genes did not show any regulation for two of the treatments, 27% and 34% PEG, and were upregulated ≥50-fold in 31% PEG (Table 4.2). Also, up to 24 (15 + 9) genes were not expressed in one treatment, 27% PEG, while overexpressed ≥50-fold in 31% and 34% PEG (Table 4.2). Overexpression of some genes during only specific PEG treatments suggested that some genes could have different roles in the dehydration response dependent of the stress severity.

Similarly, genes showed different levels of downregulation related to the level of severity of the treatment. For the first subgroup, ≥100-fold downregulation, the highest number of genes was observed at 27% PEG treatment, which was double and triple compared to the number of downregulated genes for 34% and 31% PEG treatments, respectively. Fifteen genes were common among the three treatments. The number of unique genes at 27% PEG treatment was 6 times and 13 times higher than for 34% and 31% PEG treatments, respectively (Fig. 4.8).

In the 50- to <100-fold negative regulation subgroup, the highest number of genes was still observed at 27% PEG (Fig. 4.8). Seventeen genes were common for all treatments. The number of unique genes for this subgroup was the highest at 27% PEG treatment, almost 4 times and 6 times higher than 34% and 31% PEG treatments, respectively (Fig. 4.8).

In the subgroup containing genes downregulated between 10- to <50-fold, the number of genes was much higher as compared to the previous one (Fig. 4.8).

Table 4.2. Distribution of specific differentially regulated genes.
Some of the genes were differentially regulated 50 or larger fold in treatments and not regulated in the other treatment(s).

Differential regulation (treatment)	**No regulation (treatment)**	**Number of treatment-specific upregulated genes**	**Number of treatment-specific downregulated genes**
50 – <100 fold			
27 % PEG	31% PEG, 34% PEG	39	13
31 % PEG	27% PEG, 34% PEG	52	2
34 % PEG	27% PEG, 31% PEG	34	2
27% PEG, 31% PEG	34 % PEG	5	1
27% PEG, 34% PEG	31 % PEG	5	5
31% PEG, 34% PEG	27 % PEG	15	2
≥ 100 fold			
27 % PEG	31% PEG, 34% PEG	6	17
31 % PEG	27% PEG, 34% PEG	16	1
34 % PEG	27% PEG, 31% PEG	24	1
27% PEG, 31% PEG	34 % PEG	6	2
27% PEG, 34% PEG	31 % PEG	1	2
31% PEG, 34% PEG	27 % PEG	9	4

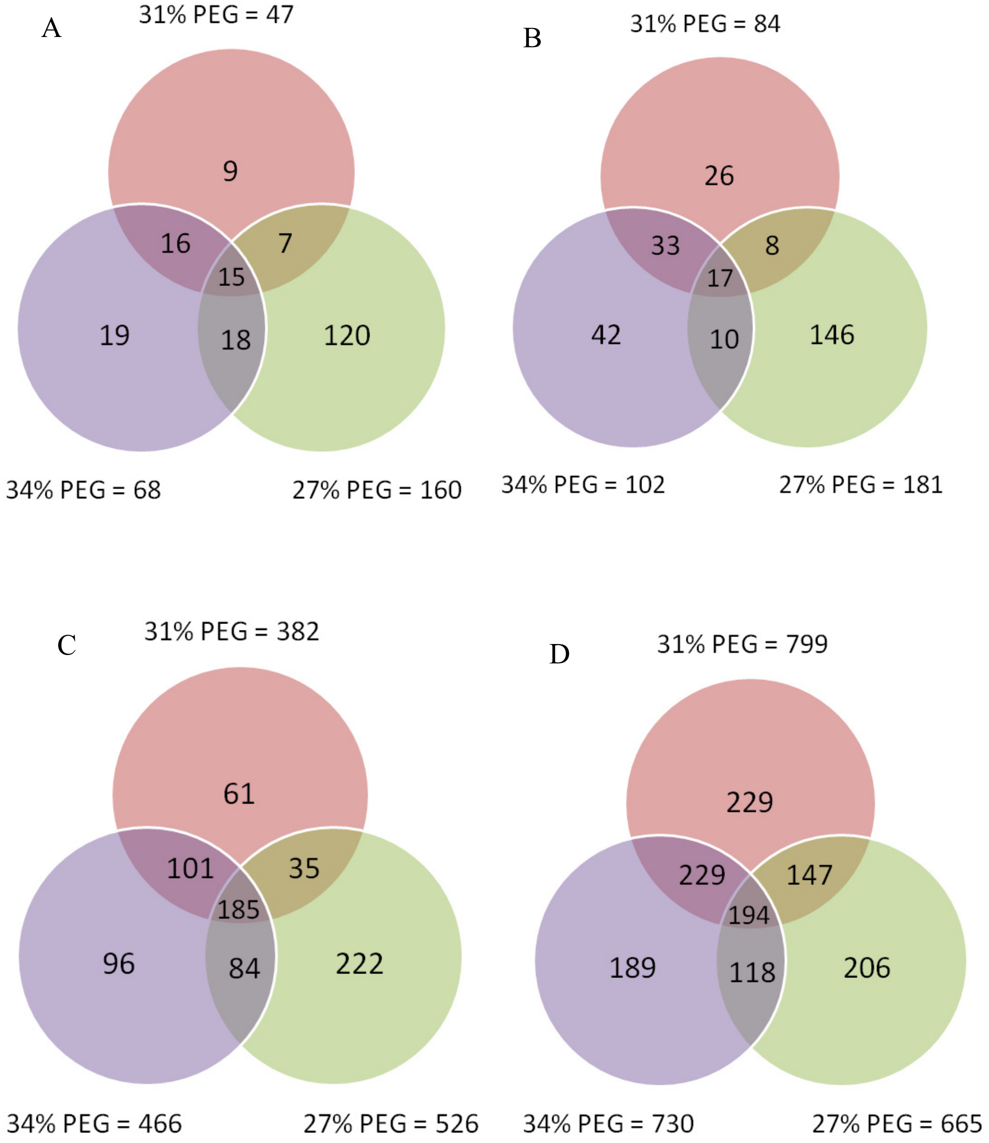


Figure 4.8. Distribution of downregulated genes 2- to >100-fold (A) ≥100-fold; B) 50- to <100-fold; C) 10- to <50-fold; and D) 2- to <10-fold) among the three PEG treatments (27%, 31% and 34% PEG) when compared to control (0% PEG).

One hundred and eighty five genes were common among all treatments. The number of unique genes at 27% PEG treatment was still higher than the other treatments, showing 222 genes compared to 96 and 61 at 34% and 31% PEG treatments, respectively (Fig. 4.8).

A high number of genes was observed, with more than 600 genes at each treatment for the 2- to <10-fold gene downregulation group. The highest number of genes was observed for the 31% PEG treatment, followed by 34% and 27% PEG treatments (Fig. 4.8). One hundred and ninety four genes were common between all treatments (Fig. 4.8). The number of unique genes was high for each treatment, the highest was observed at 31% PEG treatment, followed by 27% and 34% PEG treatments (Fig. 4.8).

The number of common genes (411) downregulated ≥2-fold is twice as high compared to the common upregulated genes. Similar as the common upregulated genes, the number of downregulated genes decreased with the severity of the dehydration stress. Annotation of both up-and downregulated common genes might reveal important information on the general stress mechanism in triticale seedlings exposed to dehydration stress.

The results also showed several unique genes downregulated ≥50-fold, that were not statistically significant (≥2-fold) in the other treatment(s) (Table 4.2). However, compared to the overexpressed genes, the number of specific downregulated genes was much smaller. The highest number of genes specific to one treatment was observed at 27% PEG treatment (13 and 17 genes) that were not regulated at 31% and 34% PEG treatments. Seven (5 + 2) genes were specific to two treatments 27% and 34% PEG, while not regulated at 31% PEG treatment (Table 4.2).

Annotation of differentially regulated genes

Knowledge of the annotated function of differentially regulated transcripts would allow a more complete view of the triticale seedling response to the three levels of dehydration stress. BLASTx of the statistically differentially regulated (≥2-fold) gene set was performed against the non redundant triticale database using in-house Linux-based BLAST software. The resulting top-hit for each gene was analyzed and annotated with Blast2GO (www.blast2go.org) and revealed a high level of coverage. A total of 3,872 genes were successfully annotated from a total of 4,467 genes, representing 87% of the total gene set. During the annotation process with Blast2GO, some of the genes had more than one annotation assigned to them. A total of 3,326 genes recorded more than 3 hits and were selected for subsequent analyses. Among the 30 species with at least four BLAST hits, the top six species with the most hits were monocots: 1,589 hits were observed in barley (*Hordeum vulgare*), followed by rice (*Oryza sativa*) with 717 hits and sorghum (*Sorghum bicolor*) with 303 hits (Fig. 4.9A). Most of the hits were observed from monocots and represented 95% of the total BLAST hits (Fig. 4.9B). The category marked “others”, which represented 2.9% of the entire distribution, is formed by species that had at least one but <4 hits. Within this category, the distribution of monocots versus dicots was almost equal, each sharing ≈ 42%; the remaining 16% represented other species (bacteria, microorganisms, animals). The category

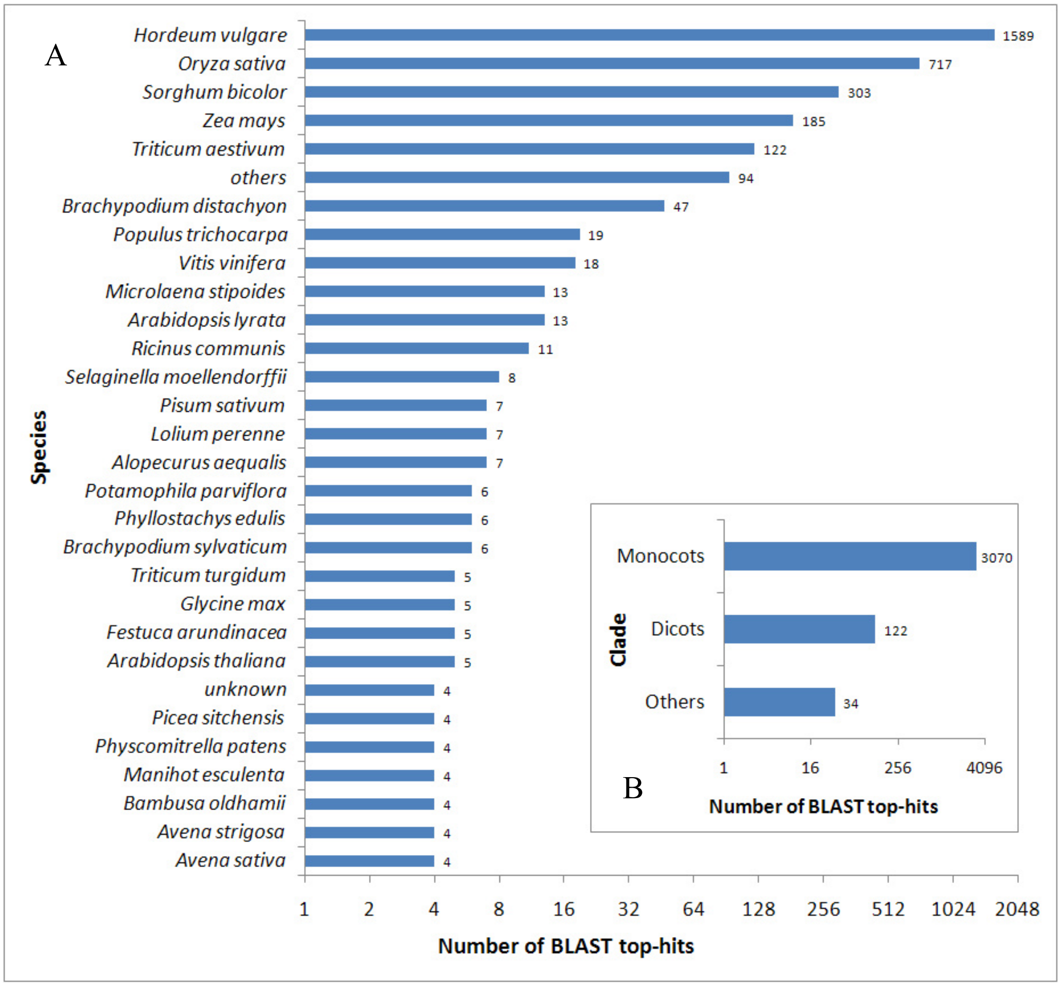


Figure 4.9. Species and genus distribution of the annotated genes based on the BLAST top-hits results of statistically differentially regulated (≥2 fold) genes of the triticale seedlings grown on solid PEG media of different concentrations.

(A) Among the thirty species with BLAST hits, the top six species are monocots. (B) Distribution based on clades shows a clear majority of genes from the monocots, as compared to dicots, showing similarity to the triticale seedling transcriptome. The category "others" represent non-specified and non-plants accessions.

named “unknown” from the species distribution represents BLAST hits where the information of the species is not available in the database (Fig. 4.9A).

The differentially regulated genes annotated using Blast2GO were classified into three categories: biological process, molecular functions and cellular components. The annotated differentially regulated genes were divided into 55 different biological processes groups (Fig. 4.10). Among all processes, some categories were overrepresented, such the ones involved in metabolism (cellular metabolic, primary, macromolecule and nitrogen compound processes), representing 36.6% of total processes. The biosynthetic process also represented 7.8% from all processes.

Three categories represented biological processes involved in the plant’s response to stress (response to stress, response to biotic and abiotic stimuli), represented 5.0% of all processes (Fig. 4.10).

When the differentially regulated genes were classified on their molecular function, they were divided into 27 categories (Fig. 4.11). Close to half of the total genes, 46.56%, were distributed into five classes, all with binding function, either nucleotide (547), ion (543), nucleic acid (452), protein (436) or nucleoside (396) binding. Two other molecular function groups with genes involved in transferase and hydrolase activity represented more than 10% each of the total distribution, with 583 (11.4%) and 558 (10.9%) genes, respectively (Fig. 4.11).

The distribution of the differentially regulated genes based on their cellular localization was divided into eight categories, the majority distributed into two categories, cell component (2,546 genes) and membrane-bounded

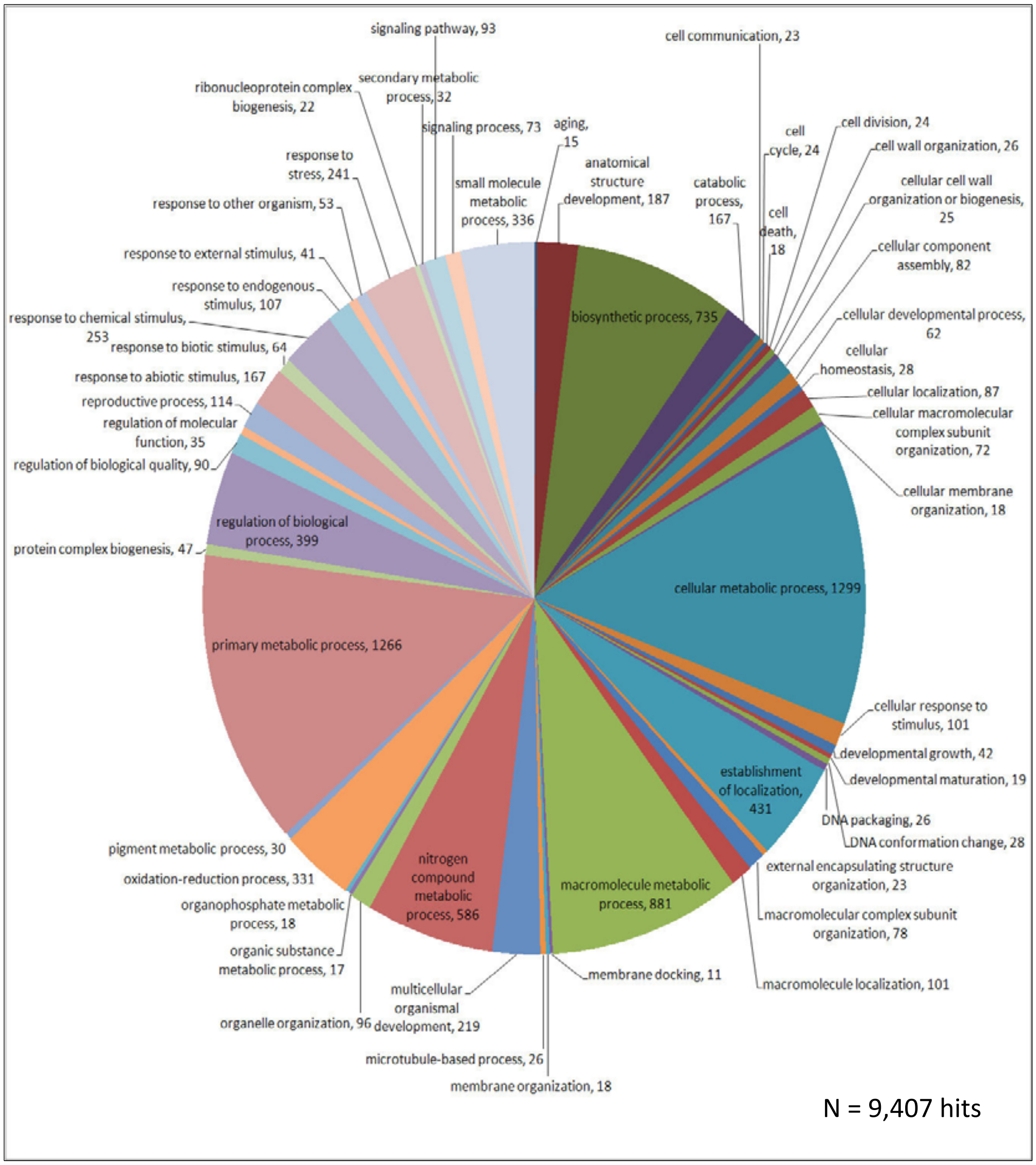


Figure 4.10. Classification of triticale seedlings transcriptome based on GO biological process.

Plant GO terms were used for classification for GO biological process. The numbers beside the processes represent the number of genes corresponding to the biologoical process. There are 55 biological processes corresponding to this GO classification.

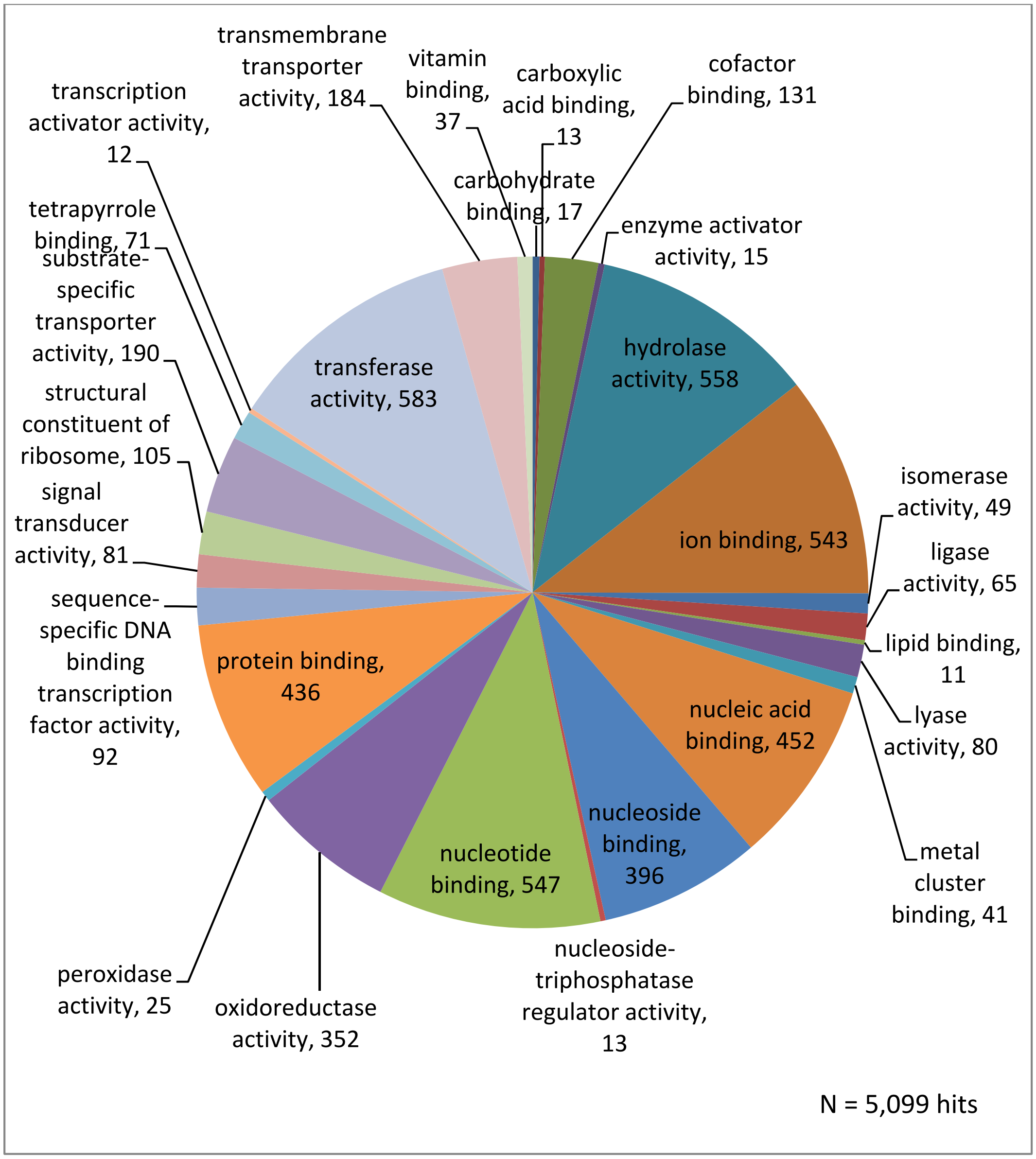


Figure 4.11. Classification of triticale seedlings transcriptome based on GO molecular function.

Plant GO terms were used for classification for GO molecular function. The numbers beside the processes represent the number of genes corresponding to the molecular function. There are 27 categories corresponding to this GO classification.

organelle (1,997 genes), covering 74.2% of the total distribution (Fig. 4.12). The remaining 25.8% was shared by six categories in the following order: organelle component (8.6%), vesicle (7.9%), protein complex (4.3%), non-membrane-bounded organelle (3.8%), apoplast (0.7%) and protein-DNA complex (0.4%) (Fig. 4.12).

A high percentage (87%) of the differentially regulated transcripts was annotated by Blast2GO which allowed for a more complete view of the triticale seedling response to the three levels of dehydration stress. Upon quantification and analysis of the differentially regulated transcripts and gene annotation, the next step was identification of the potential function of the two sets of up-and downregulated genes common to all treatments.

The combined analysis revealed that 84% (158) of the common upregulated genes had annotations (Table S4.2). Of this latter group of genes, 12% (19) were previously identified as involved in dehydration stress with roles in ABA-dependent pathway (11), proline synthesis (2), salt-related (2), leaf senescence (1), ABA-independent pathway (1), root hair differentiation (1) and as a co-chaperone anti-viral protein (1). Similarly, the results showed a very high percentage, 90%, of the common downregulated genes that were successfully annotated (Table S4.3). However, based on the literature information, only 8% (31) of the genes were identified as involved in the plants response to dehydration stress. From these 31 genes, the majority were part of the either ABA-dependent or ABA-independent pathways (Table S4.3).

According to Blast2GO annotations, the remaining 72% upregulated

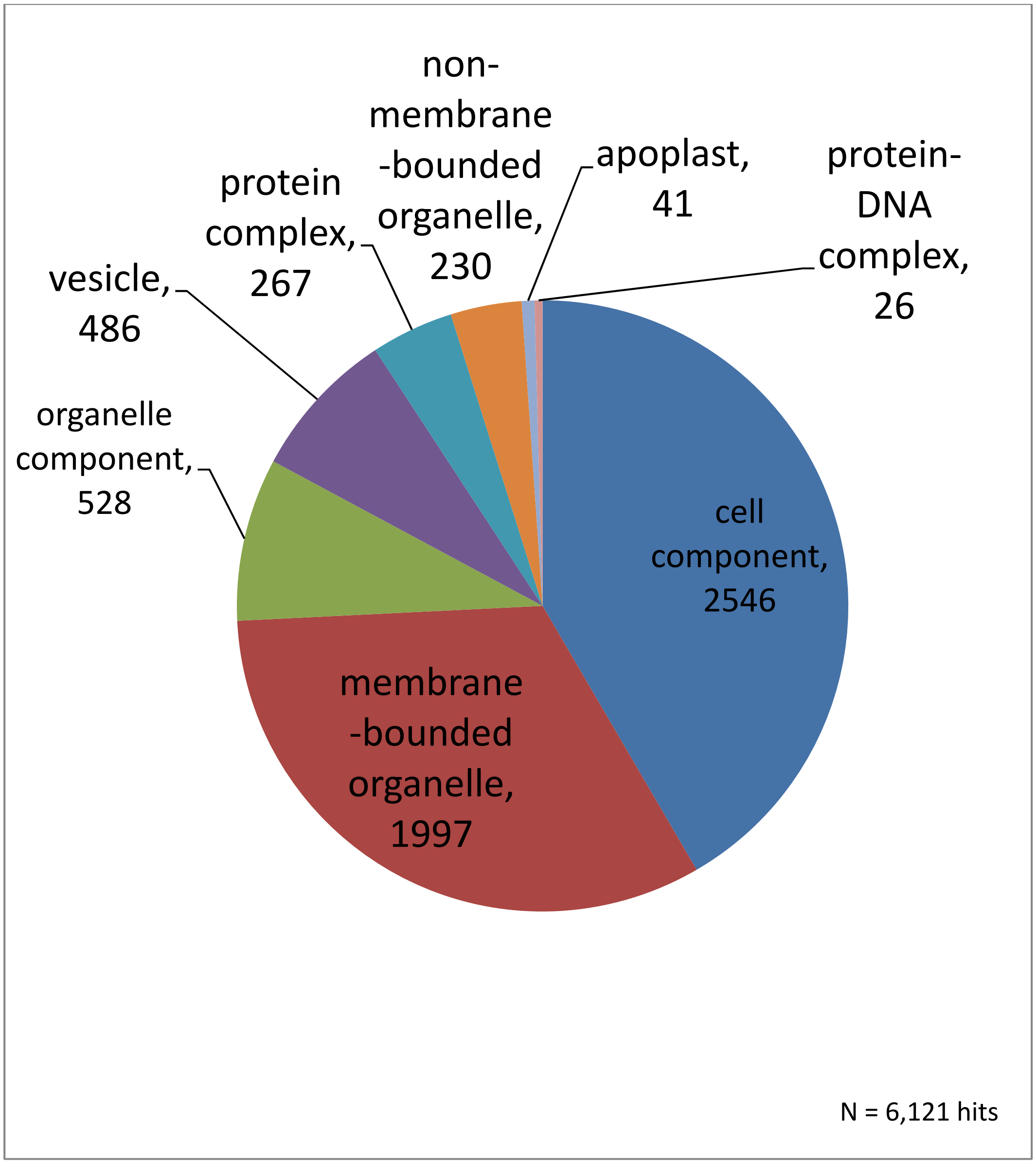


Figure 4.12. Classification of triticale seedlings transcriptome based on GO cellular component.
Plant GO terms were used for classification for GO cellular component. The numbers beside the processes represent the number of genes corresponding to the cellular component. This GO classification is divided into eight cellular component categories.

and 81% downregulated annotated genes common to all treatments were involved in metabolic, biosynthetic, and other biological processes. It is most likely that among these genes, especially the ones with high levels of differential expression have important roles in the plants general response to dehydration that have not been identified in previous studies. The fact that this study employed NGS sequencing might have helped uncover novel dehydration tolerance genes in triticale seedlings. More in-depth attention should be given to these genes and future validation studies should be considered. Moreover, there were still 72 differentially regulated (positive and negative) genes, some of which were highly regulated, that could not be annotated by the Blast2GO analysis. These also should be considered as novel dehydration tolerance gene candidates and should be validated in future studies.

Differential regulation of known transcripts involved in dehydration stress-response

Upon analysis of the results, several groups of genes of the triticale transcriptome were targeted as part of the dehydration-response mechanism of plants, based on the information available in the literature (Seki et al., 2001, 2007; Rodrigues et al., 2009; Alves et al., 2011; Medina et al., 2011). In total, 193 genes were identified as involved in dehydration stress-response and stress in general and represented ≈5% of the total number of annotated genes (Tables 4.3 - 4.5). The annotated transcripts related to dehydration stress were selected and initially separated into two groups: ABA-dependent and ABA-independent genes. These

Table 4.3. Statistically differential regulation (≥2-fold) of 103 ABA-dependent genes of triticale seedlings grown on solid PEG media of different concentrations.

		Fold change		
Gene ID	**Description**	**27% PEG**	**31% PEG**	**34% PEG**
Z-084505	PYR/PYL/RCAR proteins	1404.3	1573.8	2058.1
Z-057283	PYR/PYL/RCAR proteins	159.7	479.2	399.3
Z-002820	WRKY15	42.1	42.1	-
Z-004992	VIP3 protein	40.6	-	40.6
Z-043747	calcium-dependent protein kinase	28.8	115.2	-
Z-084415	NAC5	27.8	13.9	-
Z-085479	MYB	27.1	27.1	-
Z-033602	MYB	19.5	34.0	-
Z-042237	AKIN10	8.0	5.0	4.0
Z-078223	EAP30	6.2	-	-
Z-087218	F-box family protein	5.1	5.1	10.1
Z-074670	abscisic stress ripening protein 2	4.0	4.0	4.0
Z-080994	serine-threonine kinase	4.0	3.0	6.0
Z-085070	MYB	4.0	2.0	2.0
Z-002349	response to salt stress	3.6	3.6	3.6
Z-009386	calcium-dependent protein kinase	3.5	5.5	4.0
Z-009387	calcium-dependent protein kinase	3.5	5.5	4.0
Z-002346	response to salt stress	3.2	3.2	3.2
Z-005118	serine threonine-protein kinase	3.0	8.0	10.0
Z-076505	CBL-interacting protein kinase	3.0	3.0	3.0
Z-022604	PP2C	3.0	6.0	2.0
Z-008233	WRKY	3.0	3.0	2.0
Z-004993	WRKY39	2.0	-	-
Z-004995	WRKY39	2.0	-	-
Z-037777	serine threonine-protein phosphatase pp1	2.0	-	-
Z-001441	ABA-mediated signaling pathway	-	99.0	-
Z-021745	MYB	-	50.6	-
Z-050342	MYB	-	43.8	-
Z-028460	MYB	-	34.8	11.6
Z-059884	protein phosphatases	-	33.0	-
Z-046026	stress induced	-	22.3	-
Z-076621	ABI3	-	21.5	-
Z-048263	early salt stress and cold acclimation-induced protein 2-3	-	17.0	-
Z-055139	ER glycerol-phosphate acyltransferase	-	16.1	-
Z-076622	ABI3	-	14.8	-
Z-053395	WRKY4	-	12.1	-
Z-041659	MYB	-	11.6	11.6
Z-043924	MYB	-	9.7	9.7
Z-033599	MYB	-	7.2	-
Z-033601	MYB	-	7.2	-
Z-033913	serine-threonine kinase	-	5.0	5.0
Z-066302	ABI5	-	5.0	-
Z-066303	ABI5	-	5.0	-
Z-007614	SNRK2-8 (response to osmotic stress)	-	4.0	2.0
Z-063712	PYR/PYL/RCAR	-		58.7
Z-008074	response to oxidative stress, response to abscisic acid stimulus, response to salt stress	-	-	58.4
Z-065598	Protein phosphatases	-	-	28.5
Z-034943	NAC	-	-	24.0
Z-020335	SAPK2	-	-	18.8
Z-032306	late embryogenesis abundant protein	-	-	16.5
Z-030589	serine threonine-protein phosphatase	-	-	16.1
Z-037122	WRKY	-	-	13.7
Z-003091	RAB28	-	-	9.0
Z-021492	WRKY	-	-	2.0
Z-018665	START proteins	-	-	-5.9
Z-074881	WRKY	-	-	-3.0
Z-068436	protein kinase-like protein	-2.0	2.0	-
Z-010891	WRKY	-2.0	-2.0	-
Z-070503	MYB	-2.0	-98.7	-99.7

Z-086494	calcium-dependent protein kinase	-2.2	-	-4.5
Z-083828	serine threonine-protein kinase	-3.0	-	-9.0
Z-050748	NAC2	-3.0	-3.0	-
Z-050734	NAC2	-3.0	-3.0	-
Z-044510	NAC	-4.0	-2.0	-6.0
Z-044511	NAC	-4.0	-2.0	-6.0
Z-046226	MYB	-4.0	-4.0	-
Z-032417	WRKY7	-4.5	-3.0	-4.5
Z-041139	WRKY8	-4.5	-89.9	-90.7
Z-049804	PP2C	-5.0	-6.7	-20.0
Z-086864	WRKY	-5.0	-	-5.0
Z-013390	lectin-like receptor kinase 7	-5.9	-3.8	-
Z-076507	kinases	-6.0	-	-2.6
Z-084252	protein phosphatase	-6.1	-3.9	-4.0
Z-023561	stress induced	-8.0	-2.0	-4.0
Z-064770	map kinase kinase	-8.0	-8.0	-2.7
Z-034992	MYB	-8.1	2.0	-5.3
Z-014359	PYR/PYL/RCAR	-8.2	-5.3	-5.4
Z-015597	stress induced	-8.9	-8.9	-
Z-004994	WRKY39	-9.0	-	-
Z-004996	WRKY39	-9.0	-	-
Z-048859	MYB1	-9.3	4.0	-
Z-044427	leucine-rich receptor-like protein kinase	-12.3	-2.0	-8.0
Z-033594	calcium-dependent protein kinase	-14.7	-	-9.6
Z-044146	MYB	-16.3	-10.6	-10.7
Z-052963	WRKY25	-18.5	-12.0	-12.1
Z-076866	ABI5	-22.3	-14.4	-14.6
Z-048467	MYB	-22.4	-	-14.7
Z-063491	phosphoenolpyruvate carboxylase kinase	-28.5	-	-
Z-034741	MYB	-29.3	-19.0	-19.2
Z-044314	SAPK4	-29.6	-	-
Z-004976	CBL4	-30.6	-5.0	-5.0
Z-044370	MYB1	-31.7	-20.6	-20.8
Z-062695	NAC5	-34.2	-22.2	-22.4
Z-075502	protein phosphatases	-43.7	-	-28.6
Z-028458	MYB	-51.6	-2.0	-6.0
Z-074291	MYB4	-91.9	-59.6	-3.0
Z-074507	calcium-dependent protein kinase	-93.3	-2.2	-3.0
Z-077354	PP2AC2	-104.5	-67.8	-68.4
Z-017912	NAC	-108.2	-4.3	-70.9
Z-034012	calcium-dependent protein kinase	-140.2	-6.5	-13.0
Z-031190	protein phosphatases	-250.7	-8.0	-8.0
Z-042508	WRKY	-519.8	-16.0	-340.2
Z-076502	WRKY	-	-11.0	-11.1

“-“ no statistical difference in expression level of contig

Table 4.4. Statistically differential regulation (≥2-fold) of 36 ABA-independent genes of triticale seedlings grown on solid PEG media of different concentrations.

		Fold change		
Gene ID	**Description**	**27% PEG**	**31% PEG**	**34% PEG**
Z-076067	DREB2	15.7	47.2	36.7
Z-059815	ethylene-insensitive protein	3.7	-	-
Z-033981	DREB	-	22.8	-
Z-003663	ICE1	-	12.3	-
Z-085943	DREB1B	-	7.6	-
Z-067424	ethylene receptor	-	7.5	37.6
Z-004028	ethylene receptor	-	2.0	-
Z-038627	AP2	-	2.0	-
Z-016339	CBF	-	-	30.6
Z-064084	cold acclimation protein	-	-	-2.7
Z-013215	ERBP4	-	-3.0	-32.3
Z-056185	DREB	-	-5.0	-5.0
Z-021388	ethylene-insensitive3-like 1	-2.6	-	-
Z-021386	ethylene-insensitive3-like 1	-2.8	-3.8	-3.8
Z-021382	ethylene-insensitive3-like 1	-2.8	-3.8	-3.8
Z-021387	ethylene-insensitive3-like 1	-2.9	-3.9	-3.9
Z-021390	ethylene-insensitive3-like 1	-2.9	-3.9	-3.9
Z-002654	WCOR413	-3.0	-	-
Z-065618	ethylene receptor	-3.0	-3.0	-8.8
Z-076064	DREB2	-6.7	-2.2	-2.9
Z-066544	DREB2	-7.5	-2.5	-
Z-058139	DREB/DBF1	-8.5	-3.4	-2.4
Z-036737	AP2	-9.8	-6.4	-6.4
Z-016340	CBF	-15.7	-10.2	-10.3
Z-087043	ethylene constitutive triple response protein	-16.5	-	-10.8
Z-014948	DREB/DBF1	-22.7	-14.7	-14.9
Z-033494	EREB4	-33.7	-	-
Z-015445	AP2 ERF	-34.2	-2.0	2.5
Z-015446	AP2 ERF	-34.2	-2.0	2.5
Z-024476	EREB2	-36.5	-	-
Z-087030	EREB4	-36.8	-	-
Z-024475	EREB2	-48.9	-	-
Z-057614	DREB	-207.5	-134.6	-6.0
Z-000334	defense response to bacterium	-433.6	-2.3	-
Z-063872	AP2-related transcription factor	-484.3	-14.0	-7.0
Z-084000	cold acclimation protein	-	-4.6	-4.7

"-" no statistical difference in expression level of contig

Table 4.5. Statistically differential regulation (≥2-fold) of 54 other genes involved in dehydration stress response of triticale seedlings grown on solid PEG media of different concentrations.

			Fold change		
Gene ID	**Role**	**Description**	**27% PEG**	**31% PEG**	**34% PEG**
Z-041070	senescence	leaf senescence	211.7	317.6	317.6
Z-008424	salt	response to salt stress	210.7	210.7	126.4
Z-053745	proline	proline-rich protein	59.4	-	19.8
Z-034319	stress	universal stress protein	45.5	75.9	45.5
Z-034320	stress	universal stress protein	45.5	75.9	45.5
Z-061522	dehydrins	WDHN13	43.4	-	-
Z-061521	dehydrins	WDHN13	36.1	-	-
Z-001028	cell wall	cell wall loosening	25.0	-	25.0
Z-001234	root	root morphogenesis	24.2	-	24.2
Z-061520	dehydrins	DHN13	22.3	401.6	502.1
Z-007973	salt	RGP1-5, response to salt stress	17.0	17.0	17.0
Z-065936	proline	prolyl 4-hydroxylase alpha-2	9.7	9.7	-
Z-065935	proline	prolyl 4-hydroxylase alpha-2	9.7	9.7	-
Z-007067	proline	proline oxidase	9.6	-	19.2
Z-007028	proline	P5CS	6.4	-	-
Z-021347	stress	NAC1	6.0	-	-15.7
Z-066584	proline	P5CR	4.0	2.5	7.5
Z-066580	proline	P5CR	4.0	2.5	7.5
Z-003790	ethylene	ERF1	3.0	3.0	5.0
Z-034638	stress	DRP1	3.0	3.0	-
Z-034307	proline	P5CS	2.3	-	-
Z-002671	jasmonic acid	HPL1, response to jasmonic acid stimulus, response to wounding	2.0	4.0	5.0
Z-041870	dehydrins	DHN15	-	39.7	39.7
Z-005048	root	root hairless expressed	-	25.7	-
Z-034748	stress	universal stress protein family protein	-	21.3	21.3
Z-005046	root	root hairless expressed	-	20.5	-
Z-004880	stress	serine threonine protein kinase	-	14.4	-
Z-002526	meristem	NAM	-	12.0	-
Z-056906	proline	P5CR	-	8.2	28.8
Z-014305	dehydration	ERD4	-	2.0	2.0
Z-020052	oroline	proline transporter	-	2.0	-
Z-002969	stress	universal stress protein	-	-	5.5
Z-002971	ethylene	universal stress protein	-	-	3.2
Z-002970	ethylene	universal stress protein	-	-	3.1
Z-031420	salt	STRP_Salt tolerant protein	-	-	2.0
Z-006016	development	RPT2a, lateral root primordium development, shoot development, root cap development, meristem growth	-2.2	-4.5	-3.0
Z-005078	jasmonic acid	JRG21 (jasmonate-regulated gene 21)	-2.7	-	-
Z-000353	salt	response to salt stress	-5.0	-	-2.5
Z-030990	stress	PP2C	-9.7	-	-6.3
Z-046125	stress sensors	stress induced protein 1	-9.9	-6.4	-
Z-004508	salt	OSM34 (osmotin 34), response to salt stress, defense response to bacterium and fungus	-10.0	-2.5	-10.0
Z-004453	jasmonic acid	NPR3, defense response to bacterium, regulation of jasmonic acid mediated signaling pathway	-10.1	-	-
Z-015598	early drought	early drought induced protein	-23.2	-15.0	-15.2
Z-025692	proline	proline-rich family protein	-23.8	-	-15.6
Z-079002	stress	DRF1	-31.0	-	-
Z-007668	biotic stress	SR1 (signal responsive 1), response to biotic stimulus	-55.9	-36.3	-36.6
Z-002775	stress	protein phosphatase 2c family	-140.2	-2.5	-91.8
Z-057124	salt	STRP_Salt tolerant protein	-143.5	-7.0	-93.9
Z-057217	proline	proline oxidase	-179.3	-7.0	-14.0

Z-044131	salt	STRP_Salt tolerant protein	-	-2.7	-
Z-044132	salt	STRP_Salt tolerant protein	-	-2.7	-
Z-044133	salt	STRP_Salt tolerant protein	-	-2.7	-
Z-064504	ethylene	ethylene response protein	-	-3.0	-
Z-004163	stress	RP120	-	-	-25.6

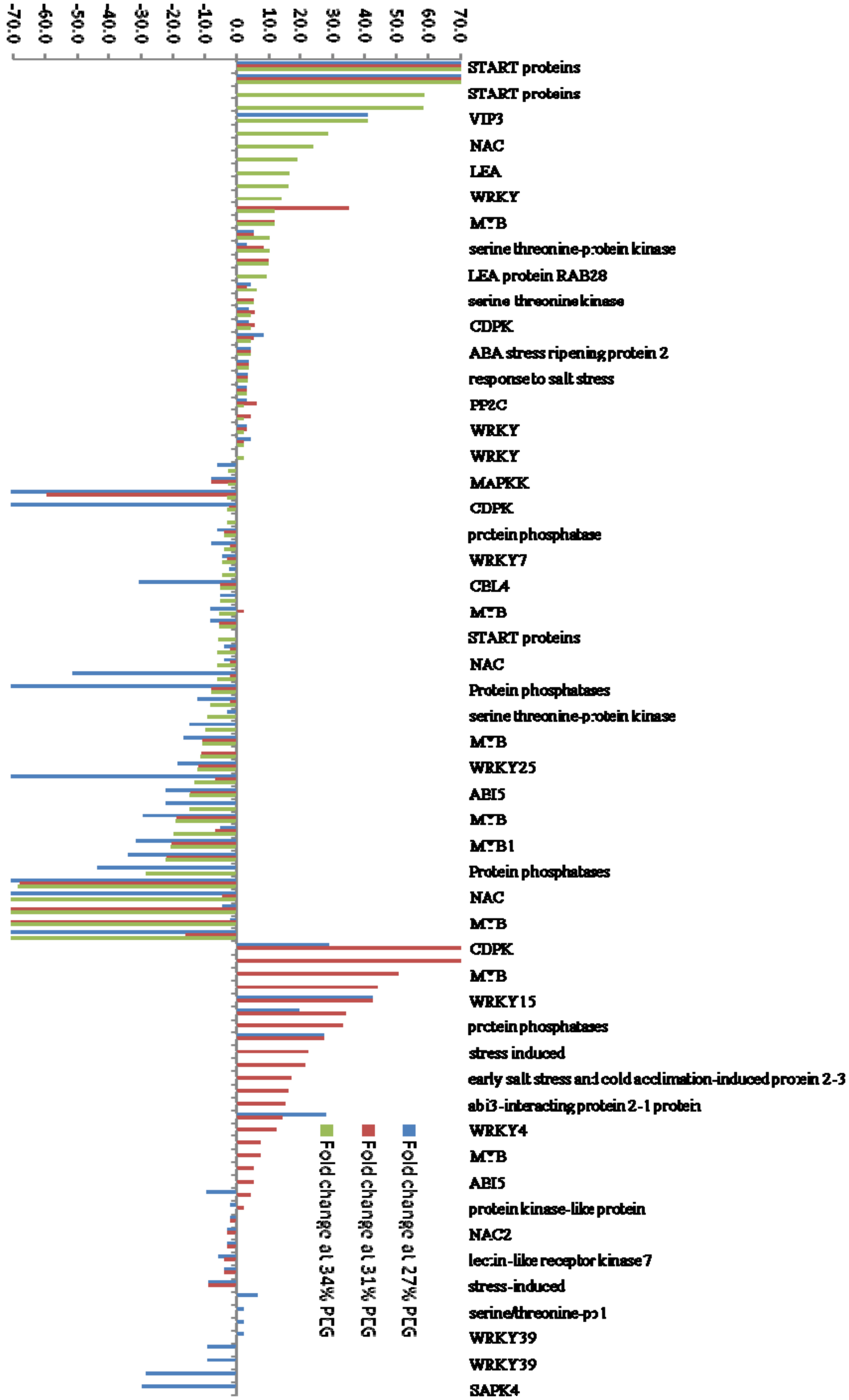
“-“ no statistical difference in expression level of contig

two pathways are the most commonly studied in dehydration experiments. However, the very high number and proportion of transcripts recovered and annotated allowed identification of other genes not directly part of the two above-mentioned pathways. Therefore, a third group of genes was added in our classification and consisted in genes identified in literature as being involved in signalling or early response to dehydration and/or stress in general that were annotated as such in our analysis (Figs. 4.13 - 4.15). Some of these genes were previously presented as common genes for all PEG treatments and will be presented in a different context in this section to emphasis their role in the plants response to dehydration stress.

Among previously identified genes in dehydration studies, 103 differentially regulated genes were classified as ABA-dependent (Fig. 4.13) and 36 genes as ABA-independent genes (Fig. 4.14). Fifty four differentially regulated genes were classified as genes with implied functions in signalling, early responses to stress in general, or dehydration stress, not included in the two previous groups (Fig. 4.15).

From the 103 ABA-dependent regulated genes, 54 were upregulated at least at one treatment; among these were the *PYR/PYL/RCAR* genes, which are the newly discovered ABA co-receptors (Fujii et al., 2009; Melcher et al., 2009; Park et al., 2009; Weiner et al., 2010; Santiago et al., 2012). Two genes (both *PYR* genes) were upregulated ≥100-fold in all the treatments, four genes (a *PYR*, a dehydration and salt dependent, a calmodulin-dependent protein kinase and a

Figure 4.13. Expression of 103 ABA-dependent differentially regulated genes in triticale seedlings grown on solid PEG media of different concentrations (0%, 27%, 31%, and 34% PEG). (visible names for 52 genes; complete list in Table 4.3)



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-40.0
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-20.0
-10.0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
START proteins
START proteins
VIP3
NAC
LEA
WRKY
MYB
serine threonine-protein kinase
LEA protein RAB28
serine threonine kinase
CDPK
ABA stress ripening protein 2
response to salt stress
PP2C
WRKY
WRKY
MAPKK
CDPK
protein phosphatase
WRKY7
CBL4
MYB
START proteins
NAC
Protein phosphatases
serine threonine-protein kinase
MYB
WRKY25
ABI5
MYB
MYB1
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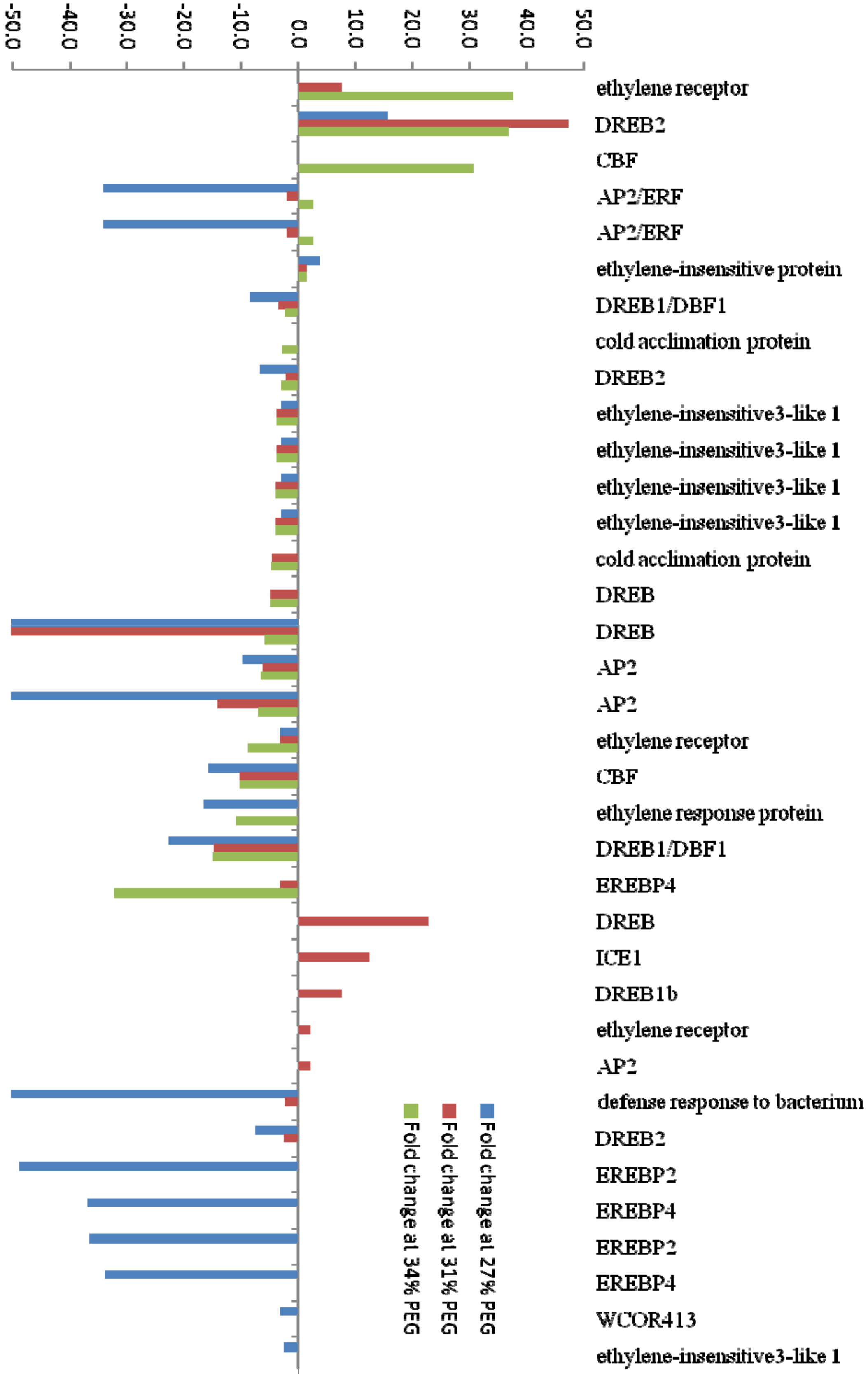
NAC
MYB
CDPK
MYB
WRKY15
protein phosphatases
stress induced
early salt stress and cold acclimation-induced protein 2-3
abi3-interacting protein 2-1 protein
WRKY4
MYB
ABI5
protein kinase-like protein
NAC2
lectin-like receptor kinase 7
stress-induced
serine/threonine-p 1
WRKY39
WRKY39
SAPK4
Fold change at 27% PEG
Fold change at 31% PEG
Fold change at 34% PEG

Figure 4.14. Expression of 36 ABA-independent differentially regulated genes in triticale seedlings grown on solid PEG media of different concentrations (0%, 27%, 31%, and 34% PEG).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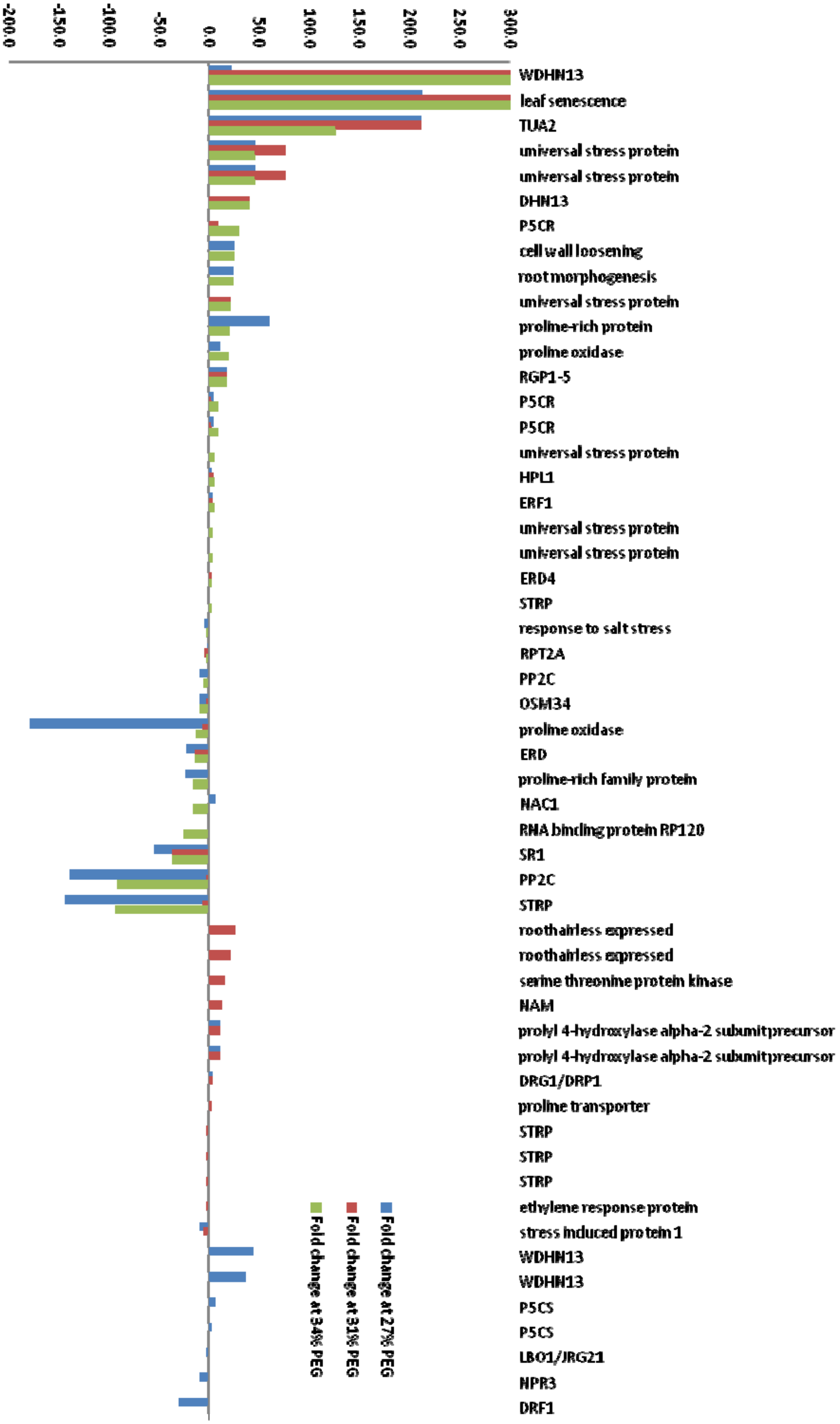
-20.0
-10.0
0.0
10.0
20.0
30.0
40.0
50.0
ethylene receptor
DREB2
CBF
AP2/ERF
AP2/ERF
ethylene-insensitive protein
DREB1/DBF1
cold acclimation protein
DREB2
ethylene-insensitive3-like 1
ethylene-insensitive3-like 1
ethylene-insensitive3-like 1
ethylene-insensitive3-like 1
cold acclimation protein
DREB
DREB
AP2
AP2
ethylene receptor
CBF
ethylene response protein
DREB1/DBF1
EREBP4
DREB
ICE1
DREB1b
ethylene receptor
AP2
defense response to bacterium
DREB2
EREBP2
EREBP4
EREBP2
EREBP4
WCOR413
ethylene-insensitive3-like 1
Fold change at 27% PEG
Fold change at 31% PEG
Fold change at 34% PEG

Figure 4.15. Expression of 54 early stress-responsive differentially regulated genes in triticale seedlings grown on solid PEG media of different concentrations (0%, 27%, 31%, and 34% PEG).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0.0
50.0
100.0
150.0
200.0
250.0
300.0
WDHN13
leaf senescence
TUA2
universal stress protein
universal stress protein
DHN13
P5CR
cell wall loosening
root morphogenesis
universal stress protein
proline-rich protein
proline oxidase
RGP1-5
P5CR
P5CR
universal stress protein
HPL1
ERF1
universal stress protein
universal stress protein
ERD4
STRP
response to salt stress
RPT2A
PP2C
OSM34
proline oxidase
ERD
proline-rich family protein
NAC1
RNA binding protein RP120
SR1
PP2C
STRP
roothairless expressed
roothairless expressed
serine threonine protein kinase
NAM
prolyl 4-hydroxylase alpha-2 subunit precursor
prolyl 4-hydroxylase alpha-2 subunit precursor
DRG1/DRP1
proline transporter
STRP
STRP
STRP
ethylene response protein
stress induced protein 1
WDHN13
WDHN13
P5CS
P5CS
LBO1/JRG21
NPR3
DRF1
🟦 Fold change at 27% PEG
🟥 Fold change at 31% PEG
🟩 Fold change at 34% PEG

MYB) were upregulated ≥50-fold in one treatment, and 30 genes (among them *WRKY*s, *MYB*s and *VIP3*) were at least 10-fold upregulated 2 or 3 different treatments. Of the entire group of upregulated transcripts, 15 (e.g. calcium-dependent and serine/threonine protein kinases) were upregulated at least 2-fold for all treatments (Table 4.3). Two ABA-dependent transcripts were positively and negatively regulated among treatments, a protein kinase (Z-068436) and a *MYB* (Z-048859), showing negative regulation at 27% PEG and upregulation at 31% PEG treatments, with no regulation at 34% PEG. A total of 49 genes from the ABA-dependent pathway were downregulated. Five of them (e.g. calcium-dependent kinase, *WRKY, PP2A, NAC*) were ≥100-fold downregulated and 3 genes (e.g. calcium-dependent kinase, *MYB*) were downregulated ≥50-fold for at least one treatment (Table 4.3). Twenty-five genes were downregulated at least 10-fold.

Similarly, a wide range of differential expression was observed for the genes related to the ABA-independent pathway (Fig. 4.14). Among the 36 differentially regulated genes, some of the genes were identified as dehydration-response element binding 1 and 2 (*DREB1*, *DREB2*). Interestingly, only nine genes were upregulated, of which only one (*DREB2*) for all three treatments, and one (ethylene receptor) for two treatments (Table 4.4). The other six upregulated genes were differentially regulated at only one treatment and two (*DREB*, *ICE1*) of them were upregulated ≥10-fold (Table 4.4). Compared to the upregulated genes, the number of downregulated genes was three times higher with 27 genes. Fourteen genes were downregulated ≥10-fold and 3 (AP2-related TF, defense response to bacterium, *DREB*) genes were ≥100-fold. Twelve genes were downregulated at least 2-fold in all PEG treatments, 8 genes at two treatments and 5 genes at only one treatment (Table 4.4). Two AP2ERF domain transcripts had dual regulation, showing >30- and 2-fold downregulation at 27% and 31% PEG, while upregulated 2.5-fold at 34% PEG (Table 4.4).

From the 54 regulated (≥2-fold) genes with involvement in early stress response, 34 were upregulated, 19 downregulated and one gene, *NAC1* (Z-021347) had positive regulation at 27% PEG and negative regulation at 34% PEG treatments (Fig. 4.15). From the 34 upregulated genes, 10 (e.g. leaf senescence, *TUA2*, *P5CR*, *HPL1, DRP4, RGP1-5,* universal stress proteins*, ERF1)* were upregulated in all PEG treatments. Three (*WDHN13*, leaf senescence, *TUA2*) transcripts were overexpressed ≥100-fold, 3 (proline and 2 universal stress proteins) were ≥50-fold and 13 (e.g. *WDHN13*, *RGP1-5*, *DHN1-5*, *NAM*, *P5CR*, root hairless, universal stress proteins) ≥10-fold (Table 4.5). The number of downregulated transcripts specific to one or two treatments (13) was almost double as compared to gene represented in all treatments (7), of which some of the genes were salt-responsive and jasmonic acid-regulated genes (Table 4.5).

Overall, from the total of 193 statistically differential expressed (≥2-fold) genes identified as dehydration-responsive, 97 genes were upregulated, 92 were downregulated, and four genes had different regulation depending on the treatment. The majority of the upregulated genes were ABA-dependent, while the lowest representation was for the ABA-independent genes.

The remaining 13% (640) of non-annotated transcripts were differentially regulated within a range of 658-fold upregulated to 553-fold downregulated. These values are high enough to be considered as genes with an important role in the plants response to dehydration in triticale seedlings.

The levels of overexpression were different from one treatment to the other. The most overexpressed transcripts was observed at 34% PEG (215), followed by 31% (204) and 27% PEG (177) (Table S4.4). The number of genes per treatment increased with the severity of the stress. Eight transcripts were overexpressed ≥100-fold in all the treatments and 6 were common and overexpressed 10- to ≤50-fold. Even if there were 85 transcripts in all treatments overexpressed between 50- to <100 fold, none of them were common among the treatments.

In contrast with the previous group, the number of downregulated genes observed at each treatment was decreasing with the increase of the stress severity as 211, 175 and 165 transcripts were present in 27%, 31%, and 34% PEG treatments, respectively (Table S4.4). Seven transcripts downregulated ≥100-fold were common to all treatments; six were common in the range of 50- to <100-fold and 19 common transcripts in the range of 10- to ≤50-fold.

The next step was to attempt to identify the function of the non-annotated genes with the help of several tools. First, the genes were clustered based on their differential regulation and sequence similarity at all PEG treatments. Therefore, non-annotated genes grouped with annotated genes with the possibility to have the same function as the later ones. This is not a highly accurate method to identify gene function and one should be aware of the limitations given by its simplicity. Subsequent analysis is needed to validate the role of the genes. There were clusters containing only non-annotated genes that could not have any function assigned. Still, based on these clusters, some of the non-annotated genes were assigned a potential role in the plants response to dehydration stress. The analysis helped to putatively identify the function of some of the non-annotated genes. However, this approach is not to be ignored because it still gives important information of the potential function and could be a first step in selecting for validation of potential novel genes with role in dehydration tolerance in triticale seedlings.

In the non-annotated group we identified a highly upregulated gene, with 658-fold at 34% PEG and, based on our clusters we identified a potential function as ribosomal protein S10. Similarly, for the lowest downregulated gene, with 552-fold at 27% PEG, we potentially identified one ABA-independent AP2-related transcription factor. However, for this set of transcripts additional studies are needed to validate their potential function.

The results show that only by looking at the annotations uncovered with Blast2GO, from the 600 genes common to all treatments, 549 (91%) could be potential novel genes with involvement in dehydration tolerance. Forty eight (8%) of these genes were differentially regulated 50-fold or more and were highly regarded as novel dehydration genes in triticale seedlings. From the total of 48 genes, 20 (42%) were non-annotated of which 40% were upregulated and the rest downregulated (Table 4.6). However, the rest of the genes (28) were successfully

Table 4.6. Potential novel dehydration tolerance genes common to all treatments

Gene ID	Gene annotation	27% PEG	31% PEG	34% PEG
Z-081475	-	610.8	166.5	445.0
Z-068270	-	264.5	163.0	185.8
Z-057282	-	263.9	197.9	230.9
Z-024119	chloroplast 30s ribosomal protein S10	203.8	101.9	237.8
Z-030725	-	179.7	119.8	658.8
Z-030793	-	175.1	116.7	525.2
Z-064168	-	155.2	103.5	155.2
Z-049948	60s ribosomal protein L11	140.5	140.5	140.5
Z-018050	-	133.9	133.9	200.8
Z-072191	-	130.1	130.1	195.1
Z-087869	ATP-dependent CLP protease	126.4	632.2	569.0
Z-063852	phagocytosis and cell motility protein	119.8	119.8	179.7
Z-065057	calcineurin b subunit	108.4	108.4	108.4
Z-030794	NADH-plastoquinone oxidoreductase subunit k	105.9	211.7	423.4
Z-024486	40s ribosomal protein	92.3	92.3	61.5
Z-063395	protein binding protein	90.5	55.7	69.6
Z-085349	embryo-specific protein 1	76.4	91.7	61.1
Z-011223	nucleolar protein	56.9	56.9	56.9
Z-088057	class III peroxidase 57 precursor	-77.6	-50.3	-50.8
Z-023309	-	-78.4	-50.8	-51.3
Z-032874	-	-78.4	-50.8	-51.3
Z-086036	-	-78.4	-50.8	-51.3
Z-084817	protein binding	-82.0	-53.2	-53.7
Z-033149	signal recognition particle 54 kda subunit	-82.0	-53.2	-53.7
Z-074819	-	-85.0	-55.1	-55.7
Z-003822	oxysterol-binding expressed	-85.9	-55.7	-56.3
Z-060515	predicted protein [Hordeum vulgare]	-85.9	-55.7	-56.3
Z-065052	proteasome maturation factor ump1 protein	-87.3	-56.7	-57.2
Z-010867	-	-88.8	-57.6	-58.1
Z-078759	-	-88.8	-57.6	-58.1
Z-063258	chitinase b	-88.8	-57.6	-58.1
Z-084396	protein	-89.8	-58.2	-58.8
Z-001295	thioredoxin	-91.1	-59.1	-59.6
Z-016039	naringenin 3-dioxygenase like protein	-93.5	-60.6	-61.2
Z-023514	mitogen-activated protein kinase kinase	-99.3	-64.4	-65.0
Z-076792	-	-159.8	-103.6	-104.6
Z-040223	-	-170.0	-110.2	-111.3
Z-023260	endonuclease exonuclease phosphatase protein	-173.7	-112.6	-113.7
Z-044109	protein	-173.7	-112.6	-113.7
Z-018620	BAX inhibitor motif-containing protein 4	-173.7	-112.6	-113.7
Z-038607	-	-177.6	-115.1	-116.2
Z-047702	-	-190.2	-123.4	-124.5
Z-040118	flavonol 3-sulfotransferase	-210.3	-136.4	-137.6
Z-088497	n-terminal domain-containing protein	-218.3	-141.6	-142.9
Z-063449	enzyme of the cupin superfamily	-259.9	-168.5	-170.1
Z-072960	s-adenosyl-l-homocysteine hydrolase	-269.0	-174.5	-176.1
Z-014669	-	-277.9	-180.3	-181.9
Z-018320	-	-326.2	-211.5	-213.5

Note: only genes differentially regulated ≥50-fold are included in this table
“-“ no annotation

annotated with Blast2GO. Ten genes were upregulated and 18 downregulated in this group and the top two upregulated genes were represented by ribosomal proteins (≥100-fold) (Table 4.6). In fact, all the annotated transcripts were identified as ubiquitous proteins that could have been easily ignored in our analysis if only seen through Blast2GO information (Table. 4.6).

4.4. Discussion

This study presents the transcriptome analysis of triticale seedlings grown on solid media with three different PEG concentrations.

The scope of this project was to identify genes involved in dehydration stress response at early stage of development in triticale using RNA-Seq, and to demonstrate the usefulness of a new system to induce dehydration stress in controlled conditions while eliminating other stresses.

The availability of the triticale seedling transcriptome is very important due to several factors. First, this species is a cross between wheat and rye which inherited abiotic and biotic resistance from rye, and high grain and biomass yield from wheat. Identifying the genes involved in dehydration tolerance will be important in understanding its response to dehydration stress, increasing the possibility to develop cereals with increased tolerance to stress, while maintaining or increasing their yield.

The 454 GS FLX Titanium system generated 1.3 million sequences with individual reads up to 500 bp. The average contig length was 595 nt, which is significantly longer than other NGS systems (Ergen et al., 2009; Franssen et al., 2011; Swarbreck et al., 2011). The analysis revealed a large number of assembled reads (>70%) which also is considered superior as compared to other studies in which on average only 50-60% of the reads were assembled (Ergen et al., 2009; Franssen et al., 2011; Swarbreck et al., 2011). These previous studies (Ergen et al., 2009; Franssen et al., 2011; Swarbreck et al., 2011), using comparison analysis against known genetic information, showed that a large percentage of the transcriptome was sequenced. Although currently it is impossible to precisely estimate the transcriptome coverage upon sequencing without a full genomic reference, comparison of our data with one of the above observations suggested that we recovered a large portion of the transcriptome. On the other hand, the number of singletons resulting from the assemblies, both pooled and individuals, was moderate at 21% and 27% in both cases, much lower than most previous studies (Ergen et al., 2009; Franssen et al., 2011; Swarbreck et al., 2011). Singletons result from different causes such as sequencing errors, template contamination or effect of assembly algorithms (Wang et al., 2009; Franssen et al., 2011). A lower number of singletons was observed in the pooled assembly compared to the individual assemblies, suggesting that some unassembled reads of the individual assemblies may be highly similar and when pooled into one library, a reduction in the number of singletons resulted.

Four thousand four hundred and sixty seven genes were statistically differentially regulated (≥2-fold) in at least one treatment. The differentially regulated genes were grouped into different categories based on their expression pattern to ensure a better analysis and understanding of the regulation of the gene expression. The eight classes, four of upregulated and four of downregulated genes revealed major differences in gene expression patterns in response to different levels of dehydration stress.

Previous studies showed that there are genes that are always regulated during dehydration stress, regardless of the severity, period of stress or even species of plant (Agarwal et al., 2007; Suprunova et al., 2007; Vendruscolo et al., 2007; Chen et al., 2008; Xu et al., 2008a, 2008b; Caruso et al., 2009; Gao et al., 2009; Hura et al., 2009a, 2009b; Li et al., 2009; Qiu and Yu, 2009; Rodrigues et al., 2009; Wang and Dong, 2009; Alcázar et al., 2010; Ashraf, 2010; Lata et al., 2010; Orellana et al., 2010; Raghavendra et al., 2010; Schramm et al., 2010; Villegas et al., 2010; Wilkinson and Davies, 2010; Yoo et al., 2010). Our study revealed 600 differentially regulated genes common to all PEG treatments. Bases on the differential regulation, some of these genes should be considered important candidates as part of a common response of triticale seedlings to dehydration stress.

The analysis of the results for 27% and 31% PEG showed a similar pattern in which there was observed a smaller number of highly regulated genes unique to a treatment and a larger number of moderately regulated genes, suggesting that the number of genes highly regulated only at specific levels or stress is small. However, there was one exception, observed at 34% PEG treatment, where there was a high number of unique genes overexpressed ≥100-fold. This could be due to the extreme level of dehydration stress on the seedlings that could have activated not only dehydration-specific genes but also genes involved in plants survival.

The annotation analysis will give a better understanding of the distribution of the genes in these groups of expression levels and would make for a much direct comparison with the current studies (Horan et al., 2008; Vandepoele et al., 2009; Eveland et al., 2010; Maheswari et al., 2010).

A high percentage (87%) of the statistically differentially regulated genes exhibited significant matches in the annotated sequences with existing genes in the public databases following Blast2GO analysis. The finding that 95% of the BLAST hits were genes identified and characterized in monocots is important, suggesting that the majority of the annotated genes involved in triticale response to dehydration were present in other monocot plants. This could have positive impact for future studies to enhance dehydration resistance in other cereals based on the triticale response to dehydration stress.

The three Blast2GO classes, biological process, molecular functions and cell localizations allowed identification of the genes with potential roles in dehydration response and classification based on their transcriptional regulatory network.

The results grouped the genes in 55 biological processes. By only looking at GO information, one can say that the majority of these genes are considered ubiquitous and not involved in dehydration stress response. However, some of these genes were highly differentially regulated and these results suggested that they might play an important role in the plant response to dehydration. These important genes could have been easily ignored and missed if we would have only used the GO information and not in combination with differential regulation results. The GO analysis showed only three categories of biological processes representing 5% of all processes and represented by 472 genes that were involved in plants stress response, either abiotic or biotic.

Based on the second GO criteria, 67% of the genes were classified as involved in binding, transferase and hydrolase activities and these also are ubiquitous genes involved in all cell function. However, the 185 genes involved in transcription activation, signal transduction and transcription factor activities could represent genes directly involved in plants response to dehydration, and were later classified as ABA-dependent, ABA-independent or genes involved in early stress response (Pérez-Rodríguez et al., 2010). Even though this represented a small number compared to the majority of the genes, we considered them important factors in identification of genes implicated in dehydration stress response.

Similarly, the last criteria, cellular localization, provided only limited information related to dehydration stress, since the majority of the genes were grouped in two categories.

However, these GO major distribution groups, allowed identification and classification of all the genes with direct involvement in dehydration stress. These differentially regulated genes were further divided into ABA-dependent, ABA-independent and other genes involved in early dehydration response (Vendruscolo et al., 2007; Qiu and Yu, 2009; Rodrigues et al., 2009; Wang and Dong, 2009;

Orellana et al., 2010; Raghavendra et al., 2010; Schramm et al., 2010; Villegas et al., 2010; Wilkinson and Davies, 2010). Interestingly, without combining both the differential regulation and GO information extracted from the results only a small percentage (10%) would have been picked as involved in dehydration stress response (according to annotation information) of the common genes and only 5% of the entire set.

Notably, from the genes related to the ABA-dependent pathway, a few were highly overexpressed at all treatments, particularly two *PYR/PYL/RCAR*s identified as START proteins (Soccio and Breslow, 2003) with expression levels ≥100-fold up to 2,058-fold. Pyrabactin resistance/ pyrabactin resistance-like / regulatory component of abscisic acid receptor (PYR/PYL/RCARs) have a role in ABA signalling *in vivo* (Fujii et al., 2009; Melcher et al., 2009; Park et al., 2009; Weiner et al., 2010; Santiago et al., 2012). Studies have shown that ABA binds to PYRs, which in turn bind and inhibit type 2C protein phosphatases (PP2C) and together they are considered to form the ABA signalling receptors (Fujii et al., 2009; Melcher et al., 2009; Park et al., 2009) (Fig. 2.4). Notably, some of the highly downregulated genes, up to 250-fold, related to ABA-dependent pathway were protein phosphatases 2A and 2C. By showing such high levels of upregulation of the START proteins and downregulation of the kinases PP2C, our study support the model of ABA signalling recently identified by Melcher et al. (2009) and Park et al. (2009). For the first time in a dehydration stress experiment, it was clearly shown that ABA co-receptor transcript abundance was regulated by an abiotic factor. This is very important, knowing that ABA is a major factor in the plant abiotic stress response, particularly dehydration stress. However, the ABA-dependent group of genes only had 9% of the total common genes upregulated during dehydration stress.

In the second category of genes grouped in the ABA-independent pathway, the majority were downregulated. This trend was anticipated because the PEG system used in this study allowed for isolation of dehydration stress from other stresses. Dehydration-responsive element binding (DREB) cis-acting elements play important roles in low-temperature (DREB1A) and dehydration (DREB2A) resistance in plants (Neale et al., 2000; Tondelli et al., 2006; Wang et al., 2006; Agarwal et al., 2007; Gutha and Reddy, 2008; Kobayashi et al., 2008; Gao et al., 2009; Nayak et al., 2009; Wei et al., 2009; Medina et al., 2011). Our study showed that from the nine upregulated genes, only one gene, identified as *DREB2*, was present in all treatments. This study confirmed that *DREB2* is involved in dehydration stress in triticale seedlings at early developmental stage which was also observed in other studies (Wang et al., 2006; Agarwal et al., 2007; Kobayashi et al., 2008; Nayak et al., 2009). From the 27 downregulated genes, two *DREB1* genes were downregulated ≥100-fold in at least one treatment. The majority of the other downregulated genes were ethylene-responsive genes, known as part of the ABA-independent pathway (Liu et al., 1998; Seki et al., 2001; Shen et al., 2003; Qin et al., 2004; Swindell, 2006; Seki et al., 2007; Gao et al., 2009; Wilkinson and Davies, 2010). Also, only 1 (0.2%) and 12 (3%) of these genes were part of the 600 differentially regulated common genes identified in the dataset.

The last group of genes involved in dehydration stress-response had mostly roles in sensing and early response to stress in general, acting as the first line of defence, before more specialized mechanisms are activated. They show differential expression to a variety of stresses, such as drought, salinity, heat, cold, diseases or fungi (Nakashima et al., 1997; Zhu, 2002; Mara et al., 2004; Tran et al., 2004; Kariola et al., 2006; Rorat, 2006; Swindell, 2006; De Leonardis et al., 2007; Mohammadi et al., 2007; Suprunova et al., 2007; Kovacs et al., 2008; Ergen et al., 2009; Hura et al., 2009a; Nakashima et al., 2009; Lata et al., 2010; Kovacs et al., 2011). The combined analyses showed that only 5% and 2% of the genes were part of the group of common genes.

The highest upregulation in this last group was observed for a dehydrin (502-fold), which is a late embryogenesis abundant (LEA) protein. These proteins have role in preserving the structural integrity of the cell by keeping the protein intact during the desiccation process in plants (Allagulova et al., 2003; Rorat, 2006; Kobayashi et al., 2008; Alves et al., 2011; Kosova et al., 2011; Medina et al., 2011). Moreover, two genes involved in proline precursors biosynthesis were upregulated. These enzymes are essential precursors in proline biosynthesis, P5C synthetase (P5CS) and P5C reductase (P5CR) (Vendruscolo et al., 2007). Upregulation of *P5CS* has been considered an efficient strategy to acquire tolerance to drought stress and this process has been successfully used in several plants such as tobacco, rice, Arabidopsis, soybean, lettuce, and wheat (Porcel et al., 2004; Ayliffe et al., 2005; Bartels and Sunkar, 2005; Vendruscolo et al., 2007). Proline is involved in osmotic adjustment, osmoprotection and protection of macromolecules from denaturation during abiotic stress (Porcel et al., 2004; Su and Wu, 2004; Ayliffe et al., 2005; Liu et al., 2007; Vendruscolo et al., 2007; Kovács et al., 2011). Notably, the results showed that, in this group, 90% of genes involved in biotic stress response, such as jasmonic acid or SR1 (signal responsive 1 to biotic stimulus) (Memelink, 2009; Qiu and Yu, 2009) were downregulated, supporting our hypothesis that we were successful in achieving controlled conditions that isolated dehydration stress from the other stresses.

The results comparison between abundance and differential regulation of the three groups of genes (ABA-dependent, ABA-independent and others) led to two hypotheses. First, the fact that the majority of ABA-dependent genes were upregulated and the majority of ABA-independent genes were downregulated suggested the existence of a strong affinity towards ABA-dependent pathway in the case of triticale's response to dehydration stress at seedling stage in disfavour of ABA-independent pathway genes. Moreover, these observations suggested that by using the system of solid PEG media and *in vitro* controlled conditions we succeeded in isolating dehydration stress from the other stresses. Secondly, the high number (640) of non-annotated differentially regulated genes present in our data set, of which only 7% were part of the group common to all treatments, suggested that by using RNA-Seq, we were able to uncover many potentially novel genes with a role in the dehydration stress-response.

Using Blast 2GO information alone, only al low percentage (9%) of genes were identified as related to dehydration stress-response which represent a rather small number knowing that the majority of them were annotated. There were a large number of differentially regulated genes common to all treatments of which many were not included in that 9%. We observed that 55 genes were highly differentially regulated 50-fold or more. Only seven of them were previously identified as related to dehydration. This suggested that, by combining the differential regulation and GO results we were able to uncover potential novel genes with a role in the dehydration tolerance. The fact that these genes were not previously identified as dehydration tolerance genes led to different conclusions. One of them is based on the fact that many of the studies that resulted in the addition of the GO term to those genes were not dehydration studies.

Another conclusion is that the early molecular gene expression studies that were carried out did not necessarily lead to the identification of all the genes involved in plant response to dehydration. Also, the dehydration response mechanism in Arabidopsis is not generally valid in all plant species and there are many genes that have not yet been identified with role in dehydration tolerance. Early studies were carried on Arabidopsis because it was easier and much more convenient to characterize and validate gene function in a small and completely sequenced genome (Denby and Gehring, 2005; Kariola et al., 2006; Livingston III et al., 2007; Swindell et al., 2007). However, the small genome size and reduced number of genes as compared to larger and more complex genomes had significantly reduced the chance to identify all the genes involved in dehydration tolerance in large genomes (Akhunov et al., 2003; Ramalingam et al., 2006). The reduced number of novel genes identified as involved in dehydration stress (other than the ones identified in Arabidopsis) could be due to the limitations of the earlier molecular gene expression methods. For example, the Gene Chip Wheat Genome Array was considered the most comprehensive and informative content for wheat gene expression research. However, the level of annotation was under 3%. Therefore, this is a clear confirmation that most of the previous dehydration studies in cereals were less informative in terms of genetic information.

Based on the depth and the large number of sequences identified, that were not considered as involved in dehydration stress-response in earlier studies according to their Blast2GO annotations, this study demonstrated that currently there is gap of knowledge concerning dehydration stress response mechanism in crop species. The results revealed that triticale seedlings have activated different sets of genes dependent on the severity level, of which only some are always part of the plant response to dehydration. It is possible that the unique genes (Table 4.2) represent additional genes involved in dehydration stress that could be unique to complex genomes or have not been identified in other studies due to the limitations of the methods. Also, they could be part of a different response mechanism specific for the level of severity where the expression of some genes increased expression of other genes useful for the plants response to dehydration.

4.5. Conclusion

In brief, using RNA-Seq for transcriptome profiling we identified 4,467 differentially expressed genes, from which, we selected and classified 193 genes that are directly involved in the dehydration stress response of triticale seedlings. Notably, 600 genes were common in all treatments, of which only a small fraction are part of the 193 genes presented above. Considering that these genes could be part of a common response to dehydration stress due to their present differential expression in all treatments we were surprised to find that only so few were identified, based on Blast2GO information, as related to dehydration stress. Incidentally, the majority (87%) of the highly differentially regulated genes were either not annotated or represented by ubiquitous proteins. Therefore, they were considered as potential novel genes of dehydration tolerance. These results suggested that using RNA-Seq we succeeded to find novel genes with important role in dehydration tolerance in triticale seedlings. They could be genes with direct involvement in the plants response to dehydration or genes with adaptation role considering that the plants have been exposed to stress for 21 days prior analysis. Future studies are needed to validate their function.

Moreover, after analyzing the results and identifying the expression levels of different differentially regulated genes involved in abiotic and biotic stresses, we demonstrated that by using solid PEG media and *in vitro* controlled conditions, we were successful in isolating dehydration stress from other stresses. This method could help isolating genes only involved in dehydration stress response. This is especially very useful when working with plants with larger genomes where the level of crosstalk among abiotic and biotic gene regulation pathways is high and could lead to misidentification of other genes. Moreover, knowing the genes involved in dehydration stress could help in identification of plants response mechanisms to the stress.

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Chapter 5. Conclusions and future directions

The results of this study demonstrated that by using RNA-Seq and an *in vitro* dehydration stress inducing system based on PEG, we were able to identify approximately 4,500 differentially regulated transcripts in a complex genome like triticale without the need for a reference sequence.

PEG solutions have been used extensively in dehydration studies to create osmotic pressure to plants tissues. Until recently it was not possible to achieve solid PEG solutions of high enough concentration to be considered a viable option for dehydration studies. In our study, we used solid PEG medium recently developed by Comeau et al. (2010). This system provided us with numerous advantages over other methods to induce dehydration stress, such as high repeatability and ability to work in sterile and controlled conditions. Our results showed that by using the solid PEG media, we were able to isolate drought stress from the other stresses and this could be of importance when studying dehydration stress as an independent factor with the purpose of validating the plant response mechanisms to this stress. We came to this conclusion when we observed that the majority of the ABA-independent genes were negatively regulated. Knowing that these genes are mainly upregulated during other abiotic stresses such as cold, freezing, salinity or biotic stresses such as diseases and pests, we considered that our method clearly separated the expression of these genes from the ones specific to drought stress.

To our knowledge, this is the first study to identify such a high number of differentially regulated genes and with such a great depth. Our study showed and confirmed the role of many gene families implicated in dehydration-stress response in triticale seedlings. Some of these genes were identified in the majority of previous dehydration studies in other plant species such as the ABA-dependent and ABA-independent pathways-related genes. Moreover, this study revealed more than 600 potentially novel genes that showed differential expression upon dehydration stress and represent valuable material for future dehydration studies. This study is the first of this type in triticale and could be considered a stepping stone for future studies for identification and validation of dehydration-related genes in seedlings and development of dehydration-specific promoters.

Our study also showed the challenges given by the high number of sequences generated by RNA-Seq. The abundance and depth of generated transcripts could be overwhelming but provided us with a very extensive collection of genes. The number of statistically differentially regulated genes 2-fold or greater represented approximately 5% of the total number of genes sequenced. Among this group, the majority of the genes in the least severe stress were downregulated at a 2:1 ratio, whereas for the more severe treatments the ratio was 1:1. Many of the differentially regulated genes had a progressive increase in abundance as the severity of the stress increased, as we expected. However, some of the genes that were highly regulated at 27% PEG, were not regulated at the other two PEG concentrations and vice versa, suggesting that the mechanisms of response to drought stress in triticale plant is more complex than model systems, knowing that cereals genomes are much larger and have a larger number of genes and gene families.

The results revealed 600 differentially regulated genes common to all treatments of which 90% were annotated. Based on the literature, only a small percentage (10%) of genes was considered related to the plant response to dehydration stress. High differential expression of many other transcripts suggested that they are many additional factors. Moreover, the fact that these transcripts were regulated in all treatments suggested that might be part of general response mechanisms to dehydration stress in triticale seedlings. Additional analyses must be carried out to validate these possible novel genes with important role in dehydration stress of triticale seedlings. Several highly regulated genes could to be selected from this study and further studied to validate their function in dehydration stress.

In this study the highest regulation was shown by an ABA co-receptor, the *PYR/PYL/RCAR* gene in all treatments. Its function in ABA regulation was determined recently by several groups and validated by many studies but none in dehydration studies. This is the first time that the ABA co-receptor is shown to be responsive to an abiotic stress. Furthermore, PP2C kinases, co-receptors in ABA signalling pathway were also present and highly downregulated. This strongly supports previous studies suggesting that a dehydration stress response is mostly regulated by ABA.

Studying triticale remains and will continue to be a challenge due to its genome size. However, in our study we clearly showed that use of RNA-Seq could reduce and even remove some of the challenges, such as the lack of a reference genome. In addition, the ability to isolate one stress from the others allowed us to concentrate our analyses on genes that respond primarily to dehydration stress rather that other stresses, knowing that triticale has excellent resistance and tolerance to most abiotic and biotic stresses. The high number of statistically differentially regulated genes identified during dehydration stress demonstrated the complexity of the triticale response to stress and revealed many possible novel genes that play important role in the dehydration response mechanisms in triticale seedlings.

In this study the differential expression of the genes was assessed after continuous exposure to dehydration stress for 21 days using three different levels of stress severity. A subsequent study would be to carry out a time course analysis from few hours to 21 days under the stress conditions to determine when the expression of these differentially regulated genes is altered.

Supplementary tables

Table S4.1. Summary of all statistically differentially regulated (≥2-fold) genes of triticale seedlings exposed to three different dehydration stress severity.

Gene ID	Fold change at 27% PEG	Fold change at 31% PEG	Fold change at 34% PEG
Z-009927	-796.4	-4.8	-3.2
Z-012882	-710.4	-4.0	-2.7
Z-002596	-576.4	-	-
Z-036731	-552.9	-11.0	-11.0
Z-017884	-537.6	-11.0	-5.5
Z-042508	-519.8	-16.0	-340.2
Z-075533	-518.0	-7.0	-339.1
Z-063872	-484.3	-14.0	-7.0
Z-044811	-453.4	-2.0	-
Z-044812	-453.4	-2.0	-
Z-066246	-452.3	-	-
Z-000334	-433.6	-2.3	-
Z-024692	-411.3	-5.2	-3.5
Z-063875	-368.8	-9.0	-3.0
Z-072333	-363.2	-2.2	-237.8
Z-037880	-359.9	-	-2.5
Z-018028	-358.6	-7.0	-
Z-012427	-355.2	-3.0	-232.5
Z-000041	-347.4	-9.0	-
Z-075932	-332.9	-2.0	-
Z-004979	-327.5	-3.0	-7.5
Z-018320	-326.2	-211.5	-213.5
Z-021484	-314.8	-204.2	-2.6
Z-076495	-302.7	-	-
Z-085094	-302.7	-	-
Z-076649	-300.7	-	-196.9
Z-073451	-292.1	-4.7	-2.3
Z-045533	-283.4	-5.0	-
Z-045791	-280.4	-8.0	-183.5
Z-014669	-277.9	-180.3	-181.9
Z-009320	-277.4	-2.0	-
Z-072960	-269.0	-174.5	-176.1
Z-063449	-259.9	-168.5	-170.1
Z-076284	-257.8	-9.0	-9.0
Z-064134	-255.6	-165.8	-167.3
Z-049650	-253.7	-10.0	-10.0
Z-031190	-250.7	-8.0	-8.0
Z-066353	-249.7	-3.0	-163.4
Z-087145	-247.8	-	-2.0
Z-037149	-242.1	-4.0	-8.0
Z-086425	-237.3	-153.9	-9.0
Z-048448	-235.0	-	-153.8
Z-085840	-233.6	-	-152.9
Z-087144	-230.8	-4.3	-2.2
Z-073131	-228.3	-3.0	-3.0
Z-022799	-226.7	-2.0	-
Z-049039	-226.7	-147.0	-2.0
Z-064688	-221.9	-11.0	-145.3
Z-076650	-221.9	-	-
Z-022579	-221.9	-3.0	-
Z-003163	-221.9	-2.0	-4.0
Z-063465	-221.9	-6.0	-145.3
Z-088497	-218.3	-141.6	-142.9
Z-002591	-213.1	-138.2	-139.5
Z-040118	-210.3	-136.4	-137.6
Z-080469	-209.7	-3.3	-2.0
Z-043183	-208.9	-2.0	-2.0
Z-034876	-208.9	-6.0	-136.7
Z-057614	-207.5	-134.6	-6.0
Z-043629	-207.2	-3.5	-4.7
Z-064335	-206.9	-4.0	-135.4
Z-028916	-204.9	-	-
Z-047702	-190.2	-123.4	-124.5

Z-065116	-183.7	-	-
Z-059484	-181.6	-	-118.9
Z-029086	-181.0	-7.0	-118.5
Z-076533	-180.6	-	-
Z-037544	-180.2	-	-
Z-056761	-179.9	-116.7	-
Z-057217	-179.3	-7.0	-14.0
Z-023618	-179.2	-4.4	-22.0
Z-038607	-177.6	-115.1	-116.2
Z-045686	-174.4	-2.7	-
Z-023260	-173.7	-112.6	-113.7
Z-044109	-173.7	-112.6	-113.7
Z-018620	-173.7	-112.6	-113.7
Z-044343	-171.8	-111.4	-2.0
Z-015499	-171.0	-7.0	-111.9
Z-040223	-170.0	-110.2	-111.3
Z-033129	-166.4	-107.9	-
Z-008248	-160.2	-4.0	-104.9
Z-076792	-159.8	-103.6	-104.6
Z-044837	-159.0	-2.7	-2.7
Z-014617	-156.7	-	-3.0
Z-049046	-156.7	-4.0	-102.5
Z-051764	-156.7	-	-
Z-011125	-154.4	-	-
Z-027168	-152.2	-98.7	-99.7
Z-062421	-152.2	-3.0	-6.0
Z-056970	-152.2	-2.0	-4.0
Z-025091	-151.3	-2.5	-5.0
Z-043896	-149.3	-96.9	-97.8
Z-077447	-147.9	-	-
Z-076846	-147.9	-96.0	-96.9
Z-020888	-145.3	-	-95.1
Z-077386	-143.9	-93.4	-94.3
Z-059855	-143.9	-2.0	-94.3
Z-059519	-143.9	-	-
Z-063425	-143.9	-2.0	-2.0
Z-078954	-143.9	-93.4	-94.3
Z-087238	-143.9	-93.4	-94.3
Z-057124	-143.5	-7.0	-93.9
Z-038127	-143.2	-92.9	-93.8
Z-014999	-142.7	-	-93.4
Z-010607	-142.3	-92.3	-93.2
Z-073784	-140.2	-90.9	-91.8
Z-065195	-140.2	-	-2.0
Z-034012	-140.2	-6.5	-13.0
Z-002775	-140.2	-2.5	-91.8
Z-012765	-140.2	-3.0	-
Z-037041	-137.4	-2.0	-
Z-023939	-134.5	-10.0	-88.1
Z-008541	-134.0	-2.7	-87.7
Z-086393	-133.1	-6.0	-87.2
Z-000928	-129.9	-84.3	-85.1
Z-009913	-129.9	-84.3	-
Z-039367	-128.9	-83.6	-84.4
Z-004227	-128.0	-83.1	-5.0
Z-035021	-127.4	-10.0	-3.3
Z-009575	-126.8	-	-4.0
Z-009576	-126.8	-	-4.0
Z-078739	-126.8	-82.3	-83.0
Z-002633	-126.8	-4.0	-4.0
Z-002634	-126.8	-4.0	-4.0
Z-058776	-125.6	-	-
Z-059198	-124.4	-80.7	-
Z-012376	-121.0	-78.5	-3.0
Z-016082	-119.9	-5.0	-3.3
Z-037557	-119.2	-3.0	-3.0
Z-022513	-119.2	-2.0	-2.0
Z-036931	-118.9	-	-5.0
Z-070883	-117.5	-76.2	-6.0
Z-032336	-117.5	-	-3.0

Z-025714	-115.8	-	-
Z-025715	-115.8	-	-
Z-079349	-115.8	-5.5	-3.7
Z-052208	-115.8	-3.0	-75.8
Z-049999	-114.9	-74.6	-6.0
Z-086740	-114.1	-74.1	-74.7
Z-068742	-113.3	-73.5	-2.0
Z-069413	-113.3	-2.0	-74.2
Z-026853	-112.1	-2.0	-73.4
Z-059866	-110.9	-	-7.0
Z-033128	-110.9	-	-3.0
Z-015829	-109.8	-2.7	-2.7
Z-043855	-108.7	-70.5	-71.2
Z-017912	-108.2	-4.3	-70.9
Z-034249	-107.9	-3.0	-
Z-080233	-106.5	-4.0	-2.0
Z-053536	-105.5	-68.4	-69.1
Z-042862	-105.5	-4.0	-69.1
Z-056990	-104.6	-9.0	-68.5
Z-016445	-104.6	-	-
Z-077354	-104.5	-67.8	-68.4
Z-085772	-104.4	-67.8	-68.4
Z-056102	-103.8	-3.0	-
Z-001793	-103.7	-67.3	-67.9
Z-078251	-102.4	-	-67.1
Z-038842	-100.5	-65.2	-2.0
Z-065554	-100.5	-65.2	-65.8
Z-023514	-99.3	-64.4	-65.0
Z-083389	-98.7	-64.0	-2.0
Z-063332	-97.8	-2.0	-
Z-021728	-93.5	-2.0	-61.2
Z-016039	-93.5	-60.6	-61.2
Z-074507	-93.3	-2.2	-3.0
Z-035123	-93.1	-4.3	-2.2
Z-074291	-91.9	-59.6	-3.0
Z-088506	-91.1	-59.1	-59.6
Z-084396	-89.8	-58.2	-58.8
Z-063258	-88.8	-57.6	-58.1
Z-060920	-88.8	-2.0	-
Z-084466	-88.8	-57.6	-3.0
Z-084467	-88.8	-57.6	-3.0
Z-010867	-88.8	-57.6	-58.1
Z-078759	-88.8	-57.6	-58.1
Z-085577	-88.5	-5.0	-57.9
Z-032570	-88.1	-	-4.0
Z-065052	-87.3	-56.7	-57.2
Z-057012	-86.9	-	-
Z-022186	-85.9	-	-
Z-025828	-85.9	-	-
Z-050823	-85.9	-2.0	-56.3
Z-003822	-85.9	-55.7	-56.3
Z-060515	-85.9	-55.7	-56.3
Z-000663	-85.9	-	-56.3
Z-086669	-85.5	-55.5	-7.0
Z-024830	-85.2	-2.7	-8.0
Z-002561	-85.0	-55.1	-
Z-074819	-85.0	-55.1	-55.7
Z-064082	-84.6	-10.0	-2.5
Z-007978	-82.9	-7.3	-3.2
Z-015567	-82.7	-2.2	-2.2
Z-006361	-82.0	-2.0	-
Z-084817	-82.0	-53.2	-53.7
Z-033149	-82.0	-53.2	-53.7
Z-085680	-81.6	-3.0	-53.4
Z-021579	-81.6	-	-53.4
Z-022536	-81.6	-3.0	-
Z-034378	-81.2	-	-53.2
Z-024409	-80.7	-	-2.0
Z-053524	-80.2	-	-2.5
Z-010916	-79.9	-3.0	-

Z-035122	-79.4	-4.3	-2.2
Z-002051	-79.3	-5.0	-51.9
Z-026774	-78.9	-51.2	-2.0
Z-084370	-78.4	-4.0	-4.0
Z-023309	-78.4	-50.8	-51.3
Z-032874	-78.4	-50.8	-51.3
Z-086036	-78.4	-50.8	-51.3
Z-033144	-78.4	-	-51.3
Z-003391	-77.8	-	-2.0
Z-004552	-77.7	-2.3	-
Z-062179	-77.6	-50.3	-3.0
Z-088057	-77.6	-50.3	-50.8
Z-022125	-77.2	-	-50.6
Z-010986	-77.2	-	-4.0
Z-063760	-77.0	-	-5.0
Z-001940	-76.1	-49.4	-49.8
Z-015018	-76.1	-49.4	-49.8
Z-001476	-76.1	-49.4	-49.8
Z-049359	-75.4	-	-
Z-025708	-74.0	-48.0	-48.4
Z-056349	-74.0	-2.0	-48.4
Z-034605	-74.0	-48.0	-48.4
Z-044216	-73.3	-	-3.0
Z-064355	-72.7	-	-
Z-001992	-72.0	-46.7	-47.1
Z-023310	-72.0	-46.7	-47.1
Z-086903	-72.0	-46.7	-47.1
Z-045418	-72.0	-	-47.1
Z-067172	-71.6	-46.5	-5.0
Z-067173	-71.6	-46.5	-5.0
Z-001239	-71.4	-3.0	-
Z-001240	-71.4	-3.0	-
Z-004553	-70.6	-2.3	-
Z-077202	-70.5	-45.7	-46.1
Z-025457	-70.1	-2.0	-45.9
Z-023308	-70.1	-2.0	-2.0
Z-049050	-70.1	-	-45.9
Z-027131	-70.1	-45.5	-45.9
Z-082402	-69.5	-3.0	-
Z-083362	-69.4	-45.0	-5.0
Z-049597	-69.2	-2.0	-2.0
Z-022574	-68.3	-	-44.7
Z-025309	-68.3	-44.3	-44.7
Z-060310	-67.7	-	-
Z-038979	-67.4	-2.0	-44.2
Z-087003	-66.6	-3.0	-2.0
Z-032332	-66.6	-43.2	-43.6
Z-037146	-66.6	-43.2	-43.6
Z-008749	-66.6	-43.2	-43.6
Z-019497	-66.6	-43.2	-
Z-056586	-65.0	-4.0	-42.5
Z-027339	-65.0	-42.1	-42.5
Z-037116	-65.0	-42.1	-42.5
Z-002551	-65.0	-2.0	-4.0
Z-002583	-64.5	-	-
Z-054911	-64.4	-41.8	-42.2
Z-028485	-64.3	-	-
Z-037924	-64.3	-41.7	-42.1
Z-009601	-64.2	-41.6	-2.0
Z-027433	-64.2	-41.6	-42.0
Z-038379	-64.2	-	-
Z-028277	-63.9	-	-3.5
Z-044335	-63.7	-	-41.7
Z-084256	-62.9	-6.0	-6.0
Z-059284	-62.9	-6.0	-2.0
Z-059286	-62.9	-6.0	-2.0
Z-035550	-62.9	-40.8	-
Z-013588	-62.8	-40.8	-5.0
Z-013605	-62.8	-40.8	-5.0
Z-013607	-62.8	-40.8	-5.0

Z-013583	-62.8	-40.8	-5.0
Z-044308	-62.7	-40.7	-41.0
Z-078110	-62.5	-2.5	-5.0
Z-057000	-62.0	-40.2	-40.6
Z-056587	-62.0	-4.0	-40.6
Z-038927	-62.0	-40.2	-40.6
Z-062710	-62.0	-40.2	-40.6
Z-076630	-61.4	-5.5	-40.2
Z-033287	-61.0	-39.6	-
Z-085584	-60.5	-39.3	-39.6
Z-075697	-60.1	-3.3	-5.0
Z-001264	-59.7	-4.0	-8.0
Z-024899	-59.6	-	-3.0
Z-006973	-59.2	-	-4.0
Z-059080	-59.2	-38.4	-38.8
Z-022999	-59.2	-38.4	-38.8
Z-008071	-59.2	-38.4	-38.8
Z-080008	-59.2	-38.4	-38.8
Z-030549	-59.2	-38.4	-38.8
Z-071826	-57.9	-37.6	-37.9
Z-066503	-57.9	-2.0	-2.0
Z-068745	-57.9	-37.6	-3.0
Z-044558	-57.9	-37.6	-
Z-018942	-57.9	-37.6	-37.9
Z-048110	-57.9	-	-37.9
Z-035229	-57.9	-	-37.9
Z-024736	-57.5	-3.0	-3.0
Z-084369	-57.3	-4.0	-4.0
Z-023649	-57.3	-2.0	-3.0
Z-035046	-57.3	-37.2	-37.5
Z-001426	-56.9	-36.9	-2.5
Z-066502	-56.7	-2.0	-2.0
Z-041052	-56.7	-36.8	-37.1
Z-018324	-56.7	-	-37.1
Z-080261	-56.0	-36.3	-36.6
Z-007668	-55.9	-36.3	-36.6
Z-044398	-55.9	-3.0	-2.0
Z-056355	-55.5	-36.0	-36.3
Z-042978	-55.5	-36.0	-36.3
Z-086437	-55.1	-35.8	-6.0
Z-040833	-55.0	-35.7	-36.0
Z-054222	-54.9	-35.6	-36.0
Z-084372	-54.9	-4.0	-2.0
Z-035179	-54.4	-35.3	-35.6
Z-050442	-54.0	-3.0	-35.4
Z-066143	-53.6	-	-
Z-018614	-53.3	-34.6	-34.9
Z-041810	-53.3	-34.6	-34.9
Z-063762	-53.3	-34.6	-34.9
Z-077169	-53.3	-5.0	-34.9
Z-077171	-53.3	-5.0	-34.9
Z-003151	-53.1	-2.5	-
Z-088188	-52.8	-	-
Z-088194	-52.8	-	-
Z-065127	-52.6	-34.1	-3.0
Z-007125	-52.6	-34.1	-3.0
Z-029775	-51.7	-33.6	-33.9
Z-064109	-51.6	-33.4	-33.8
Z-028458	-51.6	-2.0	-6.0
Z-007429	-51.2	-2.0	-
Z-012716	-51.2	-2.0	-2.0
Z-019794	-51.2	-	-
Z-083238	-51.2	-	-33.5
Z-036256	-50.9	-33.0	-3.0
Z-039814	-50.7	-32.9	-33.2
Z-084784	-50.6	-3.0	-33.1
Z-066459	-50.3	-32.6	-32.9
Z-040566	-50.0	-3.0	-32.7
Z-041624	-49.3	-	-32.3
Z-075606	-49.3	-6.0	-32.3

Z-001241	-49.3	-3.0	-
Z-001242	-49.3	-3.0	-
Z-021159	-49.3	-32.0	-2.0
Z-068889	-49.3	-	-32.3
Z-034129	-49.1	-	-
Z-024475	-48.9	-	-
Z-007658	-48.4	-2.0	-31.7
Z-076001	-48.4	-31.4	-31.7
Z-048464	-48.4	-2.0	-31.7
Z-015237	-48.4	-31.4	-31.7
Z-055768	-48.2	-4.0	-31.6
Z-009317	-47.9	-3.0	-
Z-005652	-47.6	-30.9	-31.1
Z-002788	-47.6	-30.9	-3.0
Z-078468	-47.6	-30.9	-31.1
Z-024874	-47.6	-	-
Z-078463	-47.6	-30.9	-31.1
Z-076170	-47.6	-	-31.1
Z-023967	-47.6	-	-31.1
Z-064237	-47.6	-30.9	-31.1
Z-035171	-47.6	-	-
Z-044012	-47.2	-30.6	-30.9
Z-018162	-47.0	-3.0	-
Z-058106	-46.7	-30.3	-30.6
Z-025766	-46.7	-30.3	-30.6
Z-014312	-46.7	-30.3	-2.0
Z-028298	-46.5	-30.1	-3.0
Z-036409	-46.4	-30.1	-30.4
Z-024648	-46.3	-	-30.3
Z-067066	-46.3	-	-2.0
Z-028979	-45.9	-	-30.1
Z-054160	-45.9	-29.8	-30.1
Z-024682	-45.9	-29.8	-30.1
Z-066830	-45.4	-29.5	-29.7
Z-073608	-45.2	-29.3	-29.6
Z-034328	-45.2	-	-2.0
Z-005209	-45.2	-29.3	-29.6
Z-015646	-45.2	-29.3	-
Z-010282	-44.8	-3.5	-3.5
Z-062828	-44.4	-2.0	-
Z-049840	-43.7	-28.3	-28.6
Z-013361	-43.7	-	-
Z-075502	-43.7	-	-28.6
Z-035293	-43.4	-3.0	-
Z-022118	-43.2	-28.0	-28.3
Z-030945	-43.0	-	-2.0
Z-048814	-43.0	-	-
Z-048816	-43.0	-	-
Z-053366	-43.0	-27.9	-
Z-049839	-42.3	-27.4	-27.7
Z-008926	-42.3	-	-
Z-008928	-42.3	-	-
Z-069194	-42.0	-2.5	-
Z-035496	-42.0	-2.0	-
Z-003243	-42.0	-27.2	-27.5
Z-065032	-42.0	-27.2	-2.0
Z-068532	-41.6	-27.0	-27.3
Z-074392	-41.3	-2.0	-27.0
Z-037700	-41.3	-26.8	-
Z-003689	-41.0	-26.6	-26.8
Z-008232	-41.0	-26.6	-
Z-076857	-40.8	-26.4	-26.7
Z-001993	-40.4	-26.2	-26.4
Z-014414	-40.4	-26.2	-26.4
Z-033252	-39.9	-25.8	-26.1
Z-045392	-39.8	-25.8	-26.0
Z-028880	-39.8	-2.0	-26.0
Z-043625	-39.8	-2.0	-26.0
Z-076490	-39.6	-6.0	-25.9
Z-076491	-39.6	-6.0	-25.9

Z-027443	-39.6	-25.7	-25.9
Z-075605	-39.4	-6.0	-25.8
Z-076656	-39.2	-25.4	-25.6
Z-076657	-39.2	-25.4	-25.6
Z-033174	-39.2	-	-
Z-033179	-39.2	-	-
Z-061460	-39.2	-25.4	-
Z-074296	-39.2	-2.0	-25.6
Z-083962	-38.6	-25.0	-25.3
Z-040521	-38.6	-25.0	-25.3
Z-039968	-38.6	-25.0	-25.3
Z-033271	-38.3	-	-2.0
Z-033575	-38.1	-2.0	-2.0
Z-022591	-38.1	-24.7	-24.9
Z-025011	-38.1	-24.7	-24.9
Z-059038	-38.1	-24.7	-24.9
Z-063693	-38.1	-	-24.9
Z-063695	-38.1	-	-24.9
Z-065968	-38.1	-2.0	-24.9
Z-052613	-38.1	-	-
Z-003314	-38.0	-7.6	-2.7
Z-043401	-37.8	-2.0	-2.0
Z-016824	-37.5	-24.3	-24.6
Z-030840	-37.5	-24.3	-24.6
Z-005670	-37.5	-24.3	-
Z-084885	-37.3	-24.2	-24.4
Z-063450	-37.1	-3.5	-24.3
Z-088193	-37.0	-	-
Z-043525	-37.0	-3.0	-3.0
Z-017131	-37.0	-2.0	-24.2
Z-004362	-37.0	-24.0	-24.2
Z-087030	-36.8	-	-
Z-028924	-36.6	-23.7	-23.9
Z-021733	-36.5	-23.7	-23.9
Z-024476	-36.5	-	-
Z-013006	-36.5	-	-23.9
Z-064276	-36.5	-23.7	-23.9
Z-015486	-36.5	-	-23.9
Z-087046	-36.5	-23.7	-23.9
Z-023322	-36.2	-23.5	-23.7
Z-030132	-36.0	-23.4	-23.6
Z-038122	-36.0	-23.4	-23.6
Z-038124	-36.0	-23.4	-23.6
Z-007291	-36.0	-23.4	-2.0
Z-015112	-35.7	-23.1	-23.4
Z-084649	-35.5	-23.0	-23.3
Z-034127	-35.5	-23.0	-23.3
Z-020577	-35.4	-3.0	-23.2
Z-058672	-35.1	-	-22.9
Z-080450	-35.1	-	-22.9
Z-082473	-35.1	-	-22.9
Z-040403	-35.1	-22.7	-22.9
Z-048795	-35.1	-22.7	-22.9
Z-048796	-35.1	-22.7	-22.9
Z-063845	-35.1	-22.7	-22.9
Z-047403	-35.1	-	-22.9
Z-047404	-35.1	-	-22.9
Z-032853	-34.9	-	-22.8
Z-005087	-34.6	-22.4	-22.6
Z-035351	-34.6	-22.4	-22.6
Z-068518	-34.6	-22.4	-22.6
Z-031411	-34.6	-2.0	-
Z-015445	-34.2	-2.0	2.5
Z-015446	-34.2	-2.0	2.5
Z-062695	-34.2	-22.2	-22.4
Z-041879	-34.2	-22.2	-22.4
Z-041880	-34.2	-22.2	-22.4
Z-055272	-33.7	-	-
Z-033494	-33.7	-	-
Z-074049	-33.7	-21.9	-22.1

Z-021371	-33.3	-21.6	-21.8
Z-063864	-33.3	-21.6	-21.8
Z-063865	-33.3	-21.6	-21.8
Z-069325	-33.3	-21.6	-21.8
Z-034648	-33.0	-11.0	-6.6
Z-034647	-33.0	-11.0	-6.6
Z-076056	-32.9	-21.3	-21.5
Z-081932	-32.9	-21.3	-21.5
Z-012370	-32.9	-21.3	-21.5
Z-076205	-32.5	-21.1	-21.3
Z-012371	-32.5	-21.1	-21.3
Z-069183	-32.5	-	-
Z-043752	-32.3	-20.9	-21.1
Z-005215	-32.3	-4.0	-21.1
Z-067959	-32.3	-20.9	-21.1
Z-051960	-32.1	-20.8	-21.0
Z-042630	-32.1	-20.8	-21.0
Z-087088	-32.1	-20.8	-21.0
Z-004117	-31.7	-20.6	-20.8
Z-015996	-31.7	-20.6	-2.0
Z-044370	-31.7	-20.6	-20.8
Z-072207	-31.7	-20.6	-20.8
Z-066965	-31.7	-	-
Z-024608	-31.7	-	-20.8
Z-023136	-31.7	-20.6	-20.8
Z-014450	-31.6	-20.5	-20.7
Z-014192	-31.5	-20.4	-20.6
Z-042143	-31.3	-	-20.5
Z-079657	-31.0	-20.1	-20.3
Z-079002	-31.0	-	-
Z-087045	-31.0	-	-
Z-081278	-30.6	-19.9	-20.0
Z-023945	-30.6	-2.0	-2.0
Z-004976	-30.6	-5.0	-5.0
Z-020569	-30.5	-	-3.0
Z-042412	-30.4	-4.0	-4.0
Z-085527	-30.3	-19.6	-
Z-085528	-30.3	-19.6	-
Z-056849	-30.3	-19.6	-19.8
Z-038496	-30.3	-	-
Z-083695	-30.3	-19.6	-19.8
Z-058613	-30.2	-19.6	-
Z-041995	-30.0	-	-3.0
Z-016812	-29.9	-2.0	-19.6
Z-010979	-29.9	-19.4	-19.6
Z-018863	-29.9	-19.4	-19.6
Z-045169	-29.8	-2.0	-19.5
Z-084395	-29.6	-19.2	-
Z-044314	-29.6	-	-
Z-084779	-29.6	-19.2	-19.4
Z-058105	-29.3	-	-19.2
Z-034741	-29.3	-19.0	-19.2
Z-010998	-29.0	-18.8	-19.0
Z-000533	-28.7	-5.0	-
Z-003812	-28.6	-18.6	-18.8
Z-009292	-28.6	-18.6	-18.8
Z-002522	-28.6	-18.6	-18.8
Z-024419	-28.6	-18.6	-18.8
Z-007091	-28.6	-	-18.8
Z-063491	-28.5	-	-
Z-010949	-28.5	-2.0	-18.7
Z-001788	-28.3	-18.4	-18.6
Z-013154	-28.3	-18.4	-18.6
Z-021263	-28.3		-18.6
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Z-041451	-28.0	-18.2	-3.0
Z-064087	-27.5	-	-18.0
Z-012916	-27.2	-5.0	-17.8
Z-055489	-27.2	-17.6	-17.8

Z-028304	-27.2	-17.6	-17.8
Z-028305	-27.2	-17.6	-17.8
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Z-030313	-26.9	-	-17.6
Z-001295	-26.9	-17.5	-17.6
Z-035087	-26.8	-17.4	-17.5
Z-008157	-26.6	-17.3	-17.4
Z-076500	-26.6	-17.3	-17.4
Z-043903	-26.4	-2.7	-8.0
Z-078163	-26.4	-	-
Z-040567	-26.1	-3.0	-17.1
Z-084778	-25.9	-4.0	-17.0
Z-066250	-25.9	-16.8	-16.9
Z-028200	-25.7	-16.7	-16.9
Z-061311	-25.7	-3.3	-6.4
Z-018515	-25.6	-16.6	-
Z-009582	-25.6	-16.6	-
Z-018102	-25.5	-16.5	-16.7
Z-031930	-25.0	-16.2	-16.4
Z-053412	-24.9	-16.1	-16.3
Z-061957	-24.7	-16.0	-3.0
Z-048929	-24.7	-16.0	-16.1
Z-014163	-24.7	-16.0	-16.1
Z-040449	-24.7	-	-16.1
Z-066169	-24.7	-	-16.1
Z-062875	-24.7	-16.0	-16.1
Z-062877	-24.7	-16.0	-16.1
Z-083094	-24.6	-4.0	-
Z-004853	-24.5	-15.9	-4.0
Z-048872	-24.4	-15.9	-16.0
Z-077893	-24.4	-15.9	-16.0
Z-045621	-24.4	-2.0	-2.0
Z-083383	-24.2	-15.7	-15.9
Z-022163	-24.2	-15.7	-15.9
Z-046465	-24.1	-	-
Z-086455	-24.0	-177.2	-178.8
Z-025692	-23.8		-15.6
Z-077930	-23.8	-15.4	-15.6
Z-062727	-23.8	-15.4	-15.6
Z-001925	-23.7	-2.0	-2.0
Z-062916	-23.6	-15.3	-15.4
Z-057029	-23.6	-15.3	-15.4
Z-065817	-23.5	-2.3	-3.7
Z-014882	-23.4	-15.2	-15.3
Z-076237	-23.4	-15.2	-15.3
Z-076239	-23.4	-15.2	-15.3
Z-076240	-23.4	-15.2	-15.3
Z-025258	-23.4	-	-15.3
Z-025260	-23.4	-	-15.3
Z-075305	-23.4	-15.2	-15.3
Z-032467	-23.4	-15.2	-6.0
Z-015598	-23.2	-15.0	-15.2
Z-057688	-23.2	-15.0	-15.2
Z-059081	-23.2	-15.0	-15.2
Z-038823	-23.0	-3.3	-2.9
Z-087550	-23.0	-3.5	-23.0
Z-022216	-23.0	-	-15.0
Z-031975	-23.0	-	-15.0
Z-068069	-22.9	-14.8	-15.0
Z-045068	-22.8	-14.8	-14.9
Z-034562	-22.8	-14.8	-14.9
Z-010285	-22.7	-3.5	-3.5
Z-014948	-22.7	-14.7	-14.9
Z-048467	-22.4	-	-14.7
Z-022177	-22.3	-3.0	-3.0
Z-033909	-22.3	-6.0	-3.0
Z-076866	-22.3	-14.4	-14.6
Z-008469	-22.2	-14.4	-14.5
Z-008470	-22.2	-14.4	-14.5
Z-067763	-21.8	-2.0	-2.0

Z-022504	-21.8	-14.2	-14.3
Z-080886	-21.7	-	-14.2
Z-077040	-21.7	-14.0	-14.2
Z-008153	-21.6	-14.0	-14.1
Z-049960	-21.5	-	-14.1
Z-076863	-21.5	-13.9	-14.1
Z-012579	-21.5	-13.9	-14.1
Z-024706	-21.5	-13.9	-14.1
Z-043489	-21.5	-13.9	-
Z-081296	-21.4	-	-
Z-078738	-21.4	-13.9	-
Z-008491	-21.1	-2.0	-13.8
Z-069678	-21.1	-13.7	-13.8
Z-072997	-21.0	-	-
Z-029234	-21.0	-	-3.0
Z-088390	-20.5	-	-
Z-008490	-20.5	-2.0	-13.4
Z-034155	-20.5	-13.3	-
Z-031460	-20.5	-13.3	-13.4
Z-025085	-20.3	-	-13.3
Z-042793	-20.2	-13.1	-13.2
Z-011159	-20.2	-13.1	-13.2
Z-035095	-20.0	-3.6	-40.0
Z-035476	-20.0	-5.0	-3.3
Z-018487	-20.0	-	-
Z-014415	-19.9	-	-
Z-030595	-19.9	-	-13.0
Z-078939	-19.9	-12.9	-13.0
Z-044711	-19.9	-12.9	-13.0
Z-020041	-19.7	-	-12.9
Z-007276	-19.7	-12.8	-12.9
Z-035410	-19.6	-12.7	-12.8
Z-065831	-19.6	-12.7	-
Z-065832	-19.6	-12.7	-
Z-005092	-19.6	-12.7	-12.8
Z-059012	-19.6	-	-12.8
Z-059018	-19.6	-	-12.8
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Z-059028	-19.6	-	-12.8
Z-078557	-19.4	-12.6	-12.7
Z-028086	-19.4	-2.0	-2.0
Z-028096	-19.4	-2.0	-2.0
Z-033170	-19.4	-	-
Z-033172	-19.4	-	-
Z-003070	-19.3	-	-
Z-047870	-19.2	-12.5	-12.6
Z-025358	-19.0	-3.0	-
Z-074207	-18.5	-12.0	-12.1
Z-052963	-18.5	-12.0	-12.1
Z-030159	-18.4	-5.2	-3.0
Z-030160	-18.4	-5.2	-3.0
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Z-009219	-18.2	-	-
Z-041134	-18.1	-	-11.9
Z-042732	-18.0	-2.0	-
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Z-016349	-17.6	-11.4	-11.5
Z-073388	-17.6	-11.4	-11.5
Z-051867	-17.5	-	-11.5
Z-083280	-17.4	-	-
Z-068978	-17.2	-11.1	-11.3
Z-057010	-17.2	-2.0	-2.0
Z-086351	-17.2	-11.1	-11.3
Z-033571	-17.1	-2.4	-
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Z-063829	-17.1	-11.1	-11.2
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Z-008249	-17.0	-5.7	-17.0
Z-015546	-17.0	-11.0	-11.1

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Z-021398	-16.9	-10.9	-11.0
Z-064224	-16.8	-10.9	-11.0
Z-063684	-16.6	-10.8	-10.9
Z-016447	-16.6	-	-
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Z-043524	-16.4	-10.7	-10.8
Z-044146	-16.3	-10.6	-10.7
Z-059785	-16.3	-2.7	-4.5
Z-059863	-16.2	-3.0	-3.0
Z-010316	-16.2	-	-10.6
Z-004812	-16.2	-10.5	-10.6
Z-023285	-16.0	-10.4	-10.5
Z-023286	-16.0	-10.4	-10.5
Z-012764	-16.0	-10.4	-10.5
Z-012767	-16.0	-10.4	-10.5
Z-025109	-16.0	-10.4	-10.5
Z-011842	-16.0	-	-
Z-065806	-16.0	-4.0	-8.0
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Z-048867	-15.9	-	-3.0
Z-048266	-15.7	-10.2	-10.3
Z-016340	-15.7	-10.2	-10.3
Z-037877	-15.7	-	-
Z-024300	-15.6	-10.1	-10.2
Z-069238	-15.6	-	-10.2
Z-042039	-15.5	-	-10.1
Z-042030	-15.5	-	-10.1
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Z-042037	-15.5	-	-10.1
Z-030961	-15.5	-10.0	-10.1
Z-029217	-15.5	-10.0	-10.1
Z-029218	-15.5	-10.0	-10.1
Z-040440	-15.5	-10.0	-10.1
Z-042309	-15.1	-	-9.9
Z-061358	-15.1	-	-9.9
Z-041005	-15.1	-	-9.9
Z-085815	-15.1	-9.8	-9.9
Z-063279	-15.1	-9.8	-9.9
Z-002921	-15.1	-9.9	-36.7
Z-081680	-15.1	-5.0	-9.9
Z-022711	-15.1	-9.8	-9.9
Z-003187	-15.0	-2.1	-5.0
Z-051451	-15.0	-	-6.0
Z-051452	-15.0	-	-6.0
Z-051454	-15.0	-	-6.0
Z-078679	-14.9	-	-9.7
Z-068902	-14.8	-9.6	-9.7
Z-062900	-14.8	-9.6	-9.7
Z-033594	-14.7	-	-9.6
Z-074597	-14.6	-9.4	-9.5
Z-074654	-14.6	-9.4	-9.5
Z-074658	-14.6	-9.4	-9.5
Z-011890	-14.5	-	-2.0
Z-069000	-14.5	-9.4	-9.5
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Z-024801	-14.5	-9.4	-9.5
Z-036728	-14.5	-9.4	-9.5
Z-036730	-14.5	-9.4	-9.5
Z-067492	-14.5	-9.4	-9.5
Z-067493	-14.5	-9.4	-9.5
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Z-029431	-14.3	-9.3	-9.4
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Z-085336	-14.2	-9.2	-9.3
Z-043871	-14.1	-	-9.2
Z-078532	-14.1	-2.0	-9.2
Z-081769	-14.1	-	-2.0
Z-076015	-14.1	-9.1	-9.2

Z-051571	-14.0	-	-9.2
Z-051568	-14.0	-	-9.2
Z-009593	-14.0	-14.0	-4.7
Z-025210	-14.0	-7.0	-6.0
Z-034143	-14.0	-	-2.0
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Z-024701	-13.9	-9.0	-9.1
Z-024703	-13.9	-9.0	-9.1
Z-024704	-13.9	-9.0	-9.1
Z-047722	-13.9	-9.0	-9.1
Z-064298	-13.8	-9.0	-9.0
Z-082417	-13.5	-8.8	-8.9
Z-013617	-13.5	-	-8.8
Z-043926	-13.5	-	-8.8
Z-034363	-13.5	-8.7	-8.8
Z-064997	-13.5	-	-8.8
Z-025487	-13.3	-8.6	-8.7
Z-062259	-13.3	-8.6	-8.7
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Z-042239	-13.3	-8.6	-8.7
Z-068488	-13.3	-8.6	-8.7
Z-051511	-13.0	-76.9	-77.6
Z-047733	-13.0	-8.4	-8.5
Z-020710	-13.0	-34.2	-34.6
Z-038863	-13.0	-	-
Z-045683	-13.0	-3.2	-13.0
Z-068285	-13.0	-3.2	-
Z-020712	-13.0	-34.0	-34.4
Z-086556	-13.0	-64.2	-13.0
Z-035032	-12.9	-	-3.0
Z-011172	-12.8	-4.0	-4.0
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Z-037119	-12.8	-8.3	-8.4
Z-088639	-12.8	-8.3	-
Z-088645	-12.8	-8.3	-
Z-007976	-12.7	-	-3.0
Z-015193	-12.7	-	-8.3
Z-015194	-12.7	-	-8.3
Z-061008	-12.6	-8.2	-8.2
Z-076752	-12.5	-3.1	-3.6
Z-034671	-12.5	-2.0	-8.2
Z-043536	-12.4	-8.1	-8.1
Z-030134	-12.4	-8.1	-
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Z-066542	-12.4	-8.1	-8.1
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Z-000953	-12.3	-3.4	-
Z-007977	-12.3	-	-3.0
Z-044427	-12.3	-2.0	-8.0
Z-013223	-12.2	-7.9	-2.0
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Z-040582	-12.1	-7.9	-7.9
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Z-062361	-12.0	-75.4	-12.0
Z-087624	-12.0	-	-2.0
Z-006317	-12.0	-6.0	-
Z-040230	-11.9	-7.7	-7.8
Z-040232	-11.9	-7.7	-7.8
Z-071831	-11.7	-7.6	-7.7
Z-004139	-11.7	-	-7.6
Z-054170	-11.7	-7.6	-7.6
Z-054173	-11.7	-7.6	-7.6
Z-083257	-11.5	-7.5	-7.5
Z-083258	-11.5	-7.5	-7.5
Z-056176	-11.5	-7.4	-7.5
Z-082458	-11.5	-7.4	-7.5
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Z-034672	-11.4	-	-
Z-031452	-11.4	-	-

Z-084225	-11.4	-7.4	-7.5
Z-062412	-11.3		-7.4
Z-056177	-11.3	-7.3	-7.4
Z-037793	-11.2	-7.3	-7.3
Z-065038	-11.1	-	-7.3
Z-053275	-11.0	-7.1	-7.2
Z-085237	-11.0	-190.0	-3.7
Z-021522	-11.0	-2.2	-
Z-065779	-11.0	-306.5	-5.5
Z-002950	-11.0	-11.0	-11.0
Z-003544	-11.0	-11.0	-64.8
Z-057695	-11.0	-3.7	-11.0
Z-057766	-11.0	-	-
Z-044030	-10.8	-	-
Z-044032	-10.8	-	-
Z-020065	-10.6	-6.9	-6.0
Z-013110	-10.4		-6.8
Z-009511	-10.3	-6.7	-2.0
Z-080887	-10.3	-	-
Z-069197	-10.3	-6.7	-6.7
Z-023731	-10.2	-6.6	-6.7
Z-023732	-10.2	-6.6	-6.7
Z-023733	-10.2	-6.6	-6.7
Z-023734	-10.2	-6.6	-6.7
Z-045318	-10.2	-6.6	-6.7
Z-061994	-10.2	-2.0	-6.7
Z-048985	-10.1	-6.5	-
Z-004453	-10.1	-	-
Z-087272	-10.1	-6.5	-6.6
Z-029128	-10.0	-	-2.5
Z-023557	-10.0	-10.0	-10.0
Z-064648	-10.0	-2.5	-2.5
Z-004508	-10.0	-2.5	-10.0
Z-012411	-10.0	-375.6	-10.0
Z-084774	-9.9	-6.4	-6.5
Z-006027	-9.9	-6.4	-6.5
Z-041349	-9.9	-	-6.5
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Z-085767	-9.9	-	2.5
Z-036737	-9.8	-6.4	-6.4
Z-034359	-9.8	-2.0	-6.4
Z-034362	-9.8	-2.0	-6.4
Z-071667	-9.7	-6.3	-
Z-069316	-9.7	-	-
Z-085777	-9.7	-6.3	-6.3
Z-017419	-9.7	-6.3	-6.3
Z-006640	-9.7	-	-6.3
Z-030990	-9.7	-	-6.3
Z-063578	-9.6	-6.2	-
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Z-041783	-9.6	-3.7	-4.5
Z-007547	-9.5	-6.2	-6.3
Z-014141	-9.5	-6.3	-19.0
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Z-028970	-9.4	-6.1	-6.1
Z-035050	-9.4	-6.1	-6.1
Z-048859	-9.3	4.0	-
Z-072034	-9.2	-	-6.1
Z-010529	-9.2	-6.0	-6.0
Z-050301	-9.1	-5.9	-2.0
Z-066985	-9.1	-2.0	-
Z-069544	-9.0	-2.2	-114.5
Z-001880	-9.0	-	-
Z-015817	-9.0	-4.5	-9.0
Z-037794	-9.0	-5.8	-5.9
Z-004994	-9.0	-	-
Z-004996	-9.0	-	-
Z-012665	-9.0	-57.6	-58.1
Z-023999	-9.0	-3.0	-
Z-020281	-9.0	-3.0	-3.0

Z-020284	-9.0	-3.0	-3.0
Z-057254	-9.0	-5.8	-5.9
Z-085611	-8.9	-	-
Z-015597	-8.9	-8.9	-
Z-045974	-8.8	-26.4	-3.9
Z-061546	-8.8	-	-5.7
Z-022506	-8.7	-	-5.7
Z-062687	-8.5	-5.5	-5.6
Z-055755	-8.5	-5.7	-4.2
Z-058139	-8.5	-3.4	-2.4
Z-038048	-8.5	-5.5	-5.6
Z-019684	-8.5	-	-
Z-019685	-8.4	-	-
Z-062408	-8.4	-5.5	-5.5
Z-084813	-8.3	-4.2	-2.8
Z-041849	-8.3	-5.4	-5.5
Z-084260	-8.2	-5.3	-5.4
Z-059786	-8.2	-3.3	-4.7
Z-066984	-8.2	-5.3	-5.4
Z-061549	-8.2	-	-5.4
Z-014359	-8.2	-5.3	-5.4
Z-049446	-8.2	-	-
Z-059882	-8.1	-5.3	-5.3
Z-034992	-8.1	2.0	-5.3
Z-020064	-8.0	-5.2	-4.0
Z-022491	-8.0	-5.2	-
Z-005864	-8.0	-5.2	-5.3
Z-086493	-8.0	-2.7	-2.0
Z-015859	-8.0	-4.0	-6.0
Z-015860	-8.0	-4.0	-6.0
Z-015863	-8.0	-4.0	-6.0
Z-015864	-8.0	-4.0	-6.0
Z-012350	-8.0	-276.4	-2.7
Z-009052	-8.0	-4.0	-
Z-035532	-8.0	-4.0	-2.7
Z-064770	-8.0	-8.0	-2.7
Z-023561	-8.0	-2.0	-4.0
Z-001429	-8.0	-	-
Z-063862	-8.0	-4.0	-40.3
Z-023472	-8.0	-5.2	-5.2
Z-023474	-8.0	-5.2	-5.2
Z-062883	-7.9	-4.2	-8.9
Z-061103	-7.9	-	-5.2
Z-082530	-7.7	-5.0	-5.1
Z-082531	-7.7	-5.0	-5.1
Z-082524	-7.7	-5.0	-5.1
Z-082525	-7.7	-5.0	-5.1
Z-081678	-7.7	-5.0	-5.1
Z-081679	-7.7	-5.0	-5.0
Z-032459	-7.7	-5.0	-5.0
Z-028930	-7.6	-	-5.0
Z-027371	-7.6	-	-5.0
Z-024005	-7.5	-4.9	-4.9
Z-029388	-7.5	-209.0	-15.0
Z-085488	-7.5	-	-
Z-075764	-7.5	-3.0	-
Z-066544	-7.5	-2.5	-
Z-088915	-7.4	-4.8	-
Z-014033	-7.3	-3.1	-3.7
Z-014034	-7.3	-3.1	-3.7
Z-012444	-7.3	-11.0	-3.1
Z-059078	-7.2	-	-4.7
Z-072943	-7.1	-4.6	-4.7
Z-064766	-7.1	-4.6	-
Z-045311	-7.1	-	-
Z-029687	-7.0	-4.6	-
Z-026673	-7.0	-3.5	-
Z-087082	-7.0	-	-
Z-080458	-7.0	-	-3.5
Z-068537	-7.0	-	-

Z-085687	-7.0	-	-
Z-070927	-7.0	-7.0	-7.0
Z-072317	-7.0	-2.3	-
Z-020480	-7.0	-	-
Z-020481	-7.0	-	-
Z-023982	-7.0	-	-2.0
Z-080988	-7.0	-82.3	-7.0
Z-019245	-7.0	-14.0	-7.0
Z-019248	-7.0	-14.0	-7.0
Z-088829	-7.0	-	-
Z-005211	-7.0	-2.3	-
Z-028957	-7.0	-2.3	-4.2
Z-051301	-7.0	-147.5	-148.8
Z-051574	-6.9	-	-4.5
Z-022110	-6.8	-	-
Z-022114	-6.8	-	-
Z-022108	-6.8	-	-
Z-031431	-6.8	-4.4	-4.4
Z-027468	-6.7	-2.0	-4.4
Z-062279	-6.7	-20.0	-2.5
Z-076064	-6.7	-2.2	-2.9
Z-042182	-6.5	-4.3	-13.0
Z-067652	-6.5	-	-3.2
Z-025196	-6.5	-3.2	-4.3
Z-025197	-6.5	-3.2	-4.3
Z-007469	-6.5	-3.0	-4.2
Z-068515	-6.4	-4.1	-4.2
Z-047732	-6.3	-2.4	-
Z-015609	-6.3	-4.1	-4.1
Z-085776	-6.2	-4.0	-4.1
Z-084252	-6.1	-3.9	-4.0
Z-025279	-6.0	-3.9	-3.9
Z-068474	-6.0	-3.9	-3.9
Z-012927	-6.0	-6.0	-2.0
Z-077894	-6.0	-	-4.0
Z-082542	-6.0	-76.8	-6.0
Z-086492	-6.0	-6.0	-3.0
Z-010536	-6.0	-	-2.0
Z-010542	-6.0	-	-2.0
Z-050510	-6.0	-	-3.0
Z-063294	-6.0	-3.9	-3.9
Z-066145	-6.0	-3.0	-
Z-088182	-6.0	-2.0	-12.0
Z-088280	-6.0	-2.0	-6.0
Z-085841	-6.0	-	-2.0
Z-030612	-6.0	-	-3.0
Z-030613	-6.0	-	-3.0
Z-035347	-6.0	-	-6.0
Z-003816	-6.0	-46.5	-46.9
Z-076055	-6.0	-18.0	-229.1
Z-040646	-6.0	-	-
Z-050385	-6.0	-2.0	-3.0
Z-015612	-6.0	-3.0	-15.0
Z-015613	-6.0	-3.0	-20.4
Z-022766	-6.0	-3.0	-101.5
Z-066981	-6.0	-	-2.0
Z-086446	-6.0	-195.6	-197.4
Z-038651	-6.0	-	-3.0
Z-086325	-6.0	-25.2	-25.4
Z-078340	-6.0	-6.0	-
Z-047233	-6.0	-3.0	-186.8
Z-023703	-6.0	-280.2	-282.8
Z-076507	-6.0	-	-2.6
Z-013390	-5.9	-3.8	-
Z-025212	-5.8	-3.5	-
Z-063295	-5.8	-3.8	-3.8
Z-003286	-5.8	-3.8	-3.8
Z-016108	-5.7	-9.6	-6.4
Z-031631	-5.7	-	-
Z-031632	-5.7	-	-

Z-031633	-5.7	-	-
Z-052930	-5.6	-2.1	-
Z-014355	-5.5	-	-5.5
Z-032625	-5.5	-2.7	-2.7
Z-048423	-5.5	-5.5	-278.0
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Z-074956	-5.5	-3.5	-3.6
Z-068038	-5.4	-3.5	-3.6
Z-044123	-5.3	-5.3	-8.0
Z-029567	-5.3	-	-
Z-063117	-5.3	-3.4	-3.5
Z-021280	-5.2	-21.0	-21.0
Z-031430	-5.2	-3.4	-3.4
Z-037925	-5.2	-4.1	-8.4
Z-012907	-5.2	-3.4	-3.4
Z-012533	-5.1	-3.3	-3.3
Z-047827	-5.0	-3.2	-3.3
Z-015938	-5.0	-5.0	-
Z-029679	-5.0	-	-
Z-037919	-5.0	-2.0	-3.3
Z-037920	-5.0	-2.0	-3.3
Z-014885	-5.0	-2.5	-
Z-044059	-5.0	-	-
Z-033527	-5.0	-5.0	-2.5
Z-049807	-5.0	-6.7	-20.0
Z-077054	-5.0	-	-5.0
Z-027306	-5.0	-5.0	-
Z-048096	-5.0	-5.0	-5.0
Z-023665	-5.0	-5.0	-5.0
Z-008778	-5.0	-	-2.5
Z-008766	-5.0	-	-2.5
Z-063676	-5.0	-159.9	-161.4
Z-083149	-5.0	-5.0	-5.0
Z-050406	-5.0	-	-5.0
Z-030120	-5.0	-5.0	-2.5
Z-076301	-5.0	-5.0	-
Z-078225	-5.0	-	-2.5
Z-064867	-5.0	-	-3.3
Z-007563	-5.0	-	-15.9
Z-019324	-5.0	-	-64.1
Z-019338	-5.0	-	-64.1
Z-048811	-5.0	-	-45.4
Z-083969	-5.0	-	-
Z-083970	-5.0	-	-
Z-052187	-5.0	-	-
Z-064233	-5.0	-	-2.5
Z-000353	-5.0	-	-2.5
Z-003707	-5.0	-2.5	-2.5
Z-023955	-5.0	-2.5	-74.5
Z-076071	-5.0	-	-
Z-061336	-5.0	-5.0	-70.9
Z-061333	-5.0	-5.0	-70.9
Z-029114	-5.0	-2.5	-72.7
Z-086864	-5.0	-	-5.0
Z-010661	-5.0	-	-
Z-010665	-5.0	-	-
Z-043201	-5.0	-5.0	-2.5
Z-063842	-5.0	-187.8	-5.0
Z-054247	-5.0	-157.0	-158.5
Z-049804	-5.0	-6.7	-20.0
Z-054598	-4.9	-	-
Z-085101	-4.9	-2.1	-4.3
Z-016452	-4.9		-2.3
Z-010679	-4.8	-4.8	-3.4
Z-051522	-4.8	-2.4	-
Z-044031	-4.7	-2.4	-3.2
Z-058700	-4.7	-3.1	-3.1
Z-051156	-4.7	-	-
Z-060738	-4.7	-	-14.0
Z-064868	-4.7	-3.5	-14.0

Z-031474	-4.6	-3.8	-2.2
Z-033184	-4.6	-2.1	-
Z-040302	-4.6	-3.0	-3.0
Z-009455	-4.6	-4.6	-20.5
Z-039566	-4.5	-	-4.5
Z-056870	-4.5	-9.0	-3.0
Z-027674	-4.5	-4.5	-9.0
Z-037191	-4.5	-9.0	-30.7
Z-041139	-4.5	-89.9	-90.7
Z-043826	-4.5	-	-
Z-032417	-4.5	-3.0	-4.5
Z-065349	-4.4	-2.9	-2.9
Z-065350	-4.4	-2.9	-2.9
Z-020947	-4.4	-	-
Z-009499	-4.3	-102.1	-103.0
Z-031404	-4.3	-	-
Z-063383	-4.3	-3.2	-4.3
Z-039245	-4.3	-2.2	-
Z-085801	-4.3	-10.0	-4.3
Z-009667	-4.2	-8.5	-279.6
Z-067000	-4.2	-8.5	-
Z-067001	-4.2	-8.5	-
Z-072563	-4.2	-5.7	-
Z-056062	-4.2	-	-
Z-034953	-4.2	-2.1	-3.0
Z-034954	-4.2	-2.1	-3.0
Z-086488	-4.2	-2.5	-2.8
Z-018361	-4.1	-2.7	-2.7
Z-018362	-4.1	-2.7	-2.7
Z-053374	-4.1	-2.7	-2.7
Z-084039	-4.1	-	-
Z-062713	-4.1	-	-
Z-063956	-4.1	-2.7	-2.7
Z-016494	-4.1	-2.7	-2.7
Z-039998	-4.0	-3.2	-
Z-064215	-4.0	-6.0	-4.0
Z-008746	-4.0	-4.0	-20.4
Z-022996	-4.0	-	-4.0
Z-029689	-4.0	-4.0	-
Z-029690	-4.0	-4.0	-
Z-045571	-4.0	-2.0	-2.0
Z-016701	-4.0	-147.0	-148.4
Z-076731	-4.0	-2.0	-14.4
Z-064433	-4.0	-	-
Z-062417	-4.0	-103.1	-104.1
Z-015584	-4.0	-15.7	-15.9
Z-008414	-4.0	-4.0	-2.0
Z-085896	-4.0	-58.1	-4.0
Z-030614	-4.0	-15.0	-15.2
Z-030615	-4.0	-16.6	-16.8
Z-046226	-4.0	-4.0	-
Z-044510	-4.0	-2.0	-6.0
Z-044511	-4.0	-2.0	-6.0
Z-063675	-4.0	-	-2.0
Z-066608	-4.0	-	-4.0
Z-073671	-4.0	-2.0	-65.8
Z-020862	-4.0	-2.0	-
Z-034486	-4.0	-4.0	-
Z-037080	-4.0	-	-
Z-037081	-4.0	-	-
Z-037082	-4.0	-	-
Z-037084	-4.0	-	-
Z-066600	-4.0	-4.0	-
Z-033126	-4.0	-4.0	-
Z-005748	-4.0	-	-2.0
Z-038126	-4.0	-	-2.7
Z-007732	-4.0	-38.8	-4.0
Z-027395	-4.0	-11.0	-11.1
Z-056989	-4.0	-27.9	-28.1
Z-045497	-4.0	-2.0	-122.3

Z-019325	-4.0	-	-
Z-020062	-4.0	-	-2.0
Z-043850	-4.0	-3.0	-
Z-043852	-4.0	-3.0	-
Z-055952	-4.0	-	-4.0
Z-030952	-4.0	-76.8	
Z-032573	-4.0	-	-54.5
Z-024698	-4.0	-50.8	
Z-010248	-4.0	-	-4.0
Z-044207	-4.0	-2.0	-16.0
Z-017877	-4.0	-4.0	-4.0
Z-031984	-4.0	-	-
Z-063591	-4.0	-	-
Z-005478	-4.0	-2.0	-
Z-024254	-4.0	-2.0	-
Z-048519	-4.0	-8.0	-4.0
Z-003287	-3.9	-2.5	-2.6
Z-030282	-3.8	-3.9	-2.7
Z-002712	-3.8	-	-
Z-008089	-3.8	-2.5	-
Z-008090	-3.8	-2.5	-
Z-008092	-3.8	-2.5	-
Z-068615	-3.7	-	-
Z-031902	-3.7	-4.3	-3.7
Z-031903	-3.7	-4.3	-3.7
Z-031904	-3.7	-4.3	-3.7
Z-031905	-3.7	-4.3	-3.7
Z-012550	-3.7	-3.7	-5.0
Z-064520	-3.7	-	-2.5
Z-064521	-3.7	-	-2.5
Z-022507	-3.7	-	-2.4
Z-029901	-3.7	-	-
Z-076238	-3.7	-3.7	-3.7
Z-080484	-3.7	-3.0	-6.6
Z-028959	-3.7	-11.0	-11.0
Z-001869	-3.7	-3.7	-
Z-015893	-3.7	-2.7	-
Z-015897	-3.7	-2.7	-
Z-015909	-3.7	-2.7	-
Z-015913	-3.7	-2.7	-
Z-087767	-3.7	-	-
Z-062909	-3.5	-2.2	-2.7
Z-069338	-3.5	-	-
Z-029229	-3.5	-10.5	-10.5
Z-003837	-3.5	-3.5	-3.5
Z-003842	-3.5	-3.5	-3.5
Z-005249	-3.5	-7.0	-3.5
Z-025200	-3.5	-3.5	-7.0
Z-025201	-3.5	-3.5	-7.0
Z-086292	-3.5	-7.0	-23.7
Z-041155	-3.5	-	-
Z-075572	-3.5	-2.3	-
Z-002120	-3.4	-4.9	-4.6
Z-009454	-3.4	-	-
Z-081767	-3.3	-	-
Z-081770	-3.3	-	-
Z-059264	-3.3	-2.3	-11.5
Z-059266	-3.3	-2.3	-11.5
Z-013903	-3.3	-	-
Z-076229	-3.2	-4.3	-4.3
Z-023497	-3.2	-4.0	-3.2
Z-085520	-3.2	-5.3	-16.0
Z-010633	-3.2	-	-
Z-087302	-3.1	-2.3	-
Z-071407	-3.1	-2.0	-2.0
Z-022026	-3.1	-2.3	-
Z-030555	-3.1	-	-161.2
Z-028296	-3.0	-6.0	-3.0
Z-010965	-3.0	-	-63.0
Z-029983	-3.0	-	-3.0

Z-024003	-3.0	-	-33.1
Z-010035	-3.0	-	-
Z-021533	-3.0	-	-3.0
Z-021536	-3.0	-	-3.0
Z-036846	-3.0	-	-
Z-036847	-3.0	-	-
Z-036844	-3.0	-	-
Z-048443	-3.0	-2.0	-
Z-012032	-3.0	-	-
Z-037463	-3.0	-9.0	-3.0
Z-002654	-3.0	-	-
Z-048598	-3.0	-	-3.0
Z-003927	-3.0	-3.0	-
Z-003921	-3.0	-3.0	-
Z-003924	-3.0	-3.0	-
Z-057092	-3.0	-	-
Z-014735	-3.0	-	-
Z-038473	-3.0	-	-3.0
Z-065618	-3.0	-3.0	-8.8
Z-065132	-3.0	-3.0	-
Z-037496	-3.0	-	-
Z-070880	-3.0	-70.1	-70.7
Z-067684	-3.0	-3.0	-11.5
Z-030795	-3.0	-	-
Z-068960	-3.0	-21.3	-21.5
Z-024488	-3.0	-3.0	-
Z-024490	-3.0	-3.0	-
Z-004199	-3.0	-	-73.7
Z-026801	-3.0	-	-
Z-040308	-3.0	-	-3.0
Z-036080	-3.0	-	-
Z-057091	-3.0	-	-
Z-043893	-3.0	-	-
Z-038796	-3.0	-3.0	-3.0
Z-067197	-3.0	-41.8	-3.0
Z-010736	-3.0	-56.3	-3.0
Z-010737	-3.0	-56.3	-3.0
Z-088626	-3.0	-	-54.5
Z-023316	-3.0	-	-
Z-075989	-3.0	-55.7	-56.3
Z-088516	-3.0	-	-
Z-085782	-3.0	-6.0	-58.8
Z-085987	-3.0	-	-57.5
Z-083855	-3.0	-	-
Z-083856	-3.0	-	-
Z-080090	-3.0	-	-3.0
Z-027720	-3.0	-	-
Z-027724	-3.0	-	-
Z-013146	-3.0	-3.0	-
Z-013147	-3.0	-3.0	-
Z-029453	-3.0	-121.9	-123.1
Z-068998	-3.0	-	-
Z-044975	-3.0	-33.7	-34.0
Z-044971	-3.0	-33.7	-34.0
Z-068720	-3.0	-	-
Z-057700	-3.0	-	-95.1
Z-031116	-3.0	-	-
Z-064506	-3.0	-	-
Z-084558	-3.0	-2.0	-
Z-037491	-3.0	-2.0	-
Z-037492	-3.0	-2.0	-
Z-029743	-3.0	-	-
Z-002065	-3.0	-	-3.0
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Z-049590	-3.0	-	-3.0
Z-049591	-3.0	-	-3.0
Z-007285	-3.0	-	-3.0
Z-017435	-3.0	-3.0	-65.4
Z-019734	-3.0	-136.4	-137.6

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Z-062787	-3.0	-3.0	-
Z-050748	-3.0	-3.0	-
Z-050734	-3.0	-3.0	-
Z-083828	-3.0	-	-9.0
Z-052379	-3.0	-	-2.6
Z-021387	-2.9	-3.9	-3.9
Z-021390	-2.9	-3.9	-3.9
Z-057325	-2.9	-2.5	-2.2
Z-046182	-2.9	-2.3	-
Z-046183	-2.9	-2.3	-
Z-046185	-2.9	-2.3	-
Z-057095	-2.8	-13.6	-11.3
Z-021386	-2.8	-3.8	-3.8
Z-021382	-2.8	-3.8	-3.8
Z-006169	-2.8	-8.5	-4.2
Z-059708	-2.8	-	-
Z-034949	-2.7	-2.7	-2.2
Z-063323	-2.7	-2.7	-4.4
Z-045975	-2.7	-2.2	-
Z-005078	-2.7	-	-
Z-034571	-2.7	-2.0	-2.7
Z-082424	-2.7	-	-51.5
Z-018851	-2.7	-8.0	-8.0
Z-084722	-2.7	-2.0	-2.0
Z-074456	-2.7	-2.0	-2.7
Z-022688	-2.7	-4.0	-2.7
Z-022689	-2.7	-4.0	-2.7
Z-024255	-2.7	-8.0	-2.0
Z-066494	-2.7	-2.0	-
Z-066495	-2.7	-9.4	-
Z-031473	-2.6	-3.2	-
Z-021388	-2.6	-	-
Z-007167	-2.6	-3.2	-2.2
Z-088199	-2.6	-	-2.7
Z-088202	-2.6	-	-2.7
Z-080428	-2.6	-3.3	-2.1
Z-041168	-2.6	-3.6	-4.2
Z-056632	-2.5	-57.6	-58.1
Z-025655	-2.5	-	-
Z-057141	-2.5	-	-14.6
Z-057142	-2.5	-	-14.6
Z-057143	-2.5	-	-18.2
Z-057144	-2.5	-	-18.2
Z-054234	-2.5	-	-5.0
Z-040006	-2.5	-61.3	-5.0
Z-081260	-2.5	-5.0	-5.0
Z-006961	-2.5	-10.0	-174.4
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Z-087416	-2.5	-3.3	-2.5
Z-087417	-2.5	-3.3	-2.5
Z-073798	-2.5	-5.0	-
Z-052371	-2.5	-2.5	-35.4
Z-052372	-2.5	-2.5	-35.4
Z-052373	-2.5	-2.5	-35.4
Z-028840	-2.5	-	-5.0
Z-067926	-2.5	-	-
Z-063380	-2.5	-	-
Z-022721	-2.5	-	-2.5
Z-054905	-2.5	-4.8	-5.0
Z-063828	-2.5	-5.0	-5.0
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Z-083682	-2.4	-	-
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Z-018547	-2.3	-2.0	-
Z-051509	-2.3	-42.0	-7.0
Z-065891	-2.3	-	-3.5
Z-065892	-2.3	-	-3.5
Z-063513	-2.3	-7.0	-7.0
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Z-036585	-2.3	-7.0	-164.9
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Z-012747	-2.3	-81.7	-3.5
Z-012748	-2.3	-81.7	-3.5
Z-012749	-2.3	-81.7	-3.5
Z-002204	-2.3	-3.2	-6.0
Z-046915	-2.3	-	-
Z-015818	-2.3	-	-
Z-052052	-2.3	-	-
Z-006016	-2.2	-4.5	-3.0
Z-034062	-2.2	-9.0	-3.0
Z-022494	-2.2	-	-
Z-056165	-2.2	-4.5	-2.2
Z-075929	-2.2	-3.0	-3.0
Z-084534	-2.2	-	-
Z-086494	-2.2	-	-4.5
Z-015574	-2.2	-4.8	-4.8
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Z-049646	-2.2	-	-
Z-083390	-2.2	-	-5.5
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Z-051265	-2.2	-	-2.7
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Z-084091	-2.2	-2.7	-2.6
Z-078345	-2.2	-2.6	-
Z-031117	-2.2	-3.9	-
Z-006534	-2.2	-	-
Z-025507	-2.1	-2.6	-2.8
Z-025508	-2.1	-2.6	-2.8
Z-025503	-2.1	-2.6	-2.8
Z-025504	-2.1	-2.6	-2.8
Z-066680	-2.1	-	-
Z-083351	-2.1	-7.0	-5.2
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Z-082653	-2.1	-4.7	-
Z-001586	-2.1	-	-
Z-003734	-2.0	-	-2.0
Z-003735	-2.0	-	-2.0
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Z-003737	-2.0	-	-2.0
Z-041137	-2.0	-	-
Z-045353	-2.0	-2.0	-237.8
Z-043433	-2.0	-	-
Z-040272	-2.0	-2.0	-26.8
Z-006329	-2.0	-	-19.6
Z-026224	-2.0	-6.0	-6.0
Z-007710	-2.0	-	-2.0
Z-037050	-2.0	-47.3	-47.8
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Z-062178	-2.0	-3.2	-
Z-067126	-2.0	-3.5	-
Z-043526	-2.0	-2.0	-
Z-052206	-2.0	-4.0	-
Z-007177	-2.0	-42.1	-42.5
Z-047398	-2.0	-2.0	-29.8
Z-059493	-2.0	-	-
Z-023564	-2.0	-2.0	-22.4
Z-023131	-2.0	-2.3	-
Z-056897	-2.0	-26.6	-2.0
Z-048505	-2.0	-3.0	-
Z-057773	-2.0	-5.3	-
Z-069478	-2.0	-	-23.9
Z-063488	-2.0	-	-48.4
Z-005565	-2.0	-57.6	-58.1
Z-085786	-2.0	-34.2	-34.5
Z-068220	-2.0	-	-54.5
Z-043222	-2.0	-	-3.0
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Z-029724	-2.0	-4.0	-2.0
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Z-034995	-2.0	-3.0	-3.0
Z-032989	-2.0	-11.5	-11.6
Z-020860	-2.0	-2.0	-
Z-064386	-2.0	-6.0	-
Z-034670	-2.0	-38.0	-38.3
Z-085490	-2.0	-	-
Z-045949	-2.0	-35.6	-36.0
Z-083139	-2.0	-	-
Z-048106	-2.0	-	-
Z-022083	-2.0	-	-2.0
Z-040645	-2.0	-2.0	-2.0
Z-057626	-2.0	-4.0	-2.0
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Z-047778	-2.0	-	-2.0
Z-050330	-2.0	-2.0	-2.9
Z-050331	-2.0	-2.0	-3.0
Z-037701	-2.0	-6.0	-6.0
Z-079362	-2.0	-	-21.1
Z-063387	-2.0	-2.0	-22.8
Z-074113	-2.0	-2.0	-24.7
Z-064713	-2.0	-24.9	-25.1
Z-073105	-2.0	-34.2	-34.5
Z-018820	-2.0	-	-53.7
Z-009870	-2.0	-2.0	-61.2
Z-015980	-2.0	-84.3	-85.1
Z-044299	-2.0	-4.0	-90.6
Z-047872	-2.0	-157.0	-158.5
Z-015350	-2.0	-	-
Z-029653	-2.0	-	-
Z-035907	-2.0	-2.0	-
Z-077970	-2.0	-2.0	-
Z-085837	-2.0	-18.4	-
Z-068436	-2.0	2.0	-
Z-069103	-2.0	-80.4	-81.1
Z-024738	-2.0	-21.7	-
Z-073141	-2.0	-	-
Z-013654	-2.0	-	-
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Z-012862	-2.0	-20.1	-20.3
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Z-050097	-2.0	-	-
Z-010891	-2.0	-2.0	-
Z-010688	-2.0	-73.5	-74.2
Z-064478	-2.0	-181.8	-
Z-064451	-2.0	-	-
Z-063609	-2.0	-	-
Z-063611	-2.0	-	-
Z-039971	-2.0	-	-
Z-057176	-2.0	-	-
Z-005265	-2.0	-	-
Z-041608	-2.0	-45.5	-2.0
Z-041610	-2.0	-45.5	-2.0
Z-063650	-2.0	-75.1	-2.0
Z-064655	-2.0	-181.8	-4.0
Z-023565	-2.0	-2.0	-24.9
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Z-084503	-2.0	-2.0	-51.3
Z-083355	-2.0	-98.7	-99.7
Z-087207	-2.0	-	-102.5
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Z-028408	-2.0	-	-
Z-028410	-2.0	-	-
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Z-034050	-	-2.5	-
Z-019658	-	-2.6	-2.4
Z-019659	-	-2.6	-2.4
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Z-033429	-	-3.0	-
Z-011647	-	-	-2.6
Z-017113	-	-2.7	-2.7
Z-029368	-	-4.5	-4.5
Z-029379	-	-4.5	-4.5
Z-078673	-	-2.2	-
Z-066470	-	-3.0	-241.4
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Z-037545	-	-	-3.5
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Z-031900	-	-	-2.3
Z-087424	-	-3.0	-74.2
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Z-043978	-	-	-
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Z-000306	-	-5.0	-2.5
Z-047701	-	-5.0	-2.5
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Z-047695	-	-5.0	-2.5
Z-085789	-	-2.0	-
Z-027155	-	-5.0	-260.2
Z-020567	-	-2.5	-
Z-085556	-	-	-2.5
Z-056185	-	-5.0	-5.0
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Z-048627	-	-	-5.7
Z-048628	-	-	-5.7
Z-048632	-	-	-5.7
Z-074066	-	-2.5	-10.0
Z-037108	-	-2.0	-147.7
Z-064010	-	-5.0	-
Z-016490	-	-3.0	-
Z-056569	-	-	-2.5
Z-056572	-	-	-2.5
Z-088871	-	-	-2.5
Z-050340	-	-2.5	-2.5
Z-033697	-	-5.0	-
Z-033698	-	-5.0	-
Z-033274	-	-5.0	-
Z-057802	-	-	-2.5
Z-083356	-	-	-3.0
Z-041308	-	-2.0	-
Z-054910	-	-4.8	-2.7
Z-045069	-	-2.2	-
Z-045070	-	-2.2	-
Z-042794	-	-3.8	-2.8
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Z-060915	-	-	-2.0
Z-031198	-	-2.7	-4.0
Z-035117	-	-101.6	-102.5
Z-065805	-	-2.7	-
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Z-034574	-	-2.3	-
Z-022981	-	-3.6	-
Z-050557	-	-4.6	-3.9
Z-033270	-	-	-47.8
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Z-003392	-	-	-11.1
Z-039708	-	-	-2.0
Z-084106	-	-6.0	-74.7
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Z-003393	-	-	-10.4
Z-059880	-	-2.0	-23.1
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Z-076291	-	-4.7	-
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Z-048777	-	-2.0	-
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Z-040115	-	-4.0	-4.0
Z-056173	-	-3.0	-
Z-022527	-	-3.0	-3.0
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Z-040375	-	-	-3.0
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Z-030791	-	-3.0	-
Z-003892	-	-3.0	-
Z-029730	-	-3.0	-3.0
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Z-076493	-	-12.0	-3.0
Z-033288	-	-3.0	-
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Z-062403	-	-2.0	-
Z-076190	-	-	-3.0
Z-085195	-	-9.0	-4.5
Z-035361	-	-3.0	-
Z-034974	-	-	-15.9
Z-068266	-	-	-3.0
Z-014646	-	-39.6	-
Z-040219	-	-3.0	-3.0
Z-064229	-	-3.0	
Z-074881	-	-	-3.0
Z-054276	-	-3.0	-
Z-054277	-	-3.0	-
Z-020992	-	-107.9	-109.0
Z-088323	-	-123.4	-124.5
Z-018159	-	-3.0	-134.1
Z-004090	-	-3.0	-
Z-003411	-	-3.2	-6.8
Z-014889	-	-3.8	-6.3
Z-031978	-	-	-2.0
Z-063843	-	-10.0	-2.5
Z-003410	-	-4.2	-3.6
Z-009033	-	-	-2.5
Z-009034	-	-	-2.5
Z-009035	-	-	-2.5
Z-009036	-	-	-2.5
Z-056759	-	-2.0	-14.0
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Z-075403	-	-	-3.5
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Z-016369	-	-	-15.1
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Z-001861	-	-3.0	-
Z-062104	-	-4.0	-2.0
Z-026935	-	-64.0	-64.6
Z-053843	-	-8.1	-8.2
Z-039113	-	-	-40.6
Z-055805	-	-	-3.0
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Z-010037	-	-3.0	-
Z-056063	-	-2.0	-2.0
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Z-076273	-	-3.0	-
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Z-080164	-	-2.7	-
Z-080156	-	-2.7	-
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Z-085943	-	7.6	-
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Z-055139	-	16.1	-
Z-076959	-	-	12.4
Z-032306	-	-	16.5
Z-043924	-	9.7	9.7
Z-034943	-	-	24.0
Z-076913	-	-	53.8
Z-065598	-	-	28.5
Z-059884	-	33.0	-
Z-020335	-	-	18.8
Z-030589	-	-	16.1
Z-033913	-	5.0	5.0
Z-002971	-	-	3.2
Z-002970	-	-	3.1
Z-066073	-	7.9	4.0
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Z-046026	-	22.3	-
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Z-045000	-	-2.0	-3.0
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Z-002599	-	-2.3	-
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Z-016778	-	-4.1	-3.6
Z-016779	-	-4.1	-3.6
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Z-060359	-	-3.8	-
Z-076505	-	-	-
Z-051641	-	-16.1	-16.3
Z-014305	-	2.0	2.0
Z-018665	-	-	-5.9
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Z-065337	-	-	-
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Z-011959	-	-	2.5
Z-080030	-	2.7	3.3
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Z-073117	-	-	2.4
Z-087379	-	3.5	4.0
Z-023964	-	2.0	-
Z-081365	-	12.0	9.0
Z-051297	-	-	7.0
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Z-071441	-	81.3	135.5
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Z-045738	2.0	2.0	-
Z-024618	2.0	2.0	-
Z-043152	2.0	-	-
Z-065551	2.0	-	-
Z-001920	2.0	3.0	-
Z-061835	2.0	-	-
Z-061828	2.0	-	-
Z-061832	2.0	-	-
Z-009423	2.0	-	-
Z-060706	2.0	-	-
Z-008578	2.0	-	2.0
Z-008577	2.0	-	2.0
Z-008576	2.0	-	2.0
Z-008575	2.0	-	2.0
Z-088456	2.0	-	5.0
Z-042529	2.0	-	2.0
Z-033479	2.0	-	2.0
Z-068568	2.0	-	-
Z-050518	2.0	-	2.0
Z-036584	2.0	-	-

Z-079477	2.0	-	2.0
Z-064959	2.0	-	-
Z-073676	2.0	-	-
Z-088632	2.0	-	2.0
Z-010691	2.0	-	5.0
Z-010692	2.0	-	5.0
Z-010690	2.0	-	5.0
Z-010693	2.0	-	5.0
Z-082589	2.0	-	3.0
Z-082590	2.0	-	3.0
Z-022616	2.0	-	2.0
Z-022618	2.0	-	2.0
Z-015234	2.0	-	-
Z-002671	2.0	4.0	5.0
Z-013667	2.0	2.0	2.0
Z-072406	2.0	4.0	4.0
Z-067925	2.0	2.8	2.7
Z-011997	2.0	2.0	2.0
Z-011998	2.0	2.0	2.0
Z-011991	2.0	2.0	2.0
Z-011994	2.0	2.0	2.0
Z-039830	2.0	3.0	4.0
Z-012544	2.0	2.0	7.0
Z-049901	2.0	3.0	8.0
Z-063612	2.0	2.0	2.0
Z-020797	2.0	5.0	7.0
Z-044842	2.0	2.0	2.0
Z-031225	2.0	-	-
Z-033502	2.0	-	-
Z-058374	2.1	2.1	18.6
Z-058375	2.1	2.1	18.6
Z-076242	2.1	-	-
Z-013656	2.2	2.2	3.8
Z-087652	2.2	2.2	3.8
Z-074020	2.2	-	-
Z-087872	2.2	3.6	2.8
Z-085230	2.2	-	-
Z-072959	2.2	3.8	6.2
Z-071258	2.3	-	-2.0
Z-016074	2.3	-	2.0
Z-076002	2.3	2.3	2.3
Z-034307	2.3	-	-
Z-071877	2.3	-	-
Z-010783	2.4	2.0	2.0
Z-010784	2.4	2.0	2.0
Z-018126	2.4	-	-
Z-050443	2.4	-	-
Z-021213	2.4	-	3.0
Z-076144	2.4	-	-
Z-064354	2.4	-	-
Z-008379	2.5	-	-
Z-002640	2.5	4.0	-
Z-053982	2.5		3.0
Z-024905	2.5	2.0	7.0
Z-004183	2.5	2.0	5.5
Z-039974	2.5	-	3.5
Z-023268	2.5	-	-
Z-023269	2.5	-	-
Z-044454	2.5	3.0	2.5
Z-025728	2.5	6.0	3.5
Z-025727	2.5	6.0	3.5
Z-029299	2.5	2.0	-
Z-049237	2.5	-	-
Z-064963	2.5	-	-
Z-072327	2.5	2.8	4.3
Z-029010	2.5	3.0	3.0
Z-033582	2.5	6.3	10.8
Z-023042	2.5	-	2.0
Z-023043	2.5	-	2.0
Z-023044	2.5	-	2.0

Z-023045	2.5	-	2.0
Z-073727	2.5	-	-
Z-057698	2.5	2.2	2.5
Z-054948	2.5	5.1	7.6
Z-009179	2.6	2.6	-
Z-022673	2.7	-	-
Z-022675	2.7	-	-
Z-003813	2.7	-	2.0
Z-030803	2.7	-	3.7
Z-003810	2.7	-	2.0
Z-065736	2.7	2.8	2.5
Z-065739	2.7	2.8	2.5
Z-065737	2.7	2.8	2.5
Z-050521	2.7	-	-
Z-024904	2.7	4.0	6.0
Z-081766	2.7	3.7	3.7
Z-009176	2.7	2.7	-
Z-018798	2.7	-	-
Z-009690	2.8	-	7.5
Z-042743	2.8	-	-
Z-030804	2.8	4.5	5.5
Z-007129	2.8	13.8	11.0
Z-014584	2.9	4.4	5.8
Z-014585	2.9	4.4	5.8
Z-084150	2.9	2.9	20.1
Z-084152	2.9	2.9	20.1
Z-042288	2.9	2.9	-
Z-081474	2.9	-	2.1
Z-069049	3.0	3.0	-
Z-023633	3.0	2.0	-
Z-041592	3.0	2.0	-
Z-005984	3.0	2.0	5.0
Z-052935	3.0	-	9.3
Z-073128	3.0	-	9.3
Z-006053	3.0	-	2.0
Z-059506	3.0	-	-
Z-045331	3.0	-	-
Z-045332	3.0	-	-
Z-038086	3.0	-	-
Z-043087	3.0	-	-
Z-043088	3.0	-	-
Z-000490	3.0	-	-
Z-000491	3.0	-	-
Z-009385	3.0	-	2.0
Z-055695	3.0	-	-
Z-040793	3.0	4.0	2.0
Z-031684	3.0	7.0	3.0
Z-022604	3.0	6.0	2.0
Z-008233	3.0	3.0	2.0
Z-044130	3.0	-	-
Z-047170	3.0	-	-
Z-051456	3.0	2.0	-
Z-051457	3.0	2.0	-
Z-041477	3.0	2.0	-
Z-055723	3.0	-	-
Z-021133	3.0	2.0	-
Z-043623	3.0	-	4.0
Z-070429	3.0	-	2.0
Z-024637	3.0	-	2.0
Z-007124	3.0	-	-
Z-077221	3.0	-	2.0
Z-061361	3.0	-	-
Z-079133	3.0	5.0	6.0
Z-015643	3.0	3.5	5.5
Z-068188	3.0	3.0	3.0
Z-068189	3.0	3.0	3.0
Z-003790	3.0	3.0	5.0
Z-085347	3.0	3.0	4.0
Z-005118	3.0	8.0	10.0
Z-052932	3.0	10.0	14.0

Z-083452	3.0	3.0	8.0
Z-083453	3.0	3.0	8.0
Z-074184	3.0	4.0	5.5
Z-015642	3.0	3.3	4.5
Z-030554	3.0	-	-
Z-008514	3.0	2.0	2.0
Z-034071	3.0	2.0	2.0
Z-034638	3.0	3.0	-
Z-027722	3.0	-	6.1
Z-052934	3.1	-	2.0
Z-017118	3.1	3.1	-
Z-056660	3.1	6.3	34.5
Z-042217	3.2	9.5	-
Z-020055	3.2	-	-
Z-045343	3.2	-	3.1
Z-084383	3.2	-	-
Z-084386	3.2	-	-
Z-084388	3.2	-	-
Z-002346	3.2	3.2	3.2
Z-048007	3.3	-	5.7
Z-085866	3.3	7.3	9.0
Z-012071	3.4	-	6.7
Z-012072	3.4	-	6.7
Z-081472	3.4	3.5	4.4
Z-021131	3.5	-	-
Z-021132	3.5	-	-
Z-009386	3.5	5.5	4.0
Z-009387	3.5	5.5	4.0
Z-080950	3.5	4.0	2.0
Z-080956	3.5	4.0	2.0
Z-014595	3.5	-	-
Z-030107	3.5	-	-
Z-030106	3.5	-	-
Z-014587	3.5	-	-
Z-014590	3.5	-	-
Z-000642	3.5	2.5	4.0
Z-052978	3.6	-	-
Z-002349	3.6	3.6	3.6
Z-030369	3.6	-	2.2
Z-042218	3.6	7.2	-
Z-064707	3.7	3.0	-
Z-069879	3.7	-	-
Z-078122	3.7	-	-
Z-018892	3.7	2.0	-
Z-012069	3.7	-	-
Z-012070	3.7	-	-
Z-059815	3.7	-	-
Z-023493	3.8	4.0	4.3
Z-027725	3.8	-	7.5
Z-069654	3.8	-	-
Z-057923	3.8	7.1	7.8
Z-055550	3.9	2.6	-
Z-050753	3.9	54.0	77.2
Z-020056	3.9	-	-
Z-014270	4.0	4.0	-
Z-083201	4.0	5.0	2.0
Z-066584	4.0	2.5	7.5
Z-066580	4.0	2.5	7.5
Z-083265	4.0	3.0	29.0
Z-002942	4.0	-	6.0
Z-044712	4.0	2.0	12.0
Z-083329	4.0	-	14.0
Z-073132	4.0	2.0	10.0
Z-035414	4.0	5.0	4.0
Z-013635	4.0	-	-
Z-009665	4.0	-	-
Z-009666	4.0	-	-
Z-012578	4.0	-	-
Z-060327	4.0	-	-
Z-074975	4.0	-	2.0

Z-049459	4.0	-	4.0
Z-084471	4.0	-	2.0
Z-013649	4.0	3.0	6.0
Z-080994	4.0	3.0	6.0
Z-014647	4.0	3.3	11.0
Z-085071	4.0	2.0	2.0
Z-085070	4.0	2.0	2.0
Z-010907	4.0	-	2.7
Z-012021	4.0	16.8	39.0
Z-023485	4.1	-	-
Z-051521	4.1	-	-
Z-027465	4.3	-	-
Z-058774	4.3	5.0	8.0
Z-001930	4.3	2.2	7.7
Z-027474	4.5	-	2.2
Z-029890	4.5	-	-
Z-029882	4.5	-	-
Z-041787	4.5	-	-
Z-081473	4.5	9.5	3.5
Z-050755	4.7	65.8	94.0
Z-029571	4.7	-	-
Z-048450	4.8	-	-
Z-014259	4.9	4.9	-
Z-084991	5.0	6.0	2.0
Z-004868	5.0	3.0	-
Z-004869	5.0	3.0	-
Z-072529	5.0	2.0	6.5
Z-085860	5.0	-	
Z-087760	5.0	-	2.0
Z-049433	5.0	-	-
Z-016626	5.0	3.0	-
Z-003832	5.0	13.0	12.0
Z-013670	5.0	5.0	20.0
Z-025366	5.0	2.0	2.0
Z-080419	5.0	3.0	3.0
Z-087868	5.0	3.0	2.5
Z-044492	5.0	-	5.0
Z-087218	5.1	5.1	10.1
Z-083419	5.1	-	-
Z-008580	5.1	10.3	5.1
Z-087453	5.1	30.9	41.2
Z-016498	5.2	10.5	-
Z-069962	5.4	2.6	-
Z-078703	5.5	2.0	2.0
Z-018630	5.6	28.0	-
Z-018631	5.6	28.0	-
Z-056270	5.6	2.2	-
Z-051205	5.7	-	-
Z-007473	5.8	23.2	5.8
Z-057922	5.8	8.0	9.2
Z-037054	5.9	11.8	2.9
Z-083523	5.9	5.9	-
Z-018125	6.0	-	-
Z-081468	6.0	3.3	-
Z-013373	6.0	7.0	6.0
Z-029919	6.0	2.0	
Z-021347	6.0	-	-15.7
Z-030693	6.0	6.0	-
Z-082518	6.0	-	-
Z-076814	6.0	6.0	10.5
Z-016071	6.0	6.0	33.0
Z-060329	6.0	5.0	3.0
Z-080216	6.0	3.3	3.3
Z-066158	6.1	-	48.6
Z-016454	6.1	42.5	12.1
Z-048661	6.1	-	-
Z-080476	6.1	-	-
Z-078223	6.2	-	-
Z-058764	6.3	-	6.3
Z-058765	6.3	-	6.3

Z-058770	6.3	-	6.3
Z-058771	6.3	-	6.3
Z-008155	6.3	3.2	9.5
Z-056574	6.4	-	6.4
Z-008156	6.4	3.2	9.6
Z-007028	6.4	-	-
Z-025676	6.4	-	-
Z-015361	6.5	8.0	8.5
Z-015365	6.5	8.0	8.5
Z-077556	6.5	3.0	4.5
Z-007031	6.5	-	-
Z-058820	6.6	-	-
Z-015694	6.6	7.0	4.4
Z-055702	6.7	-	2.8
Z-067423	6.7	6.6	-
Z-013485	6.8	-	6.8
Z-037921	6.8	-	-
Z-037856	6.8	2.3	4.3
Z-069093	6.8	-	-
Z-033515	6.9	-	41.1
Z-060539	6.9	-	-
Z-004283	6.9	19.0	19.8
Z-087651	6.9	-	6.9
Z-069570	6.9	-	-
Z-042734	7.0	-	-
Z-013640	7.0	-	3.1
Z-042710	7.0	3.0	9.0
Z-077044	7.0	11.0	6.0
Z-023081	7.0	6.0	9.0
Z-078125	7.0	-	-
Z-086691	7.1	-	-
Z-081471	7.1	-	-
Z-022168	7.2	4.0	2.2
Z-022169	7.2	4.0	2.2
Z-069092	7.2	-	-
Z-076535	7.2	14.4	-
Z-061474	7.2	-	7.2
Z-061466	7.2	-	7.2
Z-042733	7.2	-	-
Z-041376	7.3	-	-
Z-033507	7.3	-	43.8
Z-022170	7.3	2.0	8.0
Z-083680	7.3	2.0	8.0
Z-020063	7.3	4.7	-
Z-038121	7.4	-	-
Z-082488	7.5	-	-
Z-014786	7.5	-	15.0
Z-055677	7.5	-	2.0
Z-079997	7.5	-	2.0
Z-035497	7.5	-	7.5
Z-035498	7.5	-	7.5
Z-040743	7.7	7.7	7.7
Z-025247	7.7	-	-
Z-081500	7.8	4.1	2.2
Z-069905	7.8	-	7.8
Z-056551	7.9	7.9	-
Z-036022	7.9	47.4	7.9
Z-036015	7.9	47.4	7.9
Z-036018	7.9	47.4	7.9
Z-036019	7.9	47.4	7.9
Z-043151	8.0	-	-
Z-020836	8.0	14.0	6.0
Z-035888	8.0	-	-
Z-035889	8.0	-	-
Z-013657	8.0	6.0	14.0
Z-083126	8.0	10.0	22.0
Z-042237	8.0	5.0	4.0
Z-015594	8.1	-	8.1
Z-074961	8.1	8.1	16.2
Z-074962	8.1	8.1	16.2

Z-014439	8.3	-	8.3
Z-049812	8.3	24.9	16.6
Z-072329	8.5	3.1	2.8
Z-018124	8.6	-	-
Z-042312	8.6	-	-
Z-057911	9.0	10.0	4.0
Z-057912	9.0	10.0	4.0
Z-026018	9.0	6.0	5.0
Z-005034	9.0	-	9.0
Z-023602	9.1	9.1	-
Z-023601	9.1	9.1	-
Z-057762	9.4	-	9.4
Z-053920	9.4	3.0	3.2
Z-022713	9.5	2.3	3.0
Z-084728	9.5	-	19.0
Z-082387	9.6	-	-
Z-059431	9.6	4.8	-
Z-007067	9.6	-	19.2
Z-031088	9.6	-	9.6
Z-056552	9.7	9.7	-
Z-065936	9.7	9.7	-
Z-065935	9.7	9.7	-
Z-023051	9.8	-	-
Z-021572	9.9	19.8	19.8
Z-021573	9.9	19.8	19.8
Z-088218	9.9	-	-
Z-069089	10.0	29.9	-
Z-003323	10.0	2.0	6.0
Z-029617	10.0	10.0	-
Z-020559	10.2	-	-
Z-088936	10.3	-	-
Z-033903	10.3	12.7	17.3
Z-044051	10.7	32.1	-
Z-078370	10.7	3.6	-
Z-078224	10.7	-	10.7
Z-018489	10.9	-	175.1
Z-082029	11.0	-	11.0
Z-056419	11.0	-	-
Z-044310	11.0	-	-
Z-081470	11.1	2.6	-
Z-067422	11.1	4.7	2.7
Z-041173	11.2	5.6	5.6
Z-048016	11.4	34.1	91.0
Z-086414	11.4	-	3.8
Z-086415	11.4	-	3.8
Z-086417	11.4	-	3.8
Z-086421	11.4	-	3.8
Z-066144	11.5	-	-
Z-066146	11.5	-	-
Z-064695	11.6	-	4.3
Z-083314	11.6	11.6	-
Z-083318	11.6	11.6	-
Z-083306	11.6	11.6	-
Z-083308	11.6	11.6	-
Z-003873	11.9	23.7	-
Z-084851	11.9	-	-
Z-052157	12.0	12.0	16.6
Z-069895	12.1	24.2	-
Z-083783	12.1	-	-
Z-083784	12.1	-	-
Z-074701	12.2	-	-
Z-064692	12.2	12.2	12.2
Z-046039	12.3	-	-
Z-060719	12.4	24.7	37.1
Z-044364	12.4	-	-
Z-065054	12.4	37.3	12.4
Z-029468	12.4	49.7	12.4
Z-064696	12.5	-	4.7
Z-086721	12.6	-	-
Z-020626	12.8	-	-

Z-009444	12.9	12.9	25.9
Z-033150	13.1	-	-
Z-033151	13.1	-	-
Z-053692	13.1	6.6	-
Z-031366	13.4		13.4
Z-073799	13.4	13.4	-
Z-040340	13.4	-	-
Z-010419	13.5	-	13.5
Z-053932	13.5	13.5	-
Z-053937	13.5	13.5	-
Z-044861	13.5	-	-
Z-024872	13.5	-	-
Z-023934	13.5	13.5	108.4
Z-006330	13.7	-	-
Z-067421	13.8	2.0	2.2
Z-042449	13.8	-	-
Z-054543	14.0	-	7.0
Z-007980	14.2	14.2	-
Z-075763	14.2	-	-
Z-019187	14.4	-	-
Z-019188	14.4	-	-
Z-005090	14.5	-	-
Z-012236	14.6	-	-
Z-042847	14.6	-	-
Z-069906	14.8	29.6	59.1
Z-043755	14.8	-	4.9
Z-023318	15.0	-	-
Z-077110	15.1	15.1	22.6
Z-025301	15.1	11.3	3.8
Z-053629	15.5	15.5	-
Z-029602	15.5	31.0	15.5
Z-078914	15.6	-	-
Z-016885	15.6	15.6	15.6
Z-071449	15.6	15.6	15.6
Z-014707	15.7	-	-
Z-076067	15.7	47.2	36.7
Z-025662	15.8	-	63.2
Z-021498	15.8	15.8	31.6
Z-068462	15.9	-	-
Z-035013	16.0	-	-
Z-030268	16.0	-	16.0
Z-005730	16.1	-	8.1
Z-074371	16.3	32.5	-
Z-023320	16.3	-	-
Z-013478	16.3	-	-
Z-024654	16.5	16.5	-
Z-003522	16.5	16.5	-
Z-012026	16.5	-	-
Z-012027	16.5	-	-
Z-034063	16.6	16.6	33.2
Z-032775	16.7	-	-
Z-004511	16.7	-	-
Z-004512	16.7	-	-
Z-072114	16.7	33.5	133.9
Z-077557	16.9	16.9	22.5
Z-057770	16.9	-	-
Z-084607	17.0	-	-
Z-030165	17.0	-	-
Z-030166	17.0	-	-
Z-007973	17.0	17.0	17.0
Z-069656	17.1	-	4.3
Z-086169	17.2	-	17.2
Z-064764	17.2	-	172.4
Z-019850	17.4	34.7	34.7
Z-003320	17.6	7.6	7.6
Z-024304	17.6	35.3	-
Z-045609	17.8	-	35.6
Z-072192	17.8	-	-
Z-009184	17.8	-	-
Z-041961	17.8	-	-

Z-044004	17.8	17.8	71.1
Z-024873	18.1	-	-
Z-043391	18.1	-	-
Z-088553	18.4	-	-
Z-003896	18.4	36.7	55.1
Z-031470	18.5	-	-
Z-012658	18.5	-	-
Z-012659	18.5	-	-
Z-001811	18.5	-	-
Z-022371	18.5	92.5	296.1
Z-020102	18.7	18.7	-
Z-063330	18.7	56.0	18.7
Z-003897	18.7	37.3	56.0
Z-041932	19.0	19.0	-
Z-087454	19.0	-	-
Z-075372	19.0	-	-
Z-066550	19.0	-	-
Z-053833	19.0	9.5	9.5
Z-015368	19.0	19.0	37.9
Z-015372	19.0	19.0	37.9
Z-016880	19.0	37.9	19.0
Z-082437	19.3	-	-
Z-024331	19.3	144.7	135.0
Z-033602	19.5	34.0	-
Z-061494	19.5	-	-
Z-031369	19.5	-	-
Z-083547	19.6	-	-
Z-083572	19.6	-	-
Z-034357	19.6	39.2	19.6
Z-088171	19.6	19.6	19.6
Z-002057	19.7	39.4	34.5
Z-043136	19.8	19.8	-
Z-079023	20.0	-	-
Z-056425	20.0	-	-
Z-056426	20.0	-	-
Z-012713	20.0	-	-
Z-043806	20.1	10.1	-
Z-027158	20.2	40.5	10.1
Z-081048	20.3	-	20.3
Z-045608	20.3	-	40.6
Z-025861	20.3	20.3	-
Z-051158	20.7	-	-
Z-017135	20.7	-	-
Z-068538	20.9	-	-
Z-052155	21.1	-	7.0
Z-022044	21.3	10.6	-
Z-031597	21.5	-	-
Z-083418	21.5	-	10.7
Z-050516	21.5	-	-
Z-045369	21.7	-	21.7
Z-003729	21.9	-	21.9
Z-012600	21.9	-	-
Z-064064	21.9	-	-
Z-061520	22.3	401.6	502.1
Z-080288	22.3	89.3	-
Z-046778	22.3	-	-
Z-064505	22.3	-	-
Z-085285	22.3	11.2	33.5
Z-049010	22.8	7.6	-
Z-067196	22.8	-	22.8
Z-027726	22.8	-	-
Z-064856	22.8	-	-
Z-044545	22.8	-	-
Z-074994	22.8	-	-
Z-056523	22.8	22.8	22.8
Z-043600	22.8	22.8	45.5
Z-083510	22.9	-	-
Z-033893	23.0	-	69.0
Z-083076	23.0	23.0	23.0
Z-018937	23.2	5.8	17.4

Z-086152	23.5	-	-
Z-025776	23.5	-	-
Z-016380	23.5	-	-
Z-057242	23.5	23.5	-
Z-023048	23.6	35.5	5.9
Z-003079	24.0	24.0	-
Z-034982	24.0	24.0	59.9
Z-025779	24.2	-	24.2
Z-038115	24.2	-	72.6
Z-001234	24.2	-	24.2
Z-005751	24.2	-	-
Z-081732	24.2	-	-
Z-064177	24.2	72.6	72.6
Z-063431	24.3	48.7	158.2
Z-035935	24.5	-	24.5
Z-023641	24.5	-	-
Z-063602	24.7	99.0	-
Z-024697	24.7	-	-
Z-088165	24.7	24.7	24.7
Z-001028	25.0	-	25.0
Z-029992	25.0	-	-
Z-088374	25.0	-	-
Z-035012	25.0	75.0	66.7
Z-078302	25.3	-	25.3
Z-043801	25.3	25.3	-
Z-017433	25.3	25.3	-
Z-048642	25.3	-	-
Z-048644	25.3	-	-
Z-024299	25.3	25.3	50.6
Z-057309	25.4	-	-
Z-065336	25.4	-	12.7
Z-059451	25.5	-	-
Z-059454	25.5	-	-
Z-059466	25.5	-	-
Z-059442	25.5	-	-
Z-056046	25.6	25.6	-
Z-031185	25.6	25.6	-
Z-075373	25.6	76.7	-
Z-007094	25.6	-	-
Z-082388	25.7	4.1	2.2
Z-012325	25.9	-	25.9
Z-075571	25.9	-	51.7
Z-043849	26.2	26.2	26.2
Z-011674	26.5	13.2	-
Z-063621	26.5	26.5	-
Z-072268	26.5	-	-
Z-040995	26.8	26.8	-
Z-045505	27.1	-	81.3
Z-085479	27.1	27.1	-
Z-029632	27.1	-	-
Z-044838	27.1	-	-
Z-057370	27.1	-	-
Z-045140	27.1	-	-
Z-045141	27.1	-	-
Z-053628	27.4	-	27.4
Z-060567	27.4	27.4	82.3
Z-034626	27.4	13.7	13.7
Z-034627	27.4	13.7	13.7
Z-008737	27.4	54.8	41.1
Z-084845	27.4	-	13.7
Z-064782	27.6	27.6	55.2
Z-084415	27.8	13.9	-
Z-020443	27.8	-	27.8
Z-020446	27.8	-	27.8
Z-064876	27.8	-	27.8
Z-060589	27.8	-	-
Z-015472	28.1	-	-
Z-004477	28.1	28.1	28.1
Z-001416	28.5	-	28.5
Z-064965	28.5	85.3	-

Z-002643	28.5	28.5	34.1
Z-014586	28.6	21.5	42.9
Z-047796	28.6	14.3	28.6
Z-043747	28.8	115.2	-
Z-051455	28.8	-	-
Z-031822	28.8	-	-
Z-053333	28.8	-	-
Z-084262	28.8	-	-
Z-086786	29.0	-	-
Z-005454	29.0	-	-
Z-088950	29.2	-	29.2
Z-076720	29.2	-	-
Z-030036	29.2	-	-
Z-053258	29.2	58.4	58.4
Z-076721	29.2	-	-
Z-003847	29.4	117.5	161.5
Z-066139	29.4	19.6	9.8
Z-029601	29.6	-	-
Z-039829	29.6	29.6	29.6
Z-043106	29.9	-	149.7
Z-003080	29.9	29.9	-
Z-023053	29.9	-	-
Z-006273	29.9	-	-
Z-006274	29.9	-	-
Z-086168	29.9	-	-
Z-065058	30.3	-	30.3
Z-042481	30.8	123.0	-
Z-030675	30.8	30.8	-
Z-021201	30.8	30.8	61.5
Z-022196	30.8	61.5	30.8
Z-036021	30.8	61.5	92.3
Z-036017	30.8	61.5	92.3
Z-030670	31.2	-	31.2
Z-003195	31.2	-	-
Z-074806	31.2	-	-
Z-050271	31.2	-	-
Z-041623	31.6	31.6	-
Z-033532	31.6	-	15.8
Z-033533	31.6	-	15.8
Z-040319	32.1	-	32.1
Z-039481	32.1	-	-
Z-021874	32.1	-	-
Z-064445	32.1	32.1	96.2
Z-002645	32.1	32.1	38.6
Z-017174	32.5	-	-
Z-044061	32.5	-	-
Z-084074	32.5	32.5	48.8
Z-001943	32.5	162.6	260.1
Z-053229	32.5	162.6	260.1
Z-000600	32.7	-	16.4
Z-029264	33.0	33.0	-
Z-083532	33.0	33.0	-
Z-038196	33.0	-	-
Z-022028	33.0	33.0	131.9
Z-031417	33.5	-	-
Z-021704	33.5	-	-
Z-042548	34.0	67.9	-
Z-021574	34.0	34.0	-
Z-012369	34.0	25.5	25.5
Z-012769	34.2	17.1	51.3
Z-064786	34.5	-	34.5
Z-044214	34.5	-	-
Z-036940	34.5	34.5	17.2
Z-043608	34.7	11.6	-
Z-027480	35.0	70.0	-
Z-025652	35.0	-	-
Z-057301	35.0	-	-
Z-028267	35.0	-	-
Z-070577	35.0	-	-
Z-029734	35.0	35.0	35.0

Z-012022	35.2	19.7	35.3
Z-018943	35.3	-	-
Z-088846	35.3	17.6	17.6
Z-081494	35.6	7.0	8.9
Z-057616	35.6	-	-
Z-043181	35.9	-	-
Z-061521	36.1	-	-
Z-068536	36.1	-	108.4
Z-065901	36.1	36.1	-
Z-072526	36.1	36.1	72.3
Z-051276	36.1	72.3	108.4
Z-079990	36.4	-	-
Z-082428	36.7	-	-
Z-085248	37.3	-	37.3
Z-042518	37.3	-	37.3
Z-064313	37.3	-	-
Z-056724	37.3	-	-
Z-016443	37.6	18.8	-
Z-051883	37.9	-	19.0
Z-053337	38.6	-	-
Z-087875	38.6	173.6	77.2
Z-054449	39.0	26.0	13.0
Z-000546	39.2	-	-
Z-000547	39.2	-	-
Z-023599	39.2	29.4	14.7
Z-023600	39.2	29.4	14.7
Z-026786	39.7	13.2	26.5
Z-026789	39.7	13.2	26.5
Z-068138	39.9	39.9	-
Z-083191	39.9	-	-
Z-044976	39.9	-	-
Z-008459	39.9	-	-
Z-032659	39.9	-	-
Z-068002	39.9	79.9	159.7
Z-053951	40.3	-	40.3
Z-004992	40.6	-	40.6
Z-040435	40.6	-	-
Z-070988	40.6	-	-
Z-009612	41.4	-	41.4
Z-065961	41.4	-	-
Z-065962	41.4	-	-
Z-028075	41.8	41.8	20.9
Z-081532	42.1	-	42.1
Z-002820	42.1	42.1	-
Z-021179	42.1	-	-
Z-086008	42.1	-	-
Z-065201	42.1	84.3	168.6
Z-048635	42.4	42.4	14.1
Z-083521	42.7	42.7	42.7
Z-033291	42.9	85.9	-
Z-083658	42.9	-	-
Z-061522	43.4	-	-
Z-012023	43.5	43.5	43.5
Z-066789	43.8	-	-
Z-048092	44.5	8.9	53.3
Z-080404	44.6	-	29.8
Z-062255	44.6	44.6	-
Z-033084	44.6	-	-
Z-026745	44.6	-	-
Z-056214	44.6	-	-
Z-079005	44.6	22.3	22.3
Z-049581	45.1	-	-
Z-049009	45.5	15.2	-
Z-049014	45.5	15.2	-
Z-062277	45.5	22.8	-
Z-062278	45.5	22.8	-
Z-046914	45.5	-	-
Z-052153	45.5	45.5	74.3
Z-034319	45.5	75.9	45.5
Z-034320	45.5	75.9	45.5

Z-040038	45.5	22.8	22.8
Z-068475	45.5	-	22.8
Z-003928	46.4	23.2	-
Z-041715	46.4	-	46.4
Z-038630	46.4	46.4	-
Z-076158	46.4	23.2	46.4
Z-037117	47.4	-	-
Z-065894	47.4	94.8	94.8
Z-029593	48.2	38.6	28.9
Z-039228	48.4	-	-
Z-072866	48.4	24.2	145.3
Z-015595	48.8	16.3	97.5
Z-053716	48.9	-	-
Z-072524	49.5	-	49.5
Z-056239	49.5	-	49.5
Z-056622	49.5	49.5	-
Z-027999	49.5	-	-
Z-052942	49.5	49.5	99.0
Z-077173	49.5	-	12.4
Z-007885	50.2	50.2	16.7
Z-070614	50.6	151.7	101.2
Z-005207	50.6	50.6	-
Z-042042	50.6	-	50.6
Z-079168	50.6	-	-
Z-046208	50.6	-	-
Z-007306	50.6	-	-
Z-058616	51.1	51.1	306.9
Z-057175	51.1	-	-
Z-054528	51.7	17.2	51.7
Z-014713	51.7	-	103.5
Z-028954	51.7	-	-
Z-042833	52.0	26.0	-
Z-034747	52.3	78.5	26.2
Z-031814	52.5	52.5	35.0
Z-031815	52.5	52.5	35.0
Z-013351	52.9	92.6	39.7
Z-021491	52.9	52.9	-
Z-073151	52.9	-	-
Z-076548	52.9	-	-
Z-015397	52.9	-	-
Z-020316	54.2	-	-
Z-048620	54.2	-	-
Z-085497	54.2	-	-
Z-015641	54.8	-	-
Z-052154	54.9	28.5	34.9
Z-068273	55.5	55.5	111.0
Z-015463	55.5	-	55.5
Z-046218	55.5	-	-
Z-046220	55.5	-	-
Z-014112	55.5	-	-
Z-027968	56.2	140.5	-
Z-052158	56.9	23.3	23.3
Z-011223	56.9	56.9	56.9
Z-006294	56.9	227.6	113.8
Z-031292	56.9	56.9	-
Z-034232	56.9	-	-
Z-066780	57.6	57.6	115.2
Z-032971	58.4	29.2	29.2
Z-014762	58.4	-	-
Z-074835	58.4	-	-
Z-020832	59.1	88.7	118.2
Z-053745	59.4	-	19.8
Z-076133	59.9	-	-
Z-002778	61.0	40.6	20.3
Z-009749	61.2	12.2	36.7
Z-067787	61.5	184.5	-
Z-004478	61.5	61.5	-
Z-010770	61.5	30.8	-
Z-042735	61.5	-	-
Z-083033	62.4	31.2	218.2

Z-007060	62.4	-	-
Z-038648	63.2	189.7	189.7
Z-069987	63.2	21.1	-
Z-086640	63.2	-	-
Z-034495	63.2	-	-
Z-027514	64.1	80.1	128.2
Z-028081	65.0	-	390.2
Z-013648	65.0	-	260.1
Z-039103	65.0	-	-
Z-041716	65.0	-	-
Z-077387	66.9	-	100.4
Z-068574	66.9	-	-
Z-034347	66.9	-	-
Z-046177	66.9	-	-
Z-036251	67.6	90.1	112.7
Z-070222	67.9	237.8	67.9
Z-010226	69.0	-	-
Z-055109	71.1	35.6	71.1
Z-029371	71.1	23.7	-
Z-088643	71.1	-	47.4
Z-003281	72.3	-	-
Z-040963	73.4	-	-
Z-085349	76.4	91.7	61.1
Z-021105	77.2	115.7	115.7
Z-087587	77.4	15.5	15.5
Z-016024	77.6	-	-
Z-012549	78.5	117.7	137.3
Z-087766	78.5	-	78.5
Z-020037	80.3	26.8	26.8
Z-040994	81.3	162.6	203.2
Z-042408	81.3	-	-
Z-083809	82.3	82.3	27.4
Z-058819	82.3	-	13.7
Z-032407	84.3	126.4	42.1
Z-041114	84.3	421.5	843.0
Z-063634	85.9	-	85.9
Z-078476	85.9	-	-
Z-038629	87.5	-	-
Z-080425	88.4	22.1	22.1
Z-028666	89.3	89.3	133.9
Z-019322	89.3	89.3	-
Z-063395	90.5	55.7	69.6
Z-033481	90.5	142.3	336.2
Z-024486	92.3	92.3	61.5
Z-000287	96.9	193.7	48.4
Z-016316	96.9	48.4	-
Z-072546	99.0	148.4	99.0
Z-055383	99.0	49.5	346.3
Z-007180	99.0	49.5	-
Z-077555	99.0	-	99.0
Z-001942	99.0	-	-
Z-043098	103.5	69.0	-
Z-064780	103.5	51.7	155.2
Z-030794	105.9	211.7	423.4
Z-036252	105.9	-	52.9
Z-065057	108.4	108.4	108.4
Z-042717	111.0	166.5	-
Z-064525	113.8	-	-
Z-049818	113.8	-	-
Z-063852	119.8	119.8	179.7
Z-008740	119.8	29.9	179.7
Z-087869	126.4	632.2	569.0
Z-045385	127.9	-	51.1
Z-072191	130.1	130.1	195.1
Z-025723	133.9	-	-
Z-018050	133.9	133.9	200.8
Z-027066	136.6	182.1	-
Z-049948	140.5	140.5	140.5
Z-033585	144.1	100.8	86.4
Z-057877	146.8	110.1	-

Z-024293	149.7	119.8	-
Z-064168	155.2	103.5	155.2
Z-057283	159.7	479.2	399.3
Z-002719	162.6	-	-
Z-051518	168.6	-	-
Z-004473	173.2	99.0	99.0
Z-001875	175.1	-	-
Z-030793	175.1	116.7	525.2
Z-012395	177.8		106.7
Z-030725	179.7	119.8	658.8
Z-029575	184.5	184.5	-
Z-067420	186.8	-	60.9
Z-024119	203.8	101.9	237.8
Z-008424	210.7	210.7	126.4
Z-041070	211.7	317.6	317.6
Z-082389	227.9	86.0	-
Z-057282	263.9	197.9	230.9
Z-068270	264.5	163.0	185.8
Z-040426	317.6	105.9	52.9
Z-074159	379.3	47.4	474.2
Z-081475	610.8	166.5	445.0
Z-020170	667.1	39.2	78.5
Z-084505	1404.3	1573.8	2058.1
Z-081421	2080.9	162.6	130.1
Z-081495	2341.0	162.6	-
Z-047376	-	-2.5	-
Z-025059	-	-	-5.8
Z-025060	-	-	-5.8
Z-061970	-	-2.0	-
Z-085947	-	-2.0	-2.0
Z-030989	-	-2.0	-15.0
Z-060607	-	-13.8	-
Z-035419	-	-6.3	-6.3
Z-021727	-	-2.0	-32.9
Z-012366	-	-13.5	-13.6
Z-053246	-	-20.3	-
Z-033191	-	-24.0	-24.2
Z-064722	-	-2.0	-15.9
Z-064341	-	-	-23.6
Z-064342	-	-	-23.6
Z-070839	-	-	-32.9
Z-044323	-	-6.0	-3.0
Z-059503	-	-	-7.9
Z-047399	-	-2.0	-
Z-009602	-	-30.3	-30.6
Z-034744	-	-	-4.0
Z-059984	-	-12.0	-12.1
Z-026820	-	-32.0	-32.3
Z-020129	-	-	-2.0
Z-049599	-	-9.8	-9.9
Z-084000	-	-4.6	-4.7
Z-049238	-	-27.0	-
Z-049240	-	-27.0	-
Z-022768	-	-2.0	-2.0
Z-044111	-	-3.0	-
Z-044112	-	-3.0	-
Z-014736	-	-	-12.3
Z-014738	-	-	-12.3
Z-071115	-	-30.3	-30.6
Z-002231	-	-	-3.0
Z-064094	-	-	-18.6
Z-031219	-	-5.3	-5.3
Z-013866	-	-22.2	-22.4
Z-034175	-	-6.8	-6.8
Z-072154	-	-9.3	-9.4
Z-009367	-	-9.9	-
Z-028005	-	-3.0	-3.0
Z-028006	-	-3.0	-3.0
Z-017140	-	-12.5	-12.6
Z-062057	-	-11.8	-11.9

Z-066042	-	-37.6	-
Z-066041	-	-38.7	-
Z-009359	-	-4.1	-4.2
Z-062906	-	-	-3.0
Z-043618	-	-	-6.5
Z-086313	-	-2.0	-
Z-074183	-	-5.1	-
Z-075746	-	-	-113.7
Z-085482	-	-15.2	-15.3
Z-021672	-	-2.0	-2.0
Z-076992	-	-26.2	-26.4
Z-085937	-	-21.9	-22.1
Z-072298	-	-	-23.3
Z-051198	-	-30.9	-31.1
Z-035477	-	-62.8	-63.4
Z-065825	-	-	-17.4
Z-065827	-	-	-17.4
Z-022148	-	-2.3	-
Z-033125	-	-2.0	-
Z-020716	-	-2.5	-2.6
Z-020714	-	-2.6	-2.6
Z-076607	-	-25.3	-3.0
Z-025182	-	-4.8	-4.8
Z-066782	-	-16.3	-16.5
Z-021577	-	-	-19.8
Z-064263	-	-	-87.2
Z-085910	-	-6.3	-6.3
Z-035439	-	-32.6	-32.9
Z-073458	-	-2.6	-
Z-004163	-	-	-25.6
Z-078204	-	-2.0	-
Z-066831	-		-3.0
Z-003275	-	-2.7	-2.4
Z-005691	-	-	-18.6
Z-083978	-	-	-4.0
Z-083979	-	-	-4.0
Z-083980	-	-	-4.0
Z-083977	-	-	-4.0
Z-087291	-	-4.0	-6.0
Z-085185	-	-	-8.7
Z-072849	-	-4.9	-
Z-072853	-	-4.9	-
Z-072855	-	-4.9	-
Z-072857	-	-4.9	-
Z-026808	-	-	-12.1
Z-052231	-	-7.0	-
Z-076502	-	-11.0	-11.1
Z-079213	-	-2.7	-
Z-045761	-	-2.0	-2.0
Z-079615	-	-3.0	-3.0
Z-064389	-	-	-3.0
Z-019781	-	-6.0	-6.0
Z-084001	-	-7.1	-7.1
Z-014258	-	-10.9	-11.0
Z-014273	-	-10.9	-11.0
Z-041771	-	-20.6	-20.8
Z-077160	-	-20.6	-20.8
Z-068802	-	-26.6	-26.8
Z-025062	-	-29.3	-29.6
Z-081553	-	-34.6	-34.9
Z-040200	-	-	-38.8
Z-017458	-	-49.4	-49.8
Z-041994	-	-29.8	-

Transcripts order based on high to low regulation of 27% PEG followed by 31% and 34% PEG, respectively.
“-“ no statistical difference in expression level of contig

Table S4.2. Summary of the upregulated (≥2-fold) transcripts that were common to all PEG treatments

			Fold regulation		
Gene ID	**Dehydration-response pathway**	**Gene annotation**	**27% PEG**	**31% PEG**	**34% PEG**
Z-081421		coat protein co-chaperone	2080.9	162.6	130.1
Z-084505	ABA-dependent	PYL4_ABA receptor	1404.3	1573.8	2058.1
Z-081475	-	-	610.8	166.5	445.0
Z-068270	-	-	264.5	163.0	185.8
Z-057282	-	-	263.9	197.9	230.9
Z-041070	leaf senescence	leaf senescence	211.7	317.6	317.6
Z-008424	ABA-dependent	alpha-tubulin	210.7	210.7	126.4
Z-024119	-	ribosomal protein s10	203.8	101.9	237.8
Z-030725	-	-	179.7	119.8	658.8
Z-030793	-	-	175.1	116.7	525.2
Z-057283	ABA-dependent	PYL5_ABA receptor	159.7	479.2	399.3
Z-064168	-		155.2	103.5	155.2
Z-049948	-	60s ribosomal protein l11	140.5	140.5	140.5
Z-018050	-	-	133.9	133.9	200.8
Z-072191	-	-	130.1	130.1	195.1
Z-087869	-	ATP-dependent CLP protease	126.4	632.2	569.0
Z-063852	-	phagocytosis and cell motility protein ELMO1-like	119.8	119.8	179.7
Z-065057	-	calcineurin b subunit	108.4	108.4	108.4
Z-030794	-	NADH-plastoquinone oxidoreductase subunit k	105.9	211.7	423.4
Z-024486	-	40s ribosomal protein SA	92.3	92.3	61.5
Z-063395	-	protein binding	90.5	55.7	69.6
Z-085349	-	embryo-specific protein 1	76.4	91.7	61.1
Z-011223	-	nucleolar protein	56.9	56.9	56.9
Z-029593	-	predicted protein [*Hordeum vulgare*]	48.2	38.6	28.9
Z-076158	-	-	46.4	23.2	46.4
Z-040038	-	-	45.5	22.8	22.8
Z-079005	-	protein kinase	44.6	22.3	22.3
Z-012023	-	xyloglucan endotransglycosylase	43.5	43.5	43.5
Z-083521	-	ferredoxin- chloroplast	42.7	42.7	42.7
Z-048635	-	translation elongation factor ts	42.4	42.4	14.1
Z-028075	-	-	41.8	41.8	20.9
Z-026786	-	peroxin PEX14	39.7	13.2	26.5
Z-026789	-	peroxin PEX14	39.7	13.2	26.5
Z-023599	-	at1g68530 t26j14_10	39.2	29.4	14.7
Z-023600	-	at1g68530 t26j14_10	39.2	29.4	14.7
Z-054449	-	protein	39.0	26.0	13.0
Z-088846	-	tubby-like protein	35.3	17.6	17.6
Z-012022	-	xyloglucan endotransglycosylase	35.2	19.7	35.3
Z-029734	-	glutamyl-trna synthetase	35.0	35.0	35.0
Z-036940	-	-	34.5	34.5	17.2
Z-012369	-	arogenate dehydrogenase isoform 2	34.0	25.5	25.5
Z-084074	-	at5g46290 mpl12_7	32.5	32.5	48.8
Z-002645	-	ribosomal protein s9	32.1	32.1	38.6
Z-039829	-	NADH7	29.6	29.6	29.6
Z-014586	-	chloroplast hypothetical protein	28.6	21.5	42.9
Z-047796	-	extensin precursor-like	28.6	14.3	28.6
Z-002643	-	ribosomal protein s9	28.5	28.5	34.1
Z-004477	root hair	pattern formation	28.1	28.1	28.1
Z-034626	-	COV1-like protein	27.4	13.7	13.7

Z-034627	-	COV1-like protein	27.4	13.7	13.7
Z-043849	-	RAB8 -family small gtpase	26.2	26.2	26.2
Z-088165	-	-	24.7	24.7	24.7
Z-083076	-	protein	23.0	23.0	23.0
Z-056523	-	KH domain	22.8	22.8	22.8
Z-043600	-	ovate family	22.8	22.8	45.5
Z-085285	-	anther-specific APG	22.3	11.2	33.5
Z-027158	-	unknown [*Zea mays*]	20.2	40.5	10.1
Z-002057	-	beta-IG-H3 domain	19.7	39.4	34.5
Z-034357	-	glycosyl hydrolase family 3	19.6	39.2	19.6
Z-088171	-	Os08g0241500	19.6	19.6	19.6
Z-015368	-	NPH4 (non-phototrophic hypocotyl) DNA binding transcription activator	19.0	19.0	37.9
Z-015372	-	NPH4 (non-phototrophic hypocotyl) DNA binding transcription activator	19.0	19.0	37.9
Z-016880	-	-	19.0	37.9	19.0
Z-019850	-	blue copper protein precursor	17.4	34.7	34.7
Z-007973	salt stress	reversibly glycosylated polypeptide	17.0	17.0	17.0
Z-077557	-	protein CAPI	16.9	16.9	22.5
Z-034063	-	protein	16.6	16.6	33.2
Z-021498	-	-	15.8	15.8	31.6
Z-076067	ABA-independent	CBF14; DREB1A/1B/1C	15.7	47.2	36.7
Z-016885	-	armadillo beta-catenin repeat	15.6	15.6	15.6
Z-071449	-	DNA binding	15.6	15.6	15.6
Z-029602	-	predicted protein [*Hordeum vulgare*]	15.5	31.0	15.5
Z-077110	-	protein kinase G11A	15.1	15.1	22.6
Z-009444	-	beta-glucanase	12.9	12.9	25.9
Z-065054	-	beta-glucuronidase precursor	12.4	37.3	12.4
Z-029468	-	epoxide hydrolase	12.4	49.7	12.4
Z-060719	-	acyl carrier protein 3	12.4	24.7	37.1
Z-064692	-	protein	12.2	12.2	12.2
Z-052157	-	cellulose synthase-like protein	12.0	12.0	16.6
Z-033903	-	expansin os-EXPA2	10.3	12.7	17.3
Z-022713	-	RPL4 poly binding	9.5	2.3	3.0
Z-053920	-	conserved protein	9.4	3.0	3.2
Z-026018	-	xyloglucan endotransglucosylase hydrolase protein 32	9.0	6.0	5.0
Z-072329	-	-	8.5	3.1	2.8
Z-042237	ABA-dependent	AKIN10	8.0	5.0	4.0
Z-081500	-	hypothetical protein [*Sulfolobus solfataricus*]	7.8	4.1	2.2
Z-040743	-	protein	7.7	7.7	7.7
Z-022170	-	photosystem II phosphoprotein	7.3	2.0	8.0
Z-083680	-	photosystem II phosphoprotein	7.3	2.0	8.0
Z-022168	-	biosynthesis protein	7.2	4.0	2.2
Z-022169	-	biosynthesis protein	7.2	4.0	2.2
Z-023081	-	myosin heavy striated	7.0	6.0	9.0
Z-042710	-	TIR-NBS resistance protein	7.0	3.0	9.0
Z-037856	-	unknow protein	6.8	2.3	4.3
Z-015694	-	lipoprotein	6.6	7.0	4.4
Z-015361	-	chaperonin 21 precursor	6.5	8.0	8.5
Z-015365	-	chaperonin 21 precursor	6.5	8.0	8.5
Z-077556	-	fasciclin-like arabinogalactan-protein	6.5	3.0	4.5
Z-008156	-	protein	6.4	3.2	9.6
Z-008155	-	protein	6.3	3.2	9.5
Z-013373	-	GTP-binding protein	6.0	7.0	6.0
Z-060329	-	unknown [*Glycine max*]	6.0	5.0	3.0
Z-080216	-	SEC22	6.0	3.3	3.3
Z-057922	-	-	5.8	8.0	9.2
Z-078703	-	HSP70	5.5	2.0	2.0

Z-025366	-	glutathione s-transferase	5.0	2.0	2.0
Z-084991	-	NADH2	5.0	6.0	2.0
Z-080419	-	phenazine biosynthesis	5.0	3.0	3.0
Z-087868	-	photosystem II CP47 protein	5.0	3.0	2.5
Z-072529	-	RNA polymerase beta subunit	5.0	2.0	6.5
Z-081473	-	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	4.5	9.5	3.5
Z-001930	-	-	4.3	2.2	7.7
Z-058774	-	-	4.3	5.0	8.0
Z-080994	ABA-dependent	CIPK12	4.0	3.0	6.0
Z-085070	ABA-dependent	P5CS	4.0	2.0	2.0
Z-066584	proline	P5CR	4.0	2.5	7.5
Z-066580	proline	P5CR	4.0	2.5	7.5
Z-013649	-	CIPK12	4.0	3.0	6.0
Z-085071	-	DNA binding	4.0	2.0	2.0
Z-035414	-	protein	4.0	5.0	4.0
Z-083201	-	ubiquinol-cytochrome c reductase iron-sulfur subunit	4.0	5.0	2.0
Z-057923	-	unknown [*Zea mays*]	3.8	7.1	7.8
Z-023493	-	allene oxide synthase	3.8	4.0	4.3
Z-002349	ABA-dependent	beta chain	3.6	3.6	3.6
Z-009386	ABA-dependent	calcium-dependent protein kinase	3.5	5.5	4.0
Z-009387	ABA-dependent	calcium-dependent protein kinase	3.5	5.5	4.0
Z-080950	-	chalcone-flavanone isomerase family expressed	3.5	4.0	2.0
Z-080956	-	chalcone-flavanone isomerase family	3.5	4.0	2.0
Z-000642	-	photosystem II reaction center w protein	3.5	2.5	4.0
Z-081472	-	senescence-associated protein	3.4	3.5	4.4
Z-085866	-	hypothetical protein [*Zea mays*]	3.3	7.3	9.0
Z-002346	ABA-dependent	beta chain	3.2	3.2	3.2
Z-022604	ABA-dependent	ABI1	3.0	6.0	2.0
Z-008233	ABA-dependent	WRKY	3.0	3.0	2.0
Z-079133	-	centromere microtubule binding	3.0	5.0	6.0
Z-015643	-	conserved hypothetical protein [*Listeria monocytogenes*]	3.0	3.5	5.5
Z-040793	-	CTD-phosphatase-like protein	3.0	4.0	2.0
Z-068188	-	homocysteine s-methyltransferase	3.0	3.0	3.0
Z-068189	-	homocysteine s-methyltransferase	3.0	3.0	3.0
Z-005984	-	omega-3 fatty acid desaturase	3.0	2.0	5.0
Z-008514	-	copper ion binding	3.0	2.0	2.0
Z-003790	-	protein	3.0	3.0	5.0
Z-085347	-	protein	3.0	3.0	4.0
Z-031684	-	protein	3.0	7.0	3.0
Z-034071	-	ubiquitin protein ligase	3.0	2.0	2.0
Z-083452	-	-	3.0	3.0	8.0
Z-083453	-	-	3.0	3.0	8.0
Z-074184	-	-	3.0	4.0	5.5
Z-015642	-	-	3.0	3.3	4.5
Z-014584	-	-	2.9	4.4	5.8
Z-014585	-	-	2.9	4.4	5.8
Z-030804	-	unknown [*Glycine max*]	2.8	4.5	5.5
Z-065736	-	aquaporin	2.7	2.8	2.5
Z-065739	-	aquaporin	2.7	2.8	2.5
Z-024904	-	ORF133	2.7	4.0	6.0
Z-065737	-	PIP1 protein	2.7	2.8	2.5
Z-081766	-	pyruvate dehydrogenase e1 alpha subunit	2.7	3.7	3.7
Z-054948	-	-	2.5	5.1	7.6
Z-057698	-	-	2.5	2.2	2.5
Z-044454	-	DRG5 protein	2.5	3.0	2.5

Z-024905	-	hypothetical protein SpolCp149 [*Spinacia oleracea*]	2.5	2.0	7.0
Z-072327	-	photosystem II protein	2.5	2.8	4.3
Z-004183	-	protein	2.5	2.0	5.5
Z-029010	-	protein	2.5	3.0	3.0
Z-025728	-	unknown [*Zea mays*]	2.5	6.0	3.5
Z-025727	-	-	2.5	6.0	3.5
Z-010783	-	pyrophosphatase 1	2.4	2.0	2.0
Z-010784	-	pyrophosphatase 1	2.4	2.0	2.0
Z-076002	-	mammalian MHC III	2.3	2.3	2.3
Z-087872	-	photosystem II cytochrome B559 alpha subunit	2.2	3.6	2.8
Z-072959	-	s-adenosyl-l-homocysteine hydrolase	2.2	3.8	6.2
Z-013656	-	RNA polymerase beta subunit	2.2	2.2	3.8
Z-087652	-	RNA polymerase beta subunit	2.2	2.2	3.8
Z-002671	-	allene oxide synthase	2.0	4.0	5.0
Z-013667	-	cytochrome f	2.0	2.0	2.0
Z-072406	-	glycosyl hydrolase family 38 protein	2.0	4.0	4.0
Z-067925	-	methionine synthase	2.0	2.8	2.7
Z-011997	-	nitrogen fixation like protein	2.0	2.0	2.0
Z-011998	-	nitrogen fixation like protein	2.0	2.0	2.0
Z-011991	-	nitrogen fixation like protein	2.0	2.0	2.0
Z-011994	-	nitrogen fixation like protein	2.0	2.0	2.0
Z-044329	-	oligopeptide transporter	2.0	5.0	3.0
Z-039830	-	predicted protein [*Physcomitrella patens*]	2.0	3.0	4.0
Z-012544	-	protein	2.0	2.0	7.0
Z-049901	-	rhamnose synthase	2.0	3.0	8.0
Z-063612	-	RNA binding protein	2.0	2.0	2.0
Z-020797	-	-	2.0	5.0	7.0
Z-054666	-	-	2.0	4.0	2.0
Z-044842	-	-	2.0	2.0	2.0

“-“ no annotation available

Table S4.3. Summary of the downregulated (≥2-fold) transcripts that were common to all PEG treatments

Gene ID	Dehydration-response	Gene annotation	Fold regulation		
			27% PEG	31% PEG	34% PEG
Z-018320	-	-	-326.2	-211.5	-213.5
Z-014669	-	-	-277.9	-180.3	-181.9
Z-072960	-	s-adenosyl-l-homocysteine hydrolase	-269.0	-174.5	-176.1
Z-063449	-	enzyme of the cupin superfamily	-259.9	-168.5	-170.1
Z-064134	aquaporin	aquaporin	-255.6	-165.8	-167.3
Z-088497		HSP	-218.3	-141.6	-142.9
Z-002591	photorespiration	peroxisomal NAD-malate dehydrogenase 1, regulation of photorespiration	-213.1	-138.2	-139.5
Z-040118	-	flavonol 3-sulfotransferase	-210.3	-136.4	-137.6
Z-047702	-	-	-190.2	-123.4	-124.5
Z-038607	-	-	-177.6	-115.1	-116.2
Z-023260	-	endonuclease exonuclease phosphatase family protein	-173.7	-112.6	-113.7
Z-044109	-	protein	-173.7	-112.6	-113.7
Z-018620	-	transmembrane BAX4	-173.7	-112.6	-113.7
Z-040223	-	-	-170.0	-110.2	-111.3
Z-076792	-	-	-159.8	-103.6	-104.6
Z-023514	-	mitogen-activated protein kinase kinase	-99.3	-64.4	-65.0
Z-016039	-	naringenin 3-dioxygenase like protein	-93.5	-60.6	-61.2
Z-088506	-	thioredoxin	-91.1	-59.1	-59.6
Z-084396	-	protein	-89.8	-58.2	-58.8
Z-063258	-	chitinase B	-88.8	-57.6	-58.1
Z-010867	-	-	-88.8	-57.6	-58.1
Z-078759	-	-	-88.8	-57.6	-58.1
Z-065052	-	proteasome maturation factor UMP1	-87.3	-56.7	-57.2
Z-003822	-	oxysterol-binding expressed	-85.9	-55.7	-56.3
Z-060515	-	predicted protein [*Hordeum vulgare*]	-85.9	-55.7	-56.3
Z-074819	-	-	-85.0	-55.1	-55.7
Z-084817	-	protein binding	-82.0	-53.2	-53.7
Z-033149	-	signal recognition particle	-82.0	-53.2	-53.7
Z-023309	-	-	-78.4	-50.8	-51.3
Z-032874	-	-	-78.4	-50.8	-51.3
Z-086036	-	-	-78.4	-50.8	-51.3
Z-088057	-	TPA: class III peroxidase 57 precursor	-77.6	-50.3	-50.8
Z-076001	-	reverse transcriptase family	-48.4	-31.4	-31.7
Z-015237	-	-	-48.4	-31.4	-31.7
Z-005652	-	ABC transporter family protein	-47.6	-30.9	-31.1
Z-078468	-	HSP70	-47.6	-30.9	-31.1
Z-078463	-	pentatricopeptide repeat-containing	-47.6	-30.9	-31.1
Z-064237	-	protein	-47.6	-30.9	-31.1
Z-044012	-	soul HEME-binding family	-47.2	-30.6	-30.9
Z-058106	-	ABC transporter retinal flippase subfamily	-46.7	-30.3	-30.6
Z-025766	-	protein	-46.7	-30.3	-30.6
Z-036409	-	protein	-46.4	-30.1	-30.4
Z-054160	-	histone deacetylase	-45.9	-29.8	-30.1
Z-024682	-	nucleoporin family expressed	-45.9	-29.8	-30.1
Z-066830	-	serine endopeptidase	-45.4	-29.5	-29.7
Z-005209	cell wall	protein	-45.2	-29.3	-29.6
Z-073608	-	At5g49900 [*Oryza sativa*]	-45.2	-29.3	-29.6
Z-049840	-	mitochondrial substrate carrier family	-43.7	-28.3	-28.6
Z-022118	-	LAB11	-43.2	-28.0	-28.3
Z-049839	-	mitochondrial substrate carrier family	-42.3	-27.4	-27.7
Z-003243	-	prenylated rab acceptor family	-42.0	-27.2	-27.5
Z-068532	-	diacylglycerol kinase	-41.6	-27.0	-27.3

Z-003689	-	SORBIDRAFT [*Sorghum bicolor*]	-41.0	-26.6	-26.8
Z-076857	-	mitochondrial phosphate transporter	-40.8	-26.4	-26.7
Z-001993	embryo	replication factor	-40.4	-26.2	-26.4
Z-014414	-	-	-40.4	-26.2	-26.4
Z-033252	-	sulfite reductase	-39.9	-25.8	-26.1
Z-045392	-	aspartate semialdehyde	-39.8	-25.8	-26.0
Z-027443	-	thylakoid lumenal 15 kda chloroplast	-39.6	-25.7	-25.9
Z-076656	-	hypersensitive-induced response	-39.2	-25.4	-25.6
Z-076657	-	hypersensitive-induced response	-39.2	-25.4	-25.6
Z-083962	-	protein	-38.6	-25.0	-25.3
Z-040521	-	RAS family gtpase	-38.6	-25.0	-25.3
Z-039968	-	vacuolar processing enzyme	-38.6	-25.0	-25.3
Z-022591	-	DNA-binding protein	-38.1	-24.7	-24.9
Z-025011	-	hypothetical protein [*Oryza sativa*]	-38.1	-24.7	-24.9
Z-059038	-	nitrate reductase	-38.1	-24.7	-24.9
Z-016824	-	DNA repair	-37.5	-24.3	-24.6
Z-030840	-	-	-37.5	-24.3	-24.6
Z-084885	-	malate synthase	-37.3	-24.2	-24.4
Z-004362	-	pyruvate kinase	-37.0	-24.0	-24.2
Z-028924	-	sterol C-22 desaturase	-36.6	-23.7	-23.9
Z-021733	-	AMP-binding protein	-36.5	-23.7	-23.9
Z-064276	-	purine permease	-36.5	-23.7	-23.9
Z-087046	-	-	-36.5	-23.7	-23.9
Z-023322	-	steroid 22-alpha-hydroxylase	-36.2	-23.5	-23.7
Z-030132	-	predicted protein [*Hordeum vulgare*]	-36.0	-23.4	-23.6
Z-038122	-	predicted protein [*Hordeum vulgare*]	-36.0	-23.4	-23.6
Z-038124	-	predicted protein [*Hordeum vulgare*]	-36.0	-23.4	-23.6
Z-015112	-	RNA polymerase II mediator complex	-35.7	-23.1	-23.4
Z-084649	-	2-oxoglutarate-dependent dioxygenase	-35.5	-23.0	-23.3
Z-034127	-	cation efflux family protein	-35.5	-23.0	-23.3
Z-040403	-	-	-35.1	-22.7	-22.9
Z-048795	-	-	-35.1	-22.7	-22.9
Z-048796	-	-	-35.1	-22.7	-22.9
Z-063845	-	-	-35.1	-22.7	-22.9
Z-005087	-	calcium-transporting atpase plasma membrane- expressed	-34.6	-22.4	-22.6
Z-035351	-	cell division protein kinase	-34.6	-22.4	-22.6
Z-068518	-	galacturonosyltransferase GAUT12	-34.6	-22.4	-22.6
Z-062695	ABA-dependent	NAC5	-34.2	-22.2	-22.4
Z-041879	-	-	-34.2	-22.2	-22.4
Z-041880	-	-	-34.2	-22.2	-22.4
Z-074049	-	phosphatidylinositol kinase	-33.7	-21.9	-22.1
Z-021371	-	secondary cell wall-related glycosyltransferase family	-33.3	-21.6	-21.8
Z-063864	-	-	-33.3	-21.6	-21.8
Z-063865	-	-	-33.3	-21.6	-21.8
Z-069325	-	-	-33.3	-21.6	-21.8
Z-076056	-	chaperone protein	-32.9	-21.3	-21.5
Z-081932	-	protein	-32.9	-21.3	-21.5
Z-012370	-	-	-32.9	-21.3	-21.5
Z-076205	-	hexose transporter	-32.5	-21.1	-21.3
Z-012371	-	-	-32.5	-21.1	-21.3
Z-043752	-	CTD small phosphatase-like protein	-32.3	-20.9	-21.1
Z-067959	-	protein	-32.3	-20.9	-21.1
Z-051960	-	hypothetical protein [*Ricinus communis*]	-32.1	-20.8	-21.0
Z-042630	-	gibberellin 2-oxidase	-32.1	-20.8	-21.0
Z-087088	-	protein	-32.1	-20.8	-21.0
Z-044370	ABA-dependent	MYB1	-31.7	-20.6	-20.8
Z-004117	-	chloride channel protein CLC	-31.7	-20.6	-20.8
Z-072207	-	phospholipase A2	-31.7	-20.6	-20.8
Z-023136	-	transcription factor HBP	-31.7	-20.6	-20.8
Z-014450	-	protein kinase-like	-31.6	-20.5	-20.7

Z-014192	-	thioredoxin reductase 2	-31.5	-20.4	-20.6
Z-079657	-	predicted protein [*Hordeum vulgare*]	-31.0	-20.1	-20.3
Z-081278	-	phospholipase C	-30.6	-19.9	-20.0
Z-056849	-	protein	-30.3	-19.6	-19.8
Z-083695	-	UMP6_arath protein	-30.3	-19.6	-19.8
Z-010979	-	RNA binding	-29.9	-19.4	-19.6
Z-018863	-	short-chain dehydrogenase reductase	-29.9	-19.4	-19.6
Z-084779	-	SWI	-29.6	-19.2	-19.4
Z-034741	ABA-dependent	MYB3R	-29.3	-19.0	-19.2
Z-010998	-	-	-29.0	-18.8	-19.0
Z-003812	-	30s ribosomal protein	-28.6	-18.6	-18.8
Z-009292	-	isocitrate lyase	-28.6	-18.6	-18.8
Z-002522	-	predicted protein [*Hordeum vulgare*]	-28.6	-18.6	-18.8
Z-024419	-	protein	-28.6	-18.6	-18.8
Z-001788	-	geranyl diphosphate synthase	-28.3	-18.4	-18.6
Z-013154	-	multidrug resistance associated protein 1	-28.3	-18.4	-18.6
Z-055489	-	ARALYDRAFT [*Arabidopsis lyrata*]	-27.2	-17.6	-17.8
Z-028304	-	protein	-27.2	-17.6	-17.8
Z-028305	-	protein	-27.2	-17.6	-17.8
Z-001295	-	transcription elongation factor A protein 2	-26.9	-17.5	-17.6
Z-035087	-	cytochrome p450	-26.8	-17.4	-17.5
Z-008157	-	aspartic proteinase nepenthesin-1	-26.6	-17.3	-17.4
Z-076500	-	basic helix-loop-helix family protein	-26.6	-17.3	-17.4
Z-066250	-	transmembrane EMP24 domain	-25.9	-16.8	-16.9
Z-028200	-	ATMRP3 coupled to transmembrane	-25.7	-16.7	-16.9
Z-018102	-	protein	-25.5	-16.5	-16.7
Z-031930	-	predicted protein [*Hordeum vulgare*]	-25.0	-16.2	-16.4
Z-053412	-	complex 1 protein containing protein	-24.9	-16.1	-16.3
Z-048929	-	maize transposon MUDRA-like	-24.7	-16.0	-16.1
Z-014163	-	protein	-24.7	-16.0	-16.1
Z-062875	-	ubiquitin-associated protein	-24.7	-16.0	-16.1
Z-062877	-	ubiquitin-associated protein	-24.7	-16.0	-16.1
Z-048872	-	ribosome-like protein	-24.4	-15.9	-16.0
Z-077893	-	SNRK1	-24.4	-15.9	-16.0
Z-083383	-	hypothetical protein [*Zea mays*]	-24.2	-15.7	-15.9
Z-022163	-	-	-24.2	-15.7	-15.9
Z-077930	-	PTO1	-23.8	-15.4	-15.6
Z-062727	-	-	-23.8	-15.4	-15.6
Z-062916	-	high affinity nitrate transporter	-23.6	-15.3	-15.4
Z-057029	-	predicted protein [*Hordeum vulgare*]	-23.6	-15.3	-15.4
Z-014882	-	chloroplast protease	-23.4	-15.2	-15.3
Z-076237	-	cinnamyl alcohol dehydrogenase	-23.4	-15.2	-15.3
Z-076239	-	cinnamyl alcohol dehydrogenase	-23.4	-15.2	-15.3
Z-076240	-	cinnamyl alcohol dehydrogenase	-23.4	-15.2	-15.3
Z-075305	-	KOW domain-containing	-23.4	-15.2	-15.3
Z-015598	early drought	LTI6B - early drought induced	-23.2	-15.0	-15.2
Z-057688	-	PLATZ transcription factor	-23.2	-15.0	-15.2
Z-059081	-	protein	-23.2	-15.0	-15.2
Z-068069	-	methylase family protein	-22.9	-14.8	-15.0
Z-045068	-	BTB and TAZ domain protein	-22.8	-14.8	-14.9
Z-034562	-	histone deacetylase superfamily	-22.8	-14.8	-14.9
Z-014948	ABA-independent	CBF14	-22.7	-14.7	-14.9
Z-076866	ABA-dependent	bzip transcription factor ABI5	-22.3	-14.4	-14.6
Z-008469	-	adenylyl-sulfate kinase	-22.2	-14.4	-14.5
Z-008470	-	adenylyl-sulfate kinase	-22.2	-14.4	-14.5
Z-022504	-	-	-21.8	-14.2	-14.3
Z-077040	-	HSP	-21.7	-14.0	-14.2
Z-008153	-	indole-3-acetic acid-amido synthetase	-21.6	-14.0	-14.1
Z-076863	-	BHLH transcription factor	-21.5	-13.9	-14.1
Z-012579	-	predicted protein [Hordeum vulgare]	-21.5	-13.9	-14.1
Z-024706	-	protein	-21.5	-13.9	-14.1
Z-069678	-	CAX4	-21.1	-13.7	-13.8

Z-031460	-	glucose transporter member 8	-20.5	-13.3	-13.4
Z-042793	-	indole-3-acetic acid-amido synthetase	-20.2	-13.1	-13.2
Z-011159	-	protein	-20.2	-13.1	-13.2
Z-078939	-	protein	-19.9	-12.9	-13.0
Z-044711	-	selenoprotein precursor	-19.9	-12.9	-13.0
Z-007276	-	ac007727_10 ESTS	-19.7	-12.8	-12.9
Z-035410	-	integral membrane transporter	-19.6	-12.7	-12.8
Z-005092	-	E-loop binding protein	-19.6	-12.7	-12.8
Z-078557	-	auxin-independent growth promoter	-19.4	-12.6	-12.7
Z-047870	-	SAUR family protein	-19.2	-12.5	-12.6
Z-052963	ABA-dependent	WRKY25	-18.5	-12.0	-12.1
Z-074207	-	protein	-18.5	-12.0	-12.1
Z-016349	-	oligopeptide transporter OPT family	-17.6	-11.4	-11.5
Z-073388	-	RWP	-17.6	-11.4	-11.5
Z-068978	-	APRL6	-17.2	-11.1	-11.3
Z-086351	-	membrane protein	-17.2	-11.1	-11.3
Z-063827	-	AFC1 (arabidopsis FUS3-complementing 1)	-17.1	-11.1	-11.2
Z-063829	-	AFC1 (arabidopsis FUS3-complementing 1)	-17.1	-11.1	-11.2
Z-057243	-	nitrate chlorate expressed	-17.1	-11.1	-11.2
Z-015546	-	hepatocellular carcinoma-associated 137	-17.0	-11.0	-11.1
Z-021398	-	chaperone protein	-16.9	-10.9	-11.0
Z-064224	-	serine-threonine protein	-16.8	-10.9	-11.0
Z-063684	ABA-dependent	ABA-responsive protein	-16.6	-10.8	-10.9
Z-043524	-	Na(+) H(+)	-16.4	-10.7	-10.8
Z-044146	ABA-dependent	MYB	-16.3	-10.6	-10.7
Z-004812	-	YAB2	-16.2	-10.5	-10.6
Z-023285	-	peptide transporter	-16.0	-10.4	-10.5
Z-023286	-	peptide transporter	-16.0	-10.4	-10.5
Z-012764	-	PHG1A	-16.0	-10.4	-10.5
Z-012767	-	PHG1A	-16.0	-10.4	-10.5
Z-025109	-	transcription factor	-16.0	-10.4	-10.5
Z-002959	-	histone acetyltransferase complex component	-15.9	-10.3	-10.4
Z-016340	ABA-independent	CBF14; DREB1A/1B/1C	-15.7	-10.2	-10.3
Z-048266	-	40s ribosomal protein	-15.7	-10.2	-10.3
Z-024300	-	alcohol dehydrogenase	-15.6	-10.1	-10.2
Z-030961	-	hypothetical protein [*Oryza sativa*]	-15.5	-10.0	-10.1
Z-029217	-	protein	-15.5	-10.0	-10.1
Z-029218	-	protein	-15.5	-10.0	-10.1
Z-040440	-	RNA-binding protein 10	-15.5	-10.0	-10.1
Z-020710	-	at5g10780 t30n20_50	-13.0	-34.2	-34.6
Z-020712	-	protein	-13.0	-34.0	-34.4
Z-002950	-	oligopeptide transporter OPT family	-11.0	-11.0	-11.0
Z-023557	-	glutamine synthetase 1B	-10.0	-10.0	-10.0
Z-084774	-	NAD-dependent isocitrate dehydrogenase	-9.9	-6.4	-6.5
Z-006027	-	protein	-9.9	-6.4	-6.5
Z-036737	ABA-independent	AP2 domain containing protein	-9.8	-6.4	-6.4
Z-034359	-	glycosyl hydrolase-like protein	-9.8	-2.0	-6.4
Z-034362	-	glycosyl hydrolase-like protein	-9.8	-2.0	-6.4
Z-085777	-	af276703_1vacuolar acid invertase	-9.7	-6.3	-6.3
Z-017419	-	chalcone synthase family protein	-9.7	-6.3	-6.3
Z-041783	-	chitinase	-9.6	-3.7	-4.5
Z-007547	defense response	ring-H2 zinc finger protein	-9.5	-6.2	-6.3
Z-088083	-	UDP-glycosyltransferase	-9.5	-6.1	-6.2
Z-028970	-	4-methyl-5(b-hydroxyethyl)-thiazol monophosphate biosynthesis enzyme	-9.4	-6.1	-6.1
Z-035050	-	protein	-9.4	-6.1	-6.1
Z-010529	-	tRNA intron endonuclease	-9.2	-6.0	-6.0
Z-050301	-	protein	-9.1	-5.9	-2.0
Z-015817	-	proteasome subunit alpha	-9.0	-4.5	-9.0

Z-037794	-	tubulin family protein	-9.0	-5.8	-5.9
Z-020281	-	-	-9.0	-3.0	-3.0
Z-020284	-	-	-9.0	-3.0	-3.0
Z-057254	-	predicted protein [*Hordeum vulgare*]	-9.0	-5.8	-5.9
Z-062687	-	protein	-8.5	-5.5	-5.6
Z-055755	-	metal ion binding protein	-8.5	-5.7	-4.2
Z-058139	ABA-independent	CBF14; DREB1A/1B/1C	-8.5	-3.4	-2.4
Z-038048	-	protein	-8.5	-5.5	-5.6
Z-062408	-	gibberellin receptor GID1l2	-8.4	-5.5	-5.5
Z-084813	-	peptide methionine sulfoxide reductase	-8.3	-4.2	-2.8
Z-041849	-	NPKL2 [*Oryza sativa*]	-8.3	-5.4	-5.5
Z-084260	-	sugar transport protein 5	-8.2	-5.3	-5.4
Z-059786	-	zinc finger protein	-8.2	-3.3	-4.7
Z-066984	-	protein	-8.2	-5.3	-5.4
Z-014359	ABA-dependent	PYL9_ABA receptor	-8.2	-5.3	-5.4
Z-059882	-	3-hydroxy-3-methylglutaryl- reductase	-8.1	-5.3	-5.3
Z-020064	-	brittle culm1	-8.0	-5.2	-4.0
Z-005864	-	Os07g0694800 [*Oryza sativa*]	-8.0	-5.2	-5.3
Z-064770	ABA-dependent	CIPK12	-8.0	-8.0	-2.7
Z-023561	ABA-dependent	ERD4_Early response to dehydration	-8.0	-2.0	-4.0
Z-086493	-	calcium-dependent protein kinase	-8.0	-2.7	-2.0
Z-015859	-	DIN10 (dark inducible 10)	-8.0	-4.0	-6.0
Z-015860	-	DIN10 (dark inducible 10)	-8.0	-4.0	-6.0
Z-015863	-	DIN10 (dark inducible 10)	-8.0	-4.0	-6.0
Z-015864	-	DIN10 (dark inducible 10)	-8.0	-4.0	-6.0
Z-035532	-	lichenase-2 precursor	-8.0	-4.0	-2.7
Z-023472	-	1-o-acylceramide synthase	-8.0	-5.2	-5.2
Z-023474	-	1-o-acylceramide synthase	-8.0	-5.2	-5.2
Z-062883	-	protein	-7.9	-4.2	-8.9
Z-082530	-	uridine cytidine kinase	-7.7	-5.0	-5.1
Z-082531	-	uridine cytidine kinase	-7.7	-5.0	-5.1
Z-082524	-	uridine cytidine kinase	-7.7	-5.0	-5.1
Z-082525	-	uridine cytidine kinase	-7.7	-5.0	-5.1
Z-081678	-	protein binding protein	-7.7	-5.0	-5.1
Z-081679	-	protein binding protein	-7.7	-5.0	-5.0
Z-032459	-	-	-7.7	-5.0	-5.0
Z-024005	-	endo-beta-xylanase C	-7.5	-4.9	-4.9
Z-014033	-	predicted protein [*Hordeum vulgare*]	-7.3	-3.1	-3.7
Z-014034	-	predicted protein [*Hordeum vulgare*]	-7.3	-3.1	-3.7
Z-072943	-	cytochrome p450 family	-7.1	-4.6	-4.7
Z-070927	-	lipase precursor	-7.0	-7.0	-7.0
Z-028957	-	-	-7.0	-2.3	-4.2
Z-031431	-	protein	-6.8	-4.4	-4.4
Z-027468	-	NB-ARC domain	-6.7	-2.0	-4.4
Z-076064	ABA-independent	CBF14; DREB1A/1B/1C	-6.7	-2.2	-2.9
Z-025196	-	protein	-6.5	-3.2	-4.3
Z-025197	-	protein	-6.5	-3.2	-4.3
Z-007469	-	transposon	-6.5	-3.0	-4.2
Z-068515	-	protein kinase receptor type precursor	-6.4	-4.1	-4.2
Z-015609	-	basic helix-loop-helix	-6.3	-4.1	-4.1
Z-085776	-	soluble acid invertase	-6.2	-4.0	-4.1
Z-084252	ABA-dependent	ABI1	-6.1	-3.9	-4.0
Z-025279	-	pre-mRNA processing protein	-6.0	-3.9	-3.9
Z-068474	-	nucleoid DNA-binding CDN41	-6.0	-3.9	-3.9
Z-012927	-	2-on-2 hemoglobin	-6.0	-6.0	-2.0
Z-086492	-	calcium-dependent protein kinase	-6.0	-6.0	-3.0
Z-063294	-	constans-like protein CO5	-6.0	-3.9	-3.9
Z-088280	-	GTP cyclohydrolase I	-6.0	-2.0	-6.0
Z-050385	-	protein	-6.0	-2.0	-3.0
Z-063295	-	constans-like protein CO5	-5.8	-3.8	-3.8
Z-003286	-	myo-inositol kinase	-5.8	-3.8	-3.8
Z-016108	-	alpha-l-fucosidase 2 precursor	-5.7	-9.6	-6.4

Z-032625	-	carnitine racemase like protein	-5.5	-2.7	-2.7
Z-076474	-	protein	-5.5	-3.7	-5.5
Z-074956	-	RPT2 (root phototropism 2)	-5.5	-3.5	-3.6
Z-068038	-	protein	-5.4	-3.5	-3.6
Z-044123	-	class III acidic chitinase	-5.3	-5.3	-8.0
Z-063117	-	pentatricopeptide repeat-containing protein	-5.3	-3.4	-3.5
Z-031430	-	protein	-5.2	-3.4	-3.4
Z-037925	-	1-aminocyclopropane-1-carboxylate oxidase	-5.2	-4.1	-8.4
Z-012907	-	hexose carrier	-5.2	-3.4	-3.4
Z-012533	-	protein	-5.1	-3.3	-3.3
Z-047827	-	protein arginine n-methyltransferase	-5.0	-3.2	-3.3
Z-037919	-	ac011806_16 cullin	-5.0	-2.0	-3.3
Z-037920	-	ac011806_16 cullin	-5.0	-2.0	-3.3
Z-033527	-	beta- -glucanase	-5.0	-5.0	-2.5
Z-048096	-	GTP-binding protein	-5.0	-5.0	-5.0
Z-023665	-	integral membrane protein	-5.0	-5.0	-5.0
Z-083149	-	oligopeptide transporter OPT family	-5.0	-5.0	-5.0
Z-030120	-	polygalacturonase	-5.0	-5.0	-2.5
Z-003707	-	transmembrane and coiled-coil domains	-5.0	-2.5	-2.5
Z-043201	-	-	-5.0	-5.0	-2.5
Z-085101	-	constans-like protein CO5	-4.9	-2.1	-4.3
Z-010679	-	HSP90	-4.8	-4.8	-3.4
Z-044031	-	-	-4.7	-2.4	-3.2
Z-058700	-	s-like RNAse	-4.7	-3.1	-3.1
Z-031474	-	aspartic proteinase	-4.6	-3.8	-2.2
Z-040302	-	predicted protein [*Hordeum vulgare*]	-4.6	-3.0	-3.0
Z-032417	ABA-dependent	WRKY7	-4.5	-3.0	-4.5
Z-056870	-	protein	-4.5	-9.0	-3.0
Z-027674	-	protein	-4.5	-4.5	-9.0
Z-065349	-	protein	-4.4	-2.9	-2.9
Z-065350	-	protein	-4.4	-2.9	-2.9
Z-063383	-	TIC20-like	-4.3	-3.2	-4.3
Z-034953	-	-	-4.2	-2.1	-3.0
Z-034954	-	-	-4.2	-2.1	-3.0
Z-086488	-	calcium-dependent protein kinase	-4.2	-2.5	-2.8
Z-018361	-	ATPase-like	-4.1	-2.7	-2.7
Z-018362	-	ATPase-like	-4.1	-2.7	-2.7
Z-053374	-	protein	-4.1	-2.7	-2.7
Z-063956	-	cell division cycle protein 23	-4.1	-2.7	-2.7
Z-016494	-	protein	-4.1	-2.7	-2.7
Z-044510	ABA-dependent	NAC transcription factor	-4.0	-2.0	-6.0
Z-044511	ABA-dependent	NAC transcription factor	-4.0	-2.0	-6.0
Z-064215	-	2c-methyl-d-erythritol -cyclodiphosphate synthase	-4.0	-6.0	-4.0
Z-045571	-	adenylate family 3	-4.0	-2.0	-2.0
Z-008414	-	importin beta	-4.0	-4.0	-2.0
Z-017877	-	zinc transporter	-4.0	-4.0	-4.0
Z-048519	-	-	-4.0	-8.0	-4.0
Z-003287	-	myo-inositol kinase	-3.9	-2.5	-2.6
Z-030282	-	photosystem II CP43 chlorophyll	-3.8	-3.9	-2.7
Z-031902	-	ATP synthase beta chain	-3.7	-4.3	-3.7
Z-031903	-	ATP synthase beta chain	-3.7	-4.3	-3.7
Z-031904	-	ATP synthase beta chain	-3.7	-4.3	-3.7
Z-031905	-	ATP synthase beta chain	-3.7	-4.3	-3.7
Z-012550	-	protein	-3.7	-3.7	-5.0
Z-076238	-	cinnamyl alcohol dehydrogenase	-3.7	-3.7	-3.7
Z-080484	-	cytochrome c biogenesis protein precursor	-3.7	-3.0	-6.6
Z-062909	-	chloroplast elongation factor (EF)	-3.5	-2.2	-2.7
Z-003837	-	phytanoyl- dioxygenase domain	-3.5	-3.5	-3.5
Z-003842	-	phytanoyl- dioxygenase domain	-3.5	-3.5	-3.5
Z-005249	-	predicted protein [*Hordeum vulgare*]	-3.5	-7.0	-3.5

Z-025200	-	protein	-3.5	-3.5	-7.0
Z-025201	-	protein	-3.5	-3.5	-7.0
Z-002120	-	glycolate oxidase	-3.4	-4.9	-4.6
Z-076229	-	vacuolar iron transporter	-3.2	-4.3	-4.3
Z-023497	-	hypothetical protein [*Sorghum bicolor*]	-3.2	-4.0	-3.2
Z-071407	-	protein kinase XA21	-3.1	-2.0	-2.0
Z-065618	ABA-independent	ethylene receptor	-3.0	-3.0	-8.8
Z-028296	-	3 -n-debenzoyltaxol n-benzoyltransferase	-3.0	-6.0	-3.0
Z-037463	-	CENP-e-like kinetochore	-3.0	-9.0	-3.0
Z-038796	-	protein	-3.0	-3.0	-3.0
Z-021387	ABA-independent	ethylene-insensitive3-like 1	-2.9	-3.9	-3.9
Z-021390	ABA-independent	ethylene-insensitive3-like 1	-2.9	-3.9	-3.9
Z-057325	-	ATP synthase CF0 subunit IV	-2.9	-2.5	-2.2
Z-021386	ABA-independent	ethylene-insensitive3-like 1	-2.8	-3.8	-3.8
Z-021382	ABA-independent	ethylene-insensitive3-like 1	-2.8	-3.8	-3.8
Z-006169	-	galactinol synthase	-2.8	-8.5	-4.2
Z-034949	-	GDP dissociation inhibitor	-2.7	-2.7	-2.2
Z-063323	-	s-adenosylmethionine decarboxylase	-2.7	-2.7	-4.4
Z-034571	-	actin 1	-2.7	-2.0	-2.7
Z-018851	-	branched chain alpha-keto acid dehydrogenase E1 beta subunit	-2.7	-8.0	-8.0
Z-084722	-	copper amine oxidase	-2.7	-2.0	-2.0
Z-074456	-	fructose-bisphosphate aldolase	-2.7	-2.0	-2.7
Z-022688	-	hypothetical protein [*Oryza sativa*]	-2.7	-4.0	-2.7
Z-022689	-	hypothetical protein [*Oryza sativa*]	-2.7	-4.0	-2.7
Z-024255	-	inositol-3-phosphate synthase	-2.7	-8.0	-2.0
Z-007167	-	trafficking protein particle complex subunit 3	-2.6	-3.2	-2.2
Z-080428	-	ubiquitin-like protein 5	-2.6	-3.3	-2.1
Z-041168	-	vacuolar ATP synthase subunit	-2.6	-3.6	-4.2
Z-081260	-	hypothetical protein [*Oryza sativa*]	-2.5	-5.0	-5.0
Z-087413	-	thioredoxin H	-2.5	-3.3	-2.5
Z-087416	-	thioredoxin-like protein	-2.5	-3.3	-2.5
Z-087417	-	thioredoxin-like protein	-2.5	-3.3	-2.5
Z-063828	-	-	-2.5	-5.0	-5.0
Z-054905	-	-	-2.5	-4.8	-5.0
Z-063513	-	phenylalanine ammonia-lyase	-2.3	-7.0	-7.0
Z-002204	water stress	DRG5	-2.3	-3.2	-6.0
Z-006016	root and shoot development	26s proteasome subunit	-2.2	-4.5	-3.0
Z-034062	-	40s ribosomal protein	-2.2	-9.0	-3.0
Z-056165	-	GMC oxidoreductase family	-2.2	-4.5	-2.2
Z-075929	-	predicted protein [*Hordeum vulgare*]	-2.2	-3.0	-3.0
Z-015574	-	glutathione peroxidase	-2.2	-4.8	-4.8
Z-015575	-	glutathione peroxidase	-2.2	-4.8	-4.8
Z-084091	-	enzyme of the cupin superfamily	-2.2	-2.7	-2.6
Z-025507	-	protein	-2.1	-2.6	-2.8
Z-025508	-	protein	-2.1	-2.6	-2.8
Z-025503	-	protein	-2.1	-2.6	-2.8
Z-025504	-	protein	-2.1	-2.6	-2.8
Z-083351	-	-	-2.1	-7.0	-5.2
Z-026224	-	arsenite transport	-2.0	-6.0	-6.0
Z-033973	-	leucine zipper protein	-2.0	-2.0	-5.3
Z-029724	-	multidomain cystatin	-2.0	-4.0	-2.0
Z-034995	-	nucleic acid binding protein	-2.0	-3.0	-3.0
Z-040645	-	protein	-2.0	-2.0	-2.0
Z-057626	-	protein	-2.0	-4.0	-2.0
Z-050330	-	protein	-2.0	-2.0	-2.9
Z-050331	-	protein	-2.0	-2.0	-3.0
Z-037701	-	protein	-2.0	-6.0	-6.0

“-“ no annotation available

Table S4.4 Summary of differential regulation of the 640 non-annotated transcripts of the triticale seedlings transcriptomes

Gene ID	Fold change at 27% PEG	Fold change at 31% PEG	Fold change at 34% PEG
Z-030725	179.7	119.8	658.8
Z-048799	-	-	641.9
Z-030793	175.1	116.7	525.2
Z-061520	22.3	401.6	502.1
Z-074159	379.3	47.4	474.2
Z-081475	610.8	166.5	445.0
Z-083148	-	-	359.4
Z-019198	-	66.9	267.8
Z-001943	32.5	162.6	260.1
Z-053229	32.5	162.6	260.1
Z-057282	263.9	197.9	230.9
Z-018050	133.9	133.9	200.8
Z-072191	130.1	130.1	195.1
Z-038648	63.2	189.7	189.7
Z-006950	-	63.2	189.7
Z-068270	264.5	163.0	185.8
Z-010952	-	54.2	162.6
Z-064168	155.2	103.5	155.2
Z-064780	103.5	51.7	155.2
Z-055698	-	18.5	148.0
Z-083147	-	-	139.3
Z-037114	-	91.0	136.6
Z-057075	-	66.9	133.9
Z-072544	-	66.9	133.9
Z-022710	-	-	133.9
Z-059203	-	-	133.9
Z-075918	-	-	133.9
Z-082898	-	-	126.4
Z-017456	-	-	123.0
Z-072811	-	-	119.8
Z-054629	-	58.4	116.7
Z-086476	-	-	111.9
Z-068273	55.5	55.5	111.0
Z-023304	-	-	111.0
Z-051276	36.1	72.3	108.4
Z-004473	173.2	99.0	99.0
Z-077555	99.0	-	99.0
Z-014995	-	96.9	96.9
Z-080849	-	63.2	94.8
Z-036021	30.8	61.5	92.3
Z-036017	30.8	61.5	92.3
Z-044069	-	42.9	85.9
Z-063634	85.9	-	85.9
Z-008075	-	40.6	81.3
Z-037437	-		66.0

Z-083813	-	390.2	65.0
Z-011113	-	-	65.0
Z-042430	-	63.2	63.2
Z-010989	-	-	63.2
Z-088052	-	-	61.5
Z-042510	-	-	59.9
Z-069906	14.8	29.6	59.1
Z-074496	-	-	59.1
Z-044282	-	-	58.4
Z-003897	18.7	37.3	56.0
Z-015463	55.5	-	55.5
Z-003896	18.4	36.7	55.1
Z-056003	-	26.8	53.6
Z-075571	25.9	-	51.7
Z-054904	-	101.2	50.6
Z-056239	49.5	-	49.5
Z-079107	-	48.4	48.4
Z-079108	-	48.4	48.4
Z-068587	-	24.2	48.4
Z-088643	71.1	-	47.4
Z-028601	-	46.4	46.4
Z-076158	46.4	23.2	46.4
Z-064440	-	-	45.5
Z-004099	-	-	44.6
Z-010774	-	-	44.6
Z-010775	-	-	44.6
Z-032407	84.3	126.4	42.1
Z-083655	-	-	42.1
Z-086577	-	124.1	41.4
Z-029406	-	-	41.4
Z-045608	20.3	-	40.6
Z-088365	-	-	40.6
Z-029003	-	-	38.6
Z-002075	-	-	37.9
Z-086475	-	-	37.3
Z-057760	-	36.7	36.7
Z-009388	-	-	36.3
Z-009391	-	-	36.3
Z-031814	52.5	52.5	35.0
Z-031815	52.5	52.5	35.0
Z-064786	34.5	-	34.5
Z-034151	-	-	34.5
Z-042756	-	33.5	33.5
Z-055700	-	-	33.5
Z-073130	-	-	33.5
Z-021498	15.8	15.8	31.6
Z-001666	-	-	30.8
Z-030746	-	-	30.8
Z-059139	-	-	30.8
Z-064469	-	-	30.8
Z-057220	-	-	29.9

Z-085833	-	-	29.8
Z-085838	-	-	29.8
Z-032971	58.4	29.2	29.2
Z-001668	-	-	29.2
Z-052329	-	-	28.6
Z-030701	-	-	28.5
Z-065330	-	-	27.1
Z-065331	-	-	27.1
Z-030502	-	-	26.5
Z-064170	-	51.7	25.9
Z-088165	24.7	24.7	24.7
Z-001234	24.2	-	24.2
Z-002553	-	45.5	22.8
Z-040038	45.5	22.8	22.8
Z-083126	8.0	10.0	22.0
Z-028075	41.8	41.8	20.9
Z-064073	-	-	20.0
Z-064074	-	-	20.0
Z-004283	6.9	19.0	19.8
Z-016880	19.0	37.9	19.0
Z-024308	-	9.5	19.0
Z-087871	-	4.0	18.5
Z-000856	-	-	17.8
Z-021550	-	17.2	17.2
Z-003834	-	15.8	15.8
Z-003836	-	15.8	15.8
Z-039770	-	-	15.7
Z-009781	-	-	15.6
Z-009783	-	-	15.6
Z-023996	-	-	15.4
Z-003052	-	-	15.0
Z-003053	-	-	15.0
Z-086174	-	-	15.0
Z-052932	3.0	10.0	14.0
Z-083329	4.0	-	14.0
Z-084285	-	-	13.3
Z-084286	-	-	13.3
Z-084287	-	-	13.3
Z-065076	-	-	11.7
Z-022663	-	-	10.7
Z-022669	-	-	10.7
Z-073132	4.0	2.0	10.0
Z-052935	3.0	-	9.3
Z-073128	3.0	-	9.3
Z-043624	-	-	9.0
Z-058774	4.3	5.0	8.0
Z-083452	3.0	3.0	8.0
Z-083453	3.0	3.0	8.0
Z-055697	-	-	8.0
Z-001930	4.3	2.2	7.7
Z-081972	-	-	7.6

Z-081973	-	-	7.6
Z-054948	2.5	5.1	7.6
Z-030113	-	21.1	7.0
Z-020797	2.0	5.0	7.0
Z-051297	-	-	7.0
Z-031118	-	-	6.7
Z-030692	-	-	6.5
Z-002296	-	-	6.3
Z-037159	-	3.0	6.0
Z-014584	2.9	4.4	5.8
Z-014585	2.9	4.4	5.8
Z-074184	3.0	4.0	5.5
Z-052933	-	-	5.2
Z-018695	-	5.0	5.0
Z-026497	2.0	-	5.0
Z-010691	2.0	-	5.0
Z-010692	2.0	-	5.0
Z-010690	2.0	-	5.0
Z-010693	2.0	-	5.0
Z-013647	-	-	5.0
Z-015642	3.0	3.3	4.5
Z-069656	17.1	-	4.3
Z-028302	-	2.0	4.0
Z-007178	-	-	4.0
Z-032622	-	-	4.0
Z-025727	2.5	6.0	3.5
Z-039974	2.5	-	3.5
Z-072423	-	-	3.5
Z-013316	-	-	3.2
Z-013318	-	-	3.2
Z-013640	7.0	-	3.1
Z-082589	2.0	-	3.0
Z-082590	2.0	-	3.0
Z-001420	-	-	3.0
Z-028668	-	-	3.0
Z-028669	-	-	3.0
Z-083044	-	-	3.0
Z-051885	-	-	3.0
Z-051886	-	-	3.0
Z-072329	8.5	3.1	2.8
Z-055702	6.7	-	2.8
Z-067422	11.1	4.7	2.7
Z-013430	-	3.1	2.6
Z-015639	-	2.7	2.6
Z-015640	-	2.7	2.6
Z-057698	2.5	2.2	2.5
Z-030145	-	4.0	2.3
Z-047407	-	-	2.3
Z-055593	-	-	2.2
Z-029751	-	5.0	2.0
Z-054666	2.0	4.0	2.0

Z-049993	-	3.0	2.0
Z-078202	-	3.0	2.0
Z-044842	2.0	2.0	2.0
Z-064174	-	2.0	2.0
Z-064175	-	2.0	2.0
Z-079997	7.5	-	2.0
Z-087760	5.0	-	2.0
Z-009385	3.0	-	2.0
Z-070179	2.0	-	2.0
Z-022616	2.0	-	2.0
Z-022618	2.0	-	2.0
Z-018524	-	-	2.0
Z-046746	-	-	2.0
Z-033557	-	-	2.0
Z-033556	-	-	2.0
Z-001260	-	-	2.0
Z-031420	-	-	2.0
Z-063425	-143.9	-2.0	-2.0
Z-056063	-	-2.0	-2.0
Z-056064	-	-2.0	-2.0
Z-045761	-	-2.0	-2.0
Z-021159	-49.3	-32.0	-2.0
Z-041608	-2.0	-45.5	-2.0
Z-041610	-2.0	-45.5	-2.0
Z-063650	-2.0	-75.1	-2.0
Z-034143	-14.0	-	-2.0
Z-087145	-247.8	-	-2.0
Z-043201	-5.0	-5.0	-2.5
Z-063843	-	-10.0	-2.5
Z-022721	-2.5	-	-2.5
Z-064520	-3.7	-	-2.5
Z-064521	-3.7	-	-2.5
Z-047679	-	-	-2.5
Z-057802	-	-	-2.5
Z-051264	-2.2	-	-2.7
Z-042794	-	-3.8	-2.8
Z-034953	-4.2	-2.1	-3.0
Z-034954	-4.2	-2.1	-3.0
Z-020281	-9.0	-3.0	-3.0
Z-020284	-9.0	-3.0	-3.0
Z-073131	-228.3	-3.0	-3.0
Z-079615	-	-3.0	-3.0
Z-084466	-88.8	-57.6	-3.0
Z-084467	-88.8	-57.6	-3.0
Z-002065	-3.0	-	-3.0
Z-049588	-3.0	-	-3.0
Z-049589	-3.0	-	-3.0
Z-049590	-3.0	-	-3.0
Z-049591	-3.0	-	-3.0
Z-007285	-3.0	-	-3.0
Z-032336	-117.5	-	-3.0

Z-083356	-	-	-3.0
Z-064389	-	-	-3.0
Z-030162	-18.4	-5.2	-3.0
Z-044031	-4.7	-2.4	-3.2
Z-010282	-44.8	-3.5	-3.5
Z-012745	-2.3	-81.7	-3.5
Z-012747	-2.3	-81.7	-3.5
Z-012748	-2.3	-81.7	-3.5
Z-012749	-2.3	-81.7	-3.5
Z-045974	-8.8	-26.4	-3.9
Z-002551	-65.0	-2.0	-4.0
Z-002633	-126.8	-4.0	-4.0
Z-002634	-126.8	-4.0	-4.0
Z-040115	-	-4.0	-4.0
Z-048519	-4.0	-8.0	-4.0
Z-064655	-2.0	-181.8	-4.0
Z-010986	-77.2	-	-4.0
Z-028957	-7.0	-2.3	-4.2
Z-007293	-	-14.0	-4.7
Z-054905	-2.5	-4.8	-5.0
Z-063828	-2.5	-5.0	-5.0
Z-063842	-5.0	-187.8	-5.0
Z-032459	-7.7	-5.0	-5.0
Z-083351	-2.1	-7.0	-5.2
Z-061546	-8.8	-	-5.7
Z-019781	-	-6.0	-6.0
Z-084001	-	-7.1	-7.1
Z-056177	-11.3	-7.3	-7.4
Z-056176	-11.5	-7.4	-7.5
Z-082458	-11.5	-7.4	-7.5
Z-054170	-11.7	-7.6	-7.6
Z-054173	-11.7	-7.6	-7.6
Z-015193	-12.7	-	-8.3
Z-015194	-12.7	-	-8.3
Z-012411	-10.0	-375.6	-10.0
Z-036731	-552.9	-11.0	-11.0
Z-014258	-	-10.9	-11.0
Z-014273	-	-10.9	-11.0
Z-059012	-19.6	-	-12.8
Z-059018	-19.6	-	-12.8
Z-059025	-19.6	-	-12.8
Z-059028	-19.6	-	-12.8
Z-022504	-21.8	-14.2	-14.3
Z-062727	-23.8	-15.4	-15.6
Z-022163	-24.2	-15.7	-15.9
Z-021263	-28.3	-	-18.6
Z-010998	-29.0	-18.8	-19.0
Z-041771	-	-20.6	-20.8
Z-077160	-	-20.6	-20.8
Z-012371	-32.5	-21.1	-21.3
Z-012370	-32.9	-21.3	-21.5

Z-063864	-33.3	-21.6	-21.8
Z-063865	-33.3	-21.6	-21.8
Z-069325	-33.3	-21.6	-21.8
Z-041879	-34.2	-22.2	-22.4
Z-041880	-34.2	-22.2	-22.4
Z-040403	-35.1	-22.7	-22.9
Z-048795	-35.1	-22.7	-22.9
Z-048796	-35.1	-22.7	-22.9
Z-063845	-35.1	-22.7	-22.9
Z-047403	-35.1	-	-22.9
Z-047404	-35.1	-	-22.9
Z-087046	-36.5	-23.7	-23.9
Z-030840	-37.5	-24.3	-24.6
Z-023565	-2.0	-2.0	-24.9
Z-065968	-38.1	-2.0	-24.9
Z-074296	-39.2	-2.0	-25.6
Z-014414	-40.4	-26.2	-26.4
Z-068802	-	-26.6	-26.8
Z-025062	-	-29.3	-29.6
Z-048464	-48.4	-2.0	-31.7
Z-015237	-48.4	-31.4	-31.7
Z-068889	-49.3	-	-32.3
Z-066459	-50.3	-32.6	-32.9
Z-083238	-51.2	-	-33.5
Z-029775	-51.7	-33.6	-33.9
Z-081553	-	-34.6	-34.9
Z-035179	-54.4	-35.3	-35.6
Z-042978	-55.5	-36.0	-36.3
Z-035229	-57.9	-	-37.9
Z-030549	-59.2	-38.4	-38.8
Z-040200	-	-	-38.8
Z-038927	-62.0	-40.2	-40.6
Z-062710	-62.0	-40.2	-40.6
Z-025309	-68.3	-44.3	-44.7
Z-027131	-70.1	-45.5	-45.9
Z-049050	-70.1	-	-45.9
Z-045418	-72.0	-	-47.1
Z-029752	-2.0	-48.0	-48.4
Z-034605	-74.0	-48.0	-48.4
Z-001476	-76.1	-49.4	-49.8
Z-017458	-	-49.4	-49.8
Z-084503	-2.0	-2.0	-51.3
Z-023309	-78.4	-50.8	-51.3
Z-032874	-78.4	-50.8	-51.3
Z-086036	-78.4	-50.8	-51.3
Z-033144	-78.4	-	-51.3
Z-074819	-85.0	-55.1	-55.7
Z-000663	-85.9	-	-56.3
Z-010867	-88.8	-57.6	-58.1
Z-078759	-88.8	-57.6	-58.1
Z-017435	-3.0	-3.0	-65.4

Z-078251	-102.4	-	-67.1
Z-001793	-103.7	-67.3	-67.9
Z-069413	-113.3	-2.0	-74.2
Z-052208	-115.8	-3.0	-75.8
Z-014999	-142.7	-	-93.4
Z-078954	-143.9	-93.4	-94.3
Z-087238	-143.9	-93.4	-94.3
Z-020888	-145.3	-	-95.1
Z-076846	-147.9	-96.0	-96.9
Z-083355	-2.0	-98.7	-99.7
Z-087207	-2.0	-	-102.5
Z-076792	-159.8	-103.6	-104.6
Z-020992	-	-107.9	-109.0
Z-040223	-170.0	-110.2	-111.3
Z-038607	-177.6	-115.1	-116.2
Z-047702	-190.2	-123.4	-124.5
Z-088323	-	-123.4	-124.5
Z-018159	-	-3.0	-134.1
Z-064335	-206.9	-4.0	-135.4
Z-019734	-3.0	-136.4	-137.6
Z-002591	-213.1	-138.2	-139.5
Z-051301	-7.0	-147.5	-148.8
Z-054247	-5.0	-157.0	-158.5
Z-066353	-249.7	-3.0	-163.4
Z-014669	-277.9	-180.3	-181.9
Z-045791	-280.4	-8.0	-183.5
Z-047233	-6.0	-3.0	-186.8
Z-018320	-326.2	-211.5	-213.5
Z-023703	-6.0	-280.2	-282.8
Z-029575	184.5	184.5	-
Z-067787	61.5	184.5	-
Z-042717	111.0	166.5	-
Z-022862	-	158.8	-
Z-022863	-	158.8	-
Z-085795	-	133.9	-
Z-067963	-	111.0	-
Z-084928	-	108.4	-
Z-001441	-	99.0	-
Z-082389	227.9	86.0	-
Z-027480	35.0	70.0	-
Z-042548	34.0	67.9	-
Z-002271	-	66.9	-
Z-055386	-	66.9	-
Z-064083	-	66.9	-
Z-008225	-	63.2	-
Z-029704	-	63.2	-
Z-065826	-	63.2	-
Z-004478	61.5	61.5	-
Z-015650	-	61.5	-
Z-068803	-	61.5	-
Z-011251	-	58.4	-

Z-011252	-	58.4	-
Z-031292	56.9	56.9	-
Z-005648	-	56.9	-
Z-000868	-	56.9	-
Z-011412	-	56.9	-
Z-044981	-	55.5	-
Z-036372	-	54.2	-
Z-012077	-	52.9	-
Z-083824	-	52.9	-
Z-022784	-	51.7	-
Z-029417	-	51.7	-
Z-012567	-	50.6	-
Z-012568	-	50.6	-
Z-012878	-	49.5	-
Z-003685	-	48.4	-
Z-038138	-	48.4	-
Z-038630	46.4	46.4	-
Z-078501	-	46.4	-
Z-002077	-	45.5	-
Z-062255	44.6	44.6	-
Z-075325	-	44.6	-
Z-078180	-	44.6	-
Z-055794	-	43.8	-
Z-072267	-	42.9	-
Z-041491	-	41.4	-
Z-004811	-	40.6	-
Z-068138	39.9	39.9	-
Z-028617	-	38.6	-
Z-060125	-	37.0	-
Z-080809	-	35.6	-
Z-021574	34.0	34.0	-
Z-008460	-	34.0	-
Z-007456	-	33.5	-
Z-051544	-	33.5	-
Z-080050	-	33.0	-
Z-002222	-	30.3	-
Z-024822	-	29.9	-
Z-012711	-	29.2	-
Z-034629	-	26.5	-
Z-076202	-	26.5	-
Z-008083	-	25.9	-
Z-017433	25.3	25.3	-
Z-014799	-	24.7	-
Z-014800	-	24.7	-
Z-069895	12.1	24.2	-
Z-029371	71.1	23.7	-
Z-025795	-	23.7	-
Z-081768	-	23.5	-
Z-062277	45.5	22.8	-
Z-062278	45.5	22.8	-
Z-027539	-	22.8	-

Z-046026	-	22.3	-
Z-069987	63.2	21.1	-
Z-069727	-	20.7	-
Z-025861	20.3	20.3	-
Z-068066	-	20.0	-
Z-033837	-	19.3	-
Z-014583	-	19.0	-
Z-087289	-	18.4	-
Z-087283	-	18.4	-
Z-046222	-	15.6	-
Z-049009	45.5	15.2	-
Z-049014	45.5	15.2	-
Z-016334	-	15.1	-
Z-053932	13.5	13.5	-
Z-053937	13.5	13.5	-
Z-027538	-	12.6	-
Z-003282	-	11.4	-
Z-003285	-	11.4	-
Z-031328	-	6.3	-
Z-050920	-	6.0	-
Z-050912	-	6.0	-
Z-002640	2.5	4.0	-
Z-001920	2.0	3.0	-
Z-086561	-	3.0	-
Z-021160	-	3.0	-
Z-000601	-	3.0	-
Z-081470	11.1	2.6	-
Z-025689	-	2.5	-
Z-056270	5.6	2.2	-
Z-013054	-	2.1	-
Z-021133	3.0	2.0	-
Z-085105	-	2.0	-
Z-048980	-	2.0	-
Z-071734	-	2.0	-
Z-079306	-	2.0	-
Z-023964	-	2.0	-
Z-073697	-2.0	-2.0	-
Z-084558	-3.0	-2.0	-
Z-037491	-3.0	-2.0	-
Z-037492	-3.0	-2.0	-
Z-005478	-4.0	-2.0	-
Z-024254	-4.0	-2.0	-
Z-009320	-277.4	-2.0	-
Z-075932	-332.9	-2.0	-
Z-044811	-453.4	-2.0	-
Z-044812	-453.4	-2.0	-
Z-041308	-	-2.0	-
Z-033184	-4.6	-2.1	-
Z-039245	-4.3	-2.2	-
Z-045975	-2.7	-2.2	-
Z-046182	-2.9	-2.3	-

Z-046183	-2.9	-2.3	-
Z-046185	-2.9	-2.3	-
Z-075572	-3.5	-2.3	-
Z-078345	-2.2	-2.6	-
Z-079213	-	-2.7	-
Z-064504	-	-3.0	-
Z-062784	-3.0	-3.0	-
Z-062787	-3.0	-3.0	-
Z-023999	-9.0	-3.0	-
Z-012765	-140.2	-3.0	-
Z-010037	-	-3.0	-
Z-076273	-	-3.0	-
Z-076274	-	-3.0	-
Z-054276	-	-3.0	-
Z-054277	-	-3.0	-
Z-004090	-	-3.0	-
Z-031473	-2.6	-3.2	-
Z-039998	-4.0	-3.2	-
Z-031117	-2.2	-3.9	-
Z-088915	-7.4	-4.8	-
Z-000533	-28.7	-5.0	-
Z-045533	-283.4	-5.0	-
Z-072563	-4.2	-5.7	-
Z-078340	-6.0	-6.0	-
Z-006317	-12.0	-6.0	-
Z-015597	-8.9	-8.9	-
Z-005670	-37.5	-24.3	-
Z-008232	-41.0	-26.6	-
Z-053366	-43.0	-27.9	-
Z-015646	-45.2	-29.3	-
Z-041994	-	-29.8	-
Z-035550	-62.9	-40.8	-
Z-009913	-129.9	-84.3	-
Z-056761	-179.9	-116.7	-
Z-064478	-2.0	-181.8	-
Z-001875	175.1	-	-
Z-002719	162.6	-	-
Z-025723	133.9	-	-
Z-049818	113.8	-	-
Z-038629	87.5	-	-
Z-042408	81.3	-	-
Z-016024	77.6	-	-
Z-034347	66.9	-	-
Z-046177	66.9	-	-
Z-039103	65.0	-	-
Z-041716	65.0	-	-
Z-042735	61.5	-	-
Z-076133	59.9	-	-
Z-074835	58.4	-	-
Z-085497	54.2	-	-
Z-015397	52.9	-	-

Z-007306	50.6	-	-
Z-049581	45.1	-	-
Z-056214	44.6	-	-
Z-061522	43.4	-	-
Z-083658	42.9	-	-
Z-021179	42.1	-	-
Z-086008	42.1	-	-
Z-070988	40.6	-	-
Z-032659	39.9	-	-
Z-061521	36.1	-	-
Z-086168	29.9	-	-
Z-015472	28.1	-	-
Z-045140	27.1	-	-
Z-045141	27.1	-	-
Z-059451	25.5	-	-
Z-059454	25.5	-	-
Z-059466	25.5	-	-
Z-059442	25.5	-	-
Z-048642	25.3	-	-
Z-048644	25.3	-	-
Z-044545	22.8	-	-
Z-074994	22.8	-	-
Z-064064	21.9	-	-
Z-083572	19.6	-	-
Z-012658	18.5	-	-
Z-012659	18.5	-	-
Z-001811	18.5	-	-
Z-004511	16.7	-	-
Z-004512	16.7	-	-
Z-019187	14.4	-	-
Z-019188	14.4	-	-
Z-006330	13.7	-	-
Z-083783	12.1	-	-
Z-083784	12.1	-	-
Z-056419	11.0	-	-
Z-042733	7.2	-	-
Z-060327	4.0	-	-
Z-014587	3.5	-	-
Z-014590	3.5	-	-
Z-084383	3.2	-	-
Z-084386	3.2	-	-
Z-084388	3.2	-	-
Z-055695	3.0	-	-
Z-064963	2.5	-	-
Z-034307	2.3	-	-
Z-043152	2.0	-	-
Z-061835	2.0	-	-
Z-061828	2.0	-	-
Z-061832	2.0	-	-
Z-065551	2.0	-	-
Z-009423	2.0	-	-

Z-015234	2.0	-	-
Z-005265	-2.0	-	-
Z-063609	-2.0	-	-
Z-063611	-2.0	-	-
Z-057176	-2.0	-	-
Z-039971	-2.0	-	-
Z-064451	-2.0	-	-
Z-028408	-2.0	-	-
Z-028410	-2.0	-	-
Z-063380	-2.5	-	-
Z-067926	-2.5	-	-
Z-031116	-3.0	-	-
Z-064506	-3.0	-	-
Z-029743	-3.0	-	-
Z-041155	-3.5	-	-
Z-087767	-3.7	-	-
Z-031984	-4.0	-	-
Z-065337	-4.0	-	-
Z-063591	-4.0	-	-
Z-084039	-4.1	-	-
Z-010661	-5.0	-	-
Z-010665	-5.0	-	-
Z-018487	-20.0	-	-
Z-069183	-32.5	-	-
Z-052613	-38.1	-	-
Z-008926	-42.3	-	-
Z-008928	-42.3	-	-
Z-048814	-43.0	-	-
Z-048816	-43.0	-	-
Z-035171	-47.6	-	-
Z-038379	-64.2	-	-
Z-051764	-156.7	-	-
Z-076533	-180.6	-	-
Z-085094	-302.7	-	-

Transcripts order based on high to low regulation of 34% PEG followed by 31% and 27% PEG, respectively.
“-“ no statistical difference in expression level of contig