UNIVERSITÀ CATTOLICA DEL SACRO CUORE
Sede di Piacenza

Scuola di Dottorato per il Sistema Agro-alimentare

Doctoral School on the Agro-Food System

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S.S.D: BIO/04

PHYSIOLOGICAL AND MOLECULAR ANALYSIS OF DROUGHT RESPONSE IN SWEET SORGHUM

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Matr. n. 3810666

Academic Year 2011/2012

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Summary

One of the critical components in the production of bioenergy feedstock for renewable fuel will be water. Thusly, both drought tolerance and water-use efficiency will be critical as many of these feedstocks will be produced in marginal environments, where rainfall is limited and irrigation is either too expensive or would deplete water reserves. Sorghum (*Sorghum bicolor* (L.) Moench) is more water-use efficient than most other biomass crops. The water-use efficiency and drought tolerance in sorghum is probably due to evolutionary origins. I.E. sorghum had to adapt to the water-limited environments of Africa. Drought-resistance mechanisms in sorghum and their genetic control have been the subject of extensive researches. This has led to a relatively thorough understanding of the genetic basis of drought tolerance in this species. The tolerance is the result of several phenotypic and physiological traits that have been identified and characterized by sorghum physiologists. Traits that have been associated with drought resistance include; heat tolerance, osmotic adjustment, transpiration efficiency, rooting depth and patterns, epicuticular wax and stay-green. While all of these traits have been associated with drought tolerance in sorghum, breeders have established phenotypic methods of evaluation that, while challenging, have been effective in improving the tolerance of sorghum to drought. These sources and methodologies are easily applicable to sorghum breeding for bioenergy as well.

The physiological response related to gene regulation play a key role in plant adaptation during a drought. This study aimed at characterising the dynamics of transpiration, development, growth, as well as the expression of genes, in response to a drought during dry-down cycle. This method enables us to gravimetrically monitor bulk soil moisture content and at the same time, if soil evaporation is suppressed, whole-plant transpiration rate.

Genome wide changes in gene, expression were monitored in sorghum, following exposure of seedlings to water stress determined by the fraction of transpirable soil of water (FTSW). A sorghum cDNA microarray providing data on 21,665 probes was used to examine gene expression in shoots at three FTSW values. In growth chamber and in green house experiments, differentially expressed genes, including genes with currently unknown functions, of which a subset appear to be unique to monocots and/or sorghum, were altered in response to dehydration, at the higher stress level. The modulated sorghum genes had homology to proteins involved in regulation, growth, transport, membrane/protein turnover/repair, metabolism, dehydration protection, reactive oxygen scavenging, and plant defense. Real-time PCR was used to quantify changes in relative mRNA abundance for 8 genes that responded to water deprivation. Water stress inducible sorghum genes identified, included; heat shock proteins (HSPs), prolin reach proteins (PRPs), glutathione S transferase (GST), dehydrins (DHN), abre, delta 1-pyrroline-5-carboxylate synthetase (P5CS), inositol-1-phosphate synthase, NADP-malic enzyme and two Cytochrome P450 genes. Analysis of response profiles demonstrated the existence of a complex gene regulatory network that differentially modulates gene expression in a tissue- and kinetic-specific manner, in response to water deficit. Modulation of genes involved in signal transduction, chromatin structure, transcription, translation and RNA metabolism, contributes to sorghum’s overlapping but nevertheless distinct responses to different levels of water stress. Overall, this study provides a foundation of information on sorghum’s osmotic stress responsive gene complement that will accelerate follow up biochemical, quantitative trait locus (QTL) and comparative studies.

1. Introduction

1.1 Sorghum as bioenergy crop

The cost of energy is increasing together with the attention to the prevision that fossil fuels and gas reserves are not infinite (1 Rooney, 2007). Considering that human population is rapidly increasing, consequently, there has followed a major demand and consumption of energy. There's a worldwide necessity to improve energy and biofuel production from alternative and renewable sources. Furthermore the direct relation between increments of atmospheric CO_2, produced from the consumption of fossil fuels and global warming, drove to increase efforts to implement technologies for the production of biofuels from biomass, generating a lower carbon emission (1 Rooney, 2007). Bioenergy includes any source of heat, electrical and fuel energy derived from biological sources (Yuan *et al*., 2008). Among these are the use of agro-industrial wastes and crop residues that have the advantage of being relatively economical. The major part of biomass for bioenergy production should not come from grain crops used as feed and food but needs to be produced at low cost and with low inputs to make biofuel generations profitable and sustainable. However the need for large quantities of biomass to be converted in to energy has and will further stimulate the cultivation of dedicated crops (Perlack *et al*., 2005).

On December 17, 2008, The EU approved a directive on the promotion of the use of energy from renewable sources where sustainability criteria for biofuels are indicated. Greenhouse gas emission savings from the use of biofuels shall be at least 35% compared to the traditional non renewable fuels. Furthermore biofuels shall not be made from raw material obtained from land with high biodiversity value and from land with high carbon stock. The directive indicates default values for greenhouse emission savings for different biofuels and at the same time proposes a method for its calculation as to promote sustainable and efficient production systems. To comply with this criteria, bioenergy crops should provide high dry matter yield with low input (i.e. fertilizers, water, pesticide, energy for mechanisation), should be adapted to marginal environments and should limit the depletion of carbon stock in the soil or increase it through high below-ground biomass accumulation (De Oliveira *et al*., 2005).

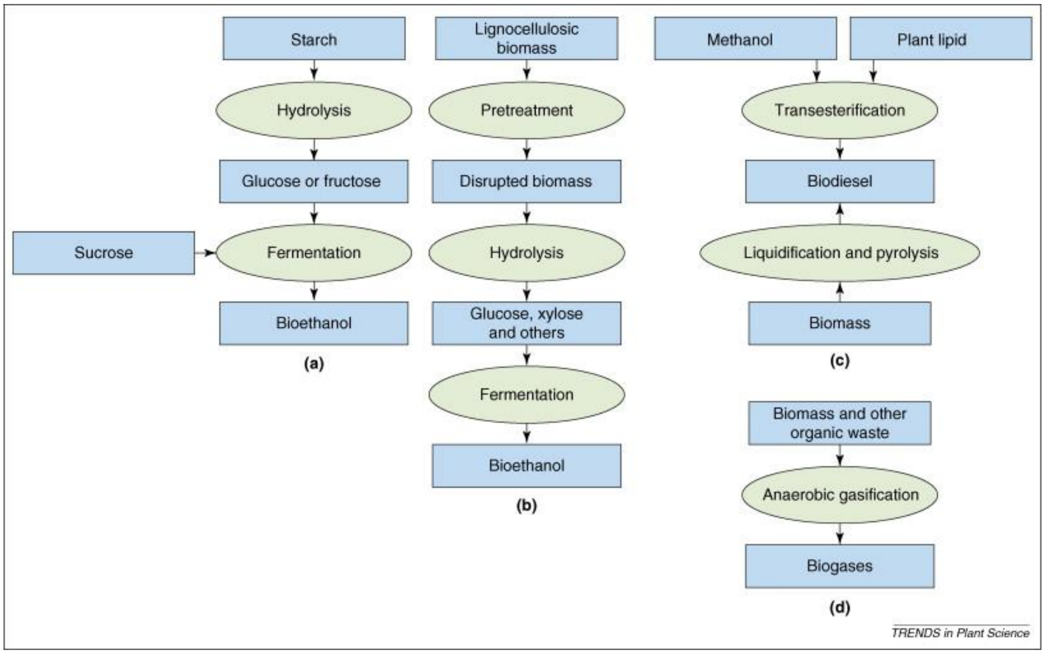
Given the current energy situation, Sweet Sorghum (*Sorghum bicolor* (L.) Moench) is now being developed as a potential bioenergy crop.

Sorghum is a C4 grass, that can be rightfully defined a multiuse crop. In fact, besides being the fifth most widely cultivated cereal crop in the world, in many regions of Africa it is grown as dual purpose crops (Awika and Rooney, 2004). It has traditional industrial applications (Berenji and Dahlberg, 2004), as a widespread forage crop and in addition, the fibre from high yielding biomass sorghum genotypes can be used for paper pulping (Amaducci *et al*., 2004).

This specific type of sorghum accumulates high levels of sugar in the stalks of the plant. The sugar's content could be converted in first generation ethanol. The lignocellulose residues could also be converted in ethanol by second generation processes, after enzymatic hydrolysis (Fig.1) (Sipos *et al*., 2008, Yuan *et al*., 2008). Sorghum biomass can also be burnt to generate heat and electricity. It can be fed into anaerobic digesters to be converted into methane and in the near future, Stover of grain sorghum, whole plants from fibre genotypes. Bagasse of sweet genotypes could be used for the production of ethanol with second generation technologies. Additional features that make sorghum a very interesting crop for bioenergy production are; high biomass yields with limited cropping inputs that result in relatively elevated, energy efficient production systems (Monti and Venturi, 2003) Sorghum allows also a large and durable accumulation of carbon in the soil which further contributes to environmental policies connected with bioenergy production (i.e. to control atmospheric CO_2 concentration) (Fernández et al., 2003).

Several studies investigated the positive performances in water use efficiency of sorghum (Mastrorilli *et al*, 1999; Steduto *et al*., 1997; Tingting *et al*., 2010), characteristics that confer drought stress tolerance to this crop, allowing its cultivation in marginal and drought-prone environments, avoiding to remove soil designate to food-feed crops production in addition to the saving of water.

Fig. 1: Biofuel production: a) Starch and sugar-based first generation ethanol; b) Lignocellulosic ethanol is believed to be the major component of the second generation of bioenergy; c) Biodiesel production; d) Biomass gasification



1.2 *Sorghum* genus and sweet sorghum: Taxonomy, morphology and anatomy.

The *Sorghum* genus belongs to the tribe Andropogonae which includes many of the tall grass genera Mischantus and Andropogon, as well as Zea, Saccharum and Sorgastrum. The *Sorghum* genus as currently proscribed consists of 25 species (Dillon *et al*., 2007), although this varies in different scientific publications confirming the dynamic nature of the classification of cultivated sorghum and its less cultivated relatives. The genus is separated into five taxonomic subgenera or sections; *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum* and *Stiposorghum*. Section *Eu-Sorghum* contains all domesticated cultivated sorghum races and varieties as *Sorghum bicolor* subsp. *bicolor*. (Doggett, 1988). All S. *bicolor* subsp. b*icolor* have *2n* = 20 chromosomes, and are described as annual, with thick culms up to 5 m in height, often branched with many tillers. They have been classified into five basic races: *bicolor, guinea*, *caudatum, kafir* and *durra,* with ten intermediate races of these also recognized (Harlan and de Wet, 1972).

The tribe Andropogoneae includes all C4 plants that thrive in mostly tropical and temperate climes. The tribe is characterized by paired spikelets, usually with one sessile and one pedicellate.

Sweet sorghums belong to tribe Andropogonae and are specific types that accumulate high levels of sugar in the stalk of the plant. These types of sorghum were identified and used as alternative sugar sources in areas where sugarcane could not be produced (Rooney *et al*., 2004).

The number of internodes produced in sweet sorghum is determined by four maturity genes controlled by photoperiod and temperature interactions. Internode length is determined by four major genetic factors as well as environmental parameters (Voigt *et al*., 1966). Stalk thickness increases with the number of internodes produced; often, late-maturing cultivars have thicker stems than early cultivars (Martin, 1970). The size of the panicle and the peduncle are often independent.

The sweet sorghums tend to produce more tillers than other sorghum types; some have the potential to produce 8 to 10 tillers and some, as few as 1. Hybrid sorghum tends to tiller more than the inbred lines. Sorghum stalks in general are characterized by relatively numerous stomates. Many sorghum cultivars have thick waxy coatings on stem surfaces, which are implicated in drought tolerance in the species. The formation of air-filled, cottony tissue has been noted in some sorghum stems as they mature. The accumulation of starch in the cells surrounding vascular bundles and in the parenchyma between the bundles occurs in sweet sorghum. The leaf midrib colar in sorghum has been associated with various characteristics such as stalk juiciness and digestibility (Kalton, 1988).

The number of leaves produced in sorghum cultivars depends on the length of the vegetative period. Typically, there are 8 to 10 nodes below the soil surface that elongate very little and the total number produced ranges from 15 to 40. Leaf angle also is affected by spacing or plant population. Because of its morphological characteristics, sweet sorghum may also be used for the production of sugar into ethanol.

1.3 The *Sorghum bicolor* genome

Sorghum and maize shared a common ancestor about 12 million years ago (Myr), when a polyploidization and repetitive DNA propagation conferred to maize a major genome complexity. It has been estimated from physical mapping that the sorghum genome is about 730 megabases, 60% larger than that of rice but only ¼ of that of maize (Paterson *et* *al*, 2005). However, sugarcane, which is more strictly phylogenetically correlated to sorghum, (about 5 Myr of divergence) has a genome larger than that of maize and 4-fold redundancy of most genes.

Sorghum has a small diploid genome and low level of gene duplication. The *Sorghum bicolor* genome has recently been sequenced providing a great boost to our knowledge of the evolution of grass genomes (Paterson *et al*., 2009). The gene order and density are similar to those of rice. Retrotransposon accumulation explains the ~75% larger genome size of sorghum compared with rice. In total, DNA transposons constitute 7.5% of the sorghum genome, between maize (2.7%) and rice (13.7%). Among 34,496 sorghum gene models, 27,460 bona fide protein-coding genes have been found. The number and size of sorghum gene families are similar to those of Arabidopsis and rice. Although gene and repetitive DNA distributions have been preserved since palaeopolyploidization ~70 million years ago, most duplicated gene sets lost one member before the sorghum-rice divergence. Concerted evolution makes one duplicated chromosomal segment appear to be only a few million years old. About 24% of genes are grass-specific and 7% are sorghum-specific. Recent genes and microRNA duplications may contribute to sorghum's drought tolerance. The characteristic adaptation of sorghum to drought may be partly related to expansion of miRNAs, upregulated during drought stress. The sbi-MIR169 subfamily comprises members of the plant nuclear factor Y Faily, linked to improved performance under drought by Arabidopsis and maize. Cytochrome P450 domain-containing genes, often involved in scavenging toxins such as those accumulated in response to stress, are abundant in sorghum. 328 cytochrome P450 genes are detected in sorghum, whereas rice has 228 such genes.

Expansins, enzymes that break hydrogen bonds and are responsible for a variety of growth responses that could be linked to the durability of sorghum, occur in 82 copies in sorghum versus 58 in rice and 40 in Arabidopsis.

Conservation of grass gene structure and order facilitates development of DNA markers to support crop improvement. About 71,000 simple-sequence repeats (SSRs) have been identified in sorghum. Conserved-intron scanning primers for 6,760 genes provide DNA markers useful across many monocotyledons. Sorghum may prove especially valuable for unravelling genome evolution in the more closely related Saccharum-Miscanthus clade:

two genome duplications since its divergence from sorghum 8-9 million years ago complicated sugar cane genetics yet Saccharum show substantially conserved gene order with sorghum.

The amenability to inbreeding and the high level of polymorphism between *Sorghum* species, permit to construct easily high density genetic maps. Thanks to these peculiar genomic traits, sorghum is used as a model for tropical grasses (C4), as *Oryza sativa* is for temperate (C3) plants (Paterson, 2008).

Thanks to the similarity of sorghum genome to other crops, genetic maps were constructed anchoring the sorghum maps to those of sugarcane (Ming *et al*, 1998), maize (Bowers *et al*, 2003) and rice (Paterson *et al*, 2004). Information derived from markers, were used to locate them on chromosomes with the aid of bacterial artificial (BAC) libraries. Sorghum was the first plant for which a BAC library was reported (Woo *et al* 1994). Physical map of both *Sorghum bicolor* and *Sorghum propinquum* have been constructed and subsequently anchored to the mapped sequenced-tagged sites (STS) loci (Bowers *et al.*, 2005). High-density maps of one intraspecific *S. bicolor* (Klein *et al.*, 2000; Menz *et al.*, 2002) and one interspecific *S. bicolor* X *Sorghum propinquum* (Chittenden *et al.*, 1994; Bowers et al, 2003) cross provide about 2600 STS and 2454 amplified fragment length polymorphisms (AFLPs).

Linkage maps were used to map genes for quantitative traits loci (QTLs). Examples of quantitative traits studies are those connected to morphology as seed size (Rami *et al.*, 1998), plant height and flowering time (Lin *et al.*, 1995), yield and composition of sugar in stem (Murray *et al.*, 2008), plant response to disease resistance (McIntyre *et al*, 2005) and stress tolerance (Knoll *et al.*, 2008). Also AFLP markers were used to link loci to agronomic traits such as biomass yield and plant height (Natoli *et al.*, 2002). Recently, the Diversity Arrays Technology (Dart) has been developed to simultaneously fingerprint the entire genome of Sorghum (Mace *et al.*, 2008).

Actually, the genome of an inbred line of sorghum is completely sequenced (Paterson, 2008), and the next step will be the assembly of contigs to order genes and to distinguish coding regions from non-coding. 204,000 ESTs information is already available at NCBI. An online resource (http://cggc.agtec.uga.edu), provides access and links to sorghum genomics informations such as EST database, genetic/physical map and polymorphism data. The IntegratedMap interface displays a graphic view of the sorghum STS-based genetic map, including polymorphisms and anchored BAC. Other sites about sorghum are those concerning functional genomics (http://fungen.botany.uga.edu), assembly of the sorghum genome (http://www.phytozome.net/sorghum) and genomics of the abiotic stress response (http://sorgblast2.tamu.edu).

While biomass yield is determined by height, stalk diameters, disease and pest resistance, biomass quality is strongly influenced by cell wall composition and structure. Genetic improvement of cell wall composition and study of genes involved in cell wall metabolism are of great importance. About 10% of the grass genome (ca. 2500 genes) is involved in construction and dynamic rearrangement of cell walls during growth (Carpita e McCann, 2008).

Over the past eight years, sorghum genome research has advanced to the point where the generation of superior biomass sorghum genotypes can be addressed using genome scale analysis in conjunction with other systems-based approaches. Several research groups have helped build integrated genetic, physical, and comparative maps of the sorghum genome, (Klein *et al*., 2000, Menz *et al*., 2002, Bowers *et al*., 2003, Klein *et al*., 2003) acquired a comprehensive understanding of chromosome and genome architecture, (Kim *et al*., 2005) validated map-based gene cloning in sorghum (Klein *et al*., 2005), carried out an in-depth Expressed Sequence Tags (EST) project, (Pratt *et al*., 2005) developed microarray and Reverse Transcription Polymerase Chain Reaction (RT-PCR) technology for analysis of gene expression, (Buchanan *et al*., 2005) and significantly advanced the knowledge of sorghum genetic diversity (Klein *et al*., 2003, Kim *et al*., 2005). Moreover, the sorghum community has advanced the ability to analyze gene action in sorghum through gene transfer technology. Most recently, the US Department of Energy's investment in an 8X whole genome shotgun sequence of sorghum, will greatly aid the completion of a high-quality sequence of the sorghum genome (Joint Genome Institute, 2006). This effort will accelerate and enable a deeper analysis of the genetic and biochemical basis of all sorghum traits including biomass generation for bioenergy. Ongoing research will utilize this emerging sorghum genome information and technology platform to advance the understanding of the genetic and biochemical basis of superior sorghum biomass generation in marginal environments and the pathways that modulate composition related to bioenergy production.

1.4 Traits providing tolerance to drought

Drought and other abiotic stresses with effects on plant water status, both in natural and agricultural settings, received particular attention from researchers and breeders. Water availability decreasing elicits a range of responses that allow plants to avoid or tolerate the water loss (Verslues *et al*., 2006) through physiological, biochemical and molecular processes. Dehydration avoidance is associated with a variety of adaptive traits that involve minimising water loss and maximising water uptake (Chaves *et al*., 2002) while dehydration tolerance involves mechanisms to avoid cellular damages caused by water loss (Verslues *et al*., 2006). Understanding water stress responses at a physiological/ molecular level is one of the most important topics in plant science (Shao *et al*., 2007). Studying the impact of physiological parameters will assist in identifying some selection criteria that might prove useful in developing drought tolerant genotypes. Several studies have been carried out to set physiological markers attributable to drought tolerance (Bibi *et al*., 2010; Ali *et al*., 2009). A survey of literature revealed that morpho-physiological traits such as leaf area (Tsuji *et al*., 2003; Karamanos and Papatheohari, 1999), root dry matter (Huang and Gao, 2000; Giuliani *et al*., 2005), whole- plant transpiration rate (Luquet *et al*., 2008; Xin *et al*., 2008) had been widely used as selection parameters for screening of water stress tolerant crop plants, such as application of genomics and molecular biology allowed elucidating the mechanisms of drought response of several plants species of agronomic interest.

1.4.1 Molecular analysis of drought tolerance in Sorghum

This approach leaded to identification of genes and QTLs associated with drought tolerance permitting the development of not conventional breeding techniques, such as Marker-assisted breeding or genetic engineering, to improve crops yield during water deficit and, consequently, in water-limited environments.

The good performance of sorghum in water deficit conditions, the sufficiently high genetic variance among the sorghum genotypes and the consequent high adaptability, made it an ideal crop model for the identification of genomic regions and genes necessary to dissect the drought tolerance complex trait. The relatively small genome size (about 730Mb), make sorghum a suitable species for functional genomics and positional cloning (Paterson *et al*., 2009; Sanchez *et al*., 2002). Sorghum genome is less complex than maize genome, and as a member of Saccharinae subtribe, it is the ideal model for its fellow members sugarcane and *Miscanthus*, both of which are polyploids that do not succumb easily to genetic studies due to sterility issues (Vermerris, 2011). Several sorghum linkage maps have been generated using different kind of DNA markers (Xu *et al*., 1994; Taramino *et al*., 1997; Rami *et al*., 1998; Kong *et al*., 2000; Menz *et al*., 2002; Mace *et al*., 2009) reaching an elevate level of density (Ashraf *et al*., 2010). Diverse genomics regions linked to resistance to pre-flowering and post-flowering drought stress were detected (Tuinstra *et al*., 1996; Kebede *et al*., 2001; Sanchez *et al*., 2002). Furthermore, recently the determination of the Sorghum bicolor (cultivar BTx623) genome sequence was completed (Paterson *et al*., 2009). This genome information allows monitoring of genome-wide gene expression profiling at a single time, in response to various abiotic stresses, through microarrays technology. Such transcriptome analysis would permit to identify sorghum drought stress responsive genes, reveal their relationships and predict also their regulatory elements (*cis* and *trans* factors). One experiment has reported the use of microarrays for gene expression analysis in sorghum under drought (Buchanan *et al*., 2005). This experiment was performed on the sorghum BTx623 genotype inducing drought stress in very young seedlings using polyethylene glycol (PEG). Even if this approach revealed the existence of a complex gene regulatory network that differentially modulates gene expression in response to water deficit (together with ABA and high salinity), it did not simulate the slow development of drought stress that occurs in field conditions. Furthermore, it may be important to investigate also post-transcriptional gene regulation after stress exposure. It’s known in fact that large part of stress-responsive genes is usually regulated at transcriptional level. Despite this the importance of post-transcriptional gene regulation has been realized with the discovery of small RNAs, divided in two major classes; microRNAs (miRNAs) and short-interfering RNAs (siRNAs). These small RNAs are known to silence genes post-transcriptionally by guiding target mRNAs for degradation or by repressing translation (Ambros, 2004; Bartel, 2004; Baulcombe, 2004; Rhoades, 2006; Mallory and Vaucheret, 2006). In plants, miRNAs complement their target mRNAs inducing their cleavage and consequently their silencing (Lewis *et al*., 2010). MiRNAs were found in plants, animals and other diverse eukaryotes as well as a number of DNA viruses. Plant miRNAs are 20-24 nucleotides (nt) long non-coding RNAs. After transcription as single-stranded RNAs they fold-up into stem-loop structures that are recognized and processed by dicer-like (DCL) enzymes. These enzymes together with other proteins act to produce the mature 21 nt long miRNAs duplexes in the nucleus. HASTY protein, an Exportin-5 homolog, transports mature miRNAs in the cytoplasm where they are assembled into RNA-induced silencing complexes (RISCs) in single-stranded form (Lewis *et al*., 2010). In both plant and animal genomes multiple precursors are found to produce similar mature miRNA products (Tanzer and Stadler, 2004; Tanzer *et al*., 2005; Maher, 2006). In facts miRNA genes are organized in gene families. In plant genomes are present fewer miRNA gene families but with a larger number of miRNA genes respect to the animal genomes (Li and Mao, 2007).

The role of miRNAs in plant development is well documented in several reviews (Mallory and Vaucheret, 2006; Rhoades, 2006; Carrington, 2003; Dugas and Bartel, 2004; Kidner and Martienssen, 2005). Considering the importance of miRNAs in post-transcriptional gene silencing, their involvement in stress regulated gene expression seemed likely (Kawaguchi *et al*., 2004; Sunkar and Zhu, 2004). The discovery that stress can regulate miRNAs levels associated with the identification of stress-induced genes as miRNAs targets, provided indications about the role of miRNAs in stress response (Sunkar *et al*., 2007, Juarez *et al*., 2004). Several studies have demonstrated that many plant miRNAs play important functions in plant resistance to abiotic and biotic stresses (Fujii *et al*., 2005; Chiou *et al*., 2006; Aung *et al*., 2006; Bari *et al*., 2006; Navarro *et al*., 2006; Sunkar *et al*., 2006; Reyes and Chua, 2007; Jung and Kang, 2007; Zhao *et al*., 2007 from Lewis *et al*., 2010) and two more recent works identified some drought-responsive miRNAs in Arabidopsis (Liu *et al*., 2008;) and rice (Jian *et al*., 2010; Zhou *et al*., 2010).

2. Aim of the work

Sweet sorghum is a multipurpose crop that, providing food, feed and fuel with a limited use of resources, responds to the criteria of modern agriculture and particularly to sustainable bioenergy production. Investigation of molecular and physiological basis of drought tolerance in sorghum and identification of candidate genes involved in water stress tolerance are useful instruments of analysis.

The identification of physiological marker combined with studies of gene expression is current research tools for the assessment of water stress in plants.

Recently, morpho-physiological traits such as leaf area, root dry matter, whole plant transpiration rate had been widely used as selection parameters for screening of water stress tolerant crop plants. Likewise, gene espression analysis in young seedlings of sorghum under water stress using microarray, were described. However, papers have not, thus far, reported a combined evaluation of physiologiocal-molecular parameters in seedlings in a more advanced phenological stage.

Gene expression analysis and post transcriptional gene regulation have not yet been described in young plants of sorghum to the phenological stage of 10-12 leaves, subjected to different phases of water stress.

During open field evaluation of several sweet sorghum genotypes under drought conditions, sweet sorghum genotype IS19453 was selected as drought tolerant line, based on its high pre- and post-flowering drought tolerance in field trials (Cirad, personal communication). IS33350 genotype was previously classified as a senescent stay- green source as opposed to the genotype tolerant SDS19483 (Habyarimana *et al*., 2004).

In this work the physiological and molecular responses to water deficit of different sweet sorghum genotypes were evaluated after application of a “dry-down” pot experimental procedure in controlled environment (growth chamber and green house). Drought-related genes and their regulation were identified through transcriptome analysis by using large scale microarray. Post-transcription modulation due to miRNA genes has been evaluated making use of a dedicated microRNA microarray. Candidate genes involved in drought tolerance will be available for molecular-assisted breeding or genetic engineering. Introduction of the water stress tolerance in more productive cultivar in terms of sugar and biomass will allow to obtain high yields without irrigation and also in drought-prone regions.

In this work, physiological and molecular assays were developped to define the drought stress degree in IS19453, BR501, BR505, SDS 19483, IS33350, Mpwekwa genotypes and identify a gene response-related.

The specific objectives of the research are:

- Conduct tests in controlled environment to assess and identify sorghum genotypes characterized by specific tolerance to stress conditions due to water depletion.
- Identify structural genes, regulatory, and micro-RNA involved in drought tolerant genotypes identified during the testing.

The overall objective of this study was to characterize different sorghum genotypes that differ in their response to drought stress. The characterization was so focused on both physiological and molecular aspects of this comlex genetic trait.

To identify useful traits in sweet sorghum for bioethanol production, and characterize their physiological and genetic basis, dry down experiments were developped in order to characterize physiological parameters. Equally, gene expression analysis and post transcriptional gene regulation analysis were carried out with the aim to verify the eventual association with the physiological response.

3. Materials and Methods

3.1 Plant material

Tolerant and sensitive sweet sorghum genotypes were checked in growth chamber and in green house conditions at the Università Cattolica del Sacro Cuore (Piacenza) and in farm Eridano (Podenzano).

Sweet sorghum genotype IS19453 was selected as drought tolerant line, based on its high pre- and post-flowering drought tolerance in field trials (Bracconier, CIRAD, personal communication).

Tolerant genotype (Mwekwa) was choosen on the basis of biomass performance at the seedling growth stage in glasshouse and in rain out shelter condition (Habyarimana *et al.*, 2002). A stay-green source (SDS19483) along with a senescent type (IS33350) was previously identified (Habyarimana *et al.*, 2004) (Tab.1).

BR501, BR505 genotypes were evaluated for their different behavior to drought stress in field conditions, evaluated for brix, total sugars in % of juice, sugar extracted, percent fiber of fresh biomass, percent water in the biomass (Schaffert, EMBRAPA, personal communication). In this work BR genotypes were used to better understand the physiological-molecular differences.

The study was approved and enhanced by Sweetfuel Project supported by the European Commission in the 7th Framework Programme.

Tab. 1: Analyzed genotypes: DMYS: above ground dry mass stability. Adaptability index was derived from the coefficient of a linear regression line obtained by plotting the mean aboveground dry mass yield. Stay green: in SDS19483 genotype retained more than 70% of green leaf area at harvest, while the senescent type IS33350 kept an average of 20%. na: not analyzed (data from Habyarimana *et al.*, 2004).

Genotypes	DMYS	Adaptability index	Stay green
Mpwekwa	0.6	1.51	2.7
SDS19483	0.9	0.82	1.9
IS33350	0.5	1.4	3.7
IS19453	na	na	na

3.2 Dry down experiments: experimental conditions in growth chamber

In a first experimental approach, sweet sorghum genotype IS19453 was grown in climatic chamber for the measurement of physiological traits and gene expression.

Seeds were germinated during 2 days in an incubator at 30°C. After germination (hypocotyl appearance), seedlings were transplanted in pots (capacity 1037 cm^3) filled with 1.2 Kg of sterile zeolite and fertilized with 0.87g/pot of NPK fertilizer containing 15% N, 9% P, 15% K. Zeolite was chosen because presents water holding capacity of about 55% (550g of water per Kg), and permits good cleaning of roots. 50 plants, 1 plant per pot, were grown with 13 h daylight at 27 °C, humidity 50-60% and 11 h dark period at 24 °C, humidity 60-70% under controlled conditions.

At the developmental stage of four leaves, plants were irrigated (600 water plus 0.5g/l of soluble fertilizer NPK 20-20-20 with nutritional microelements) until field capacity. Then the pots were covered with PVC bags following the protocol for "dry-down" experiment (Luquet *et al*., 2008). This kind of procedure permits to estimate water loss through transpiration by the plant, eliminating evaporation. Water stress was applied immediately by stopping irrigation after the achievement of field capacity. Plants measurements such as leaf area (LA), daily plant transpiration (PT), plant growth rate, and biomass parameters like root dry mass (RDM), were performed. During the test twelve plants were irrigated as positive controls. Seedlings samples were collected for the RNA extraction both from irrigated and not irrigated pots, at different value of FTSW (fraction of transpirable soil water) 0.37, 0.25 and 0.15, corresponding to 25, 27 and 30 days after emergence (DAE), respectively. Leaf area was measured with an optical area meter (LI-3000, Inc.). Roots were dried in an oven at 105 °C for 24 h. Leaf area and root dry mass data were related to FTSW.

All data were subjected to analysis of variance using MSTAT-C Statistical Package developed by Michigan State University. The least significant difference (LSD) test was used for comparing treatments' means.

3.2.1 Dry down experiments in greenhouse

In a subsequent experimental approach, sweet sorghum genotypes Mpwekwa, IS19483, IS33350, BR501, BR505 were grown in greenhouse to expand the analysis of physiological traits and gene expression in a more advanced phenology stage of 12 leaves. Seeds were germinated during 2 days in an incubator at 30°C. After germination, seedlings were transplanted in pots (80000 cm^3) filled with 10 Kg of soil fertilized with 2.5 g/pot of soluble fertilizer NPK 20-20-20 with nutritional microelements. In this experiment soil contained 12% of sand, 67% of silt and 22% of clay and it was chosen to simulate the field condition. 160 plants, 1 plant per pot, were grown with 13 h daylight at 35°C, humidity 50-60% and 11 h dark period at 25°C, humidity 60-70% in green house.
At the developmental stage of ten leaves plants were irrigated until field capacity. Pots were covered with PVC bags. For each genotype four plants were used to determine wilting point (WP). Water stress was applied immediately by stopping irrigation after the achievement of field capacity. Plants measurements such as daily plant transpiration (PT), plant growth rate, were performed. During the test three plants for each genotype, were irrigated as positive controls. Seedlings samples were collected for the RNA extraction both from irrigated and not irrigated pots. Irrigated pots remained at a FTSW value of 0.70 equal to 70% of awailable water (AW). Not irrigated plants were maintained for three days at FTSW 0.30, and 0.10. Samplings have been performed on 41, 42 and 44 DAE, respectively.

3.3 Determination of soil available water content

Hydraulic characteristics of zeolite and soil were determined as follows: soil moisture at field capacity (FC) was determined for each pot reaching water saturation, and wilting point (WP), defined as the water content not available to the plant, was calculated in a previous dry-down experiment. The single-pot FC values and the mean WP value (90.8g±5.08; 1000g±3.24 for plants cultivated in zeolite and terreno, respectively) obtained were used to determine the Fraction of Transpirable Soil Water (FTSW).

FTSW value ranges from 1.0 at FC to 0.0 when water became unavailable for transpiration. The FTSW values represent the portion of remaining volumetric soil water available for transpiration on each day of the experiment and were used as the indicator of stress (Ritchie, 1981). FTSW was calculated as: (day *n* pot weight) - (weight at WP)/ (weight at FC)- (weight at WP). Pots were weighed twice a day (for 30 day after emergence (DAE) in growth chamber and for 44 DAE in green house) to monitor daily water transpiration rate, expressed as g of water loss/day for each plant. This approach permitted to identify FTSW values at which plant transpiration decreased, indicating the beginning and subsequent phases of water deficit stress.

Measurement of photosynthetic parameters

Gas exchange characteristics were measured on young fully-expanded leaves of seedlings of both stressed and non-stressed treatments using a CIRAS-2 portable photosynthesis system (PP System, Amesbury, USA). Measurements were initiated when CO_2 concentration in the leaf chamber approached ambient concentration and the photosynthetic active radiation (PAR) reached the value of 600 µmol photons m^{-2} s^{-1}. Net photosynthetic rate (Pn) was calculated in IS19453, Keller, Mpwekwa genotypes in a previous dry down experiment.

3.4 Microarray construction and analysis

20331 unique TC sequences of sorghum, including 509 relative to drought stress, were downloaded from the public database plant gene index at http://compbio.dfci.harvard.edu/tgi/plant.html (ID numbers from TC111339 to TC134779). In addiction, from the same database were selected sequences of rice and maize (313 (ID from TC284308 to TC360769)) and 1021 (ID from TC458185 to TC570293 and other IDs starting with different letters), respectively representing genes involved in response to drought but not present in Sorghum database. These were obtained after BLAST (basic local alignment search tool) of rice and maize water stress genes sequences against those of sorghum. Sequences with absent or low homology with the sorghum ones were chosen. All the sequences obtained were sent to NimbleGen Company for probes synthesis and microarray assembly. NimbleGen technology produces very dense array, 1620k spots. Each 12X135K NimbleGen array was divided into 12 chambers, containing 21,665 probes, 13 negative controls (both replicated 6 times) and 10649 positive controls each.

Total RNA was isolated from 0.5g of leaves tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Quality of integrity (absence of degradation) of total RNAs was checked by electrophoretic assay using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA), while quantity was measured using NanoDrop spectrophotometer (Thermo Fisher Scientific Wilmington, DE, USA). In growth chamber experiment, RNA was extracted from 4 biological replicates for each condition (irrigated used as positive control, and not irrigated plants) and for each different level of drought stress (T0.37, T0.25 and T0.15). In green house experiment, RNA was extracted from 3 biological replicates (given the good similarity between replies; Spearman correlation > 0.9) for irrigated and not-irrigated plants for each different level of stress (T0.30, T0.10)

10µg of Total RNA was reverse transcribed to cDNA using the SuperScript Double–Stranded cDNA Synthesis kit (Invitrogen). After adding 100 pmol/µl of oligo dT Primers solution, total RNA was incubated at 70°C for 10 min and then placed on ice for 5 min. The 18 µl final volume of reaction mix included 5X First-Strand Buffer, 0.1 M DTT, 10 mM dNTP Mix. Incubation was performed in a thermocycler for 2 min at 42°C. 2 µl of SuperScriptII RT was added and solution was incubated at 42°C for 60 min obtaining First Strand cDNA. To the first strand reaction, 5X Second Strand Buffer, 10 mMdNTP Mix, 10 U/ µl DNA ligase, 10 U/µl DNA Polymerase, 2 U/µl RNase H, were added to reach a volume of 150 µl and solution was incubated at 16° for 2 hours. 2 µl of 5 U/µl T4 DNA polymerase were added to each reaction and samples incubated at 16°C for 5 min. cDNA was then purified with the RNase A Cleanup protocol (Invitrogen) according to the manufacturer's instruction. Purified cDNA was then precipitated in 500 µl of ice-cold 80% ethanol, centrifuged at 12000 g for 5 min, repeating twice the washing steps. Dried pellet was rehydrated with 20 µl of nuclease-free. The amount and quality of the cDNA were evaluated by NanoDrop spectrophotometer and Bioanalyzer 2100, respectively.

To obtain a higher amount and quality of labelled cDNA, the NimbleGen One-Color DNA Labeling kit was applied. After adding 40 µl of diluted Cy3-Random Nonamers primers, 1 µg of cDNA was denatured in a thermocycler at 98°C for 10 min. The 100 µl final volume of reaction mix included 10 mM dNTP Mix, 50 U/ µl Klenow fragment, 80 µl of cDNA- Cy3-Random Nonamers primers mix. Incubation was performed in a thermocycler for 2 hours at 37°C. The labelled cDNA was then precipitated in 500 µl of ice-cold 80% ethanol, centrifuged at 12000 g for 10 min, repeating twice the washing steps. Dried pellet was rehydrated with 25 µl of nuclease-free water. The amount of Cy3-labeled cDNA was determined by NanoDrop spectrophotometer. 4 µg of Cy3-labeled cDNA were dried in a vacuum concentrator and resuspended in the hybridization solution master mix to a final volume of 12 µl according to the manufacturer's instructions.

The Cy3-labeled cDNA targets denatured at 95°C for 5 min, were hybridized on the glass slide. A coverglass (Mixer, Nimblegen) was positioned on the spotted area and the mixer-slide was put in a hybridization chamber (Hybridization System, Nimblegen). Hybridization was performed at 42°C for 17.5 hours. Washing of hybridized arrays was performed with NimbleGen Wash Buffer kit according to the manufacturer's instruction. After hybridization, slides were disassembled from mixer and washed twice at 42°C with wash solution I for 1 min, once with wash solution II for 1 min, once with wash solution III for 5 sec and spinned for 1 min. Finally, slides were scanned by using Axon Gene pix 4400A scanner (Axon Instruments, Union City, CA, USA) with a resolution of 2µm.

Accurate microarray quantification is best realized when there is a linear relationship between fluorescence and RNA concentration. For any microarray, there are two parameters that define its dynamic range: the range of fluorescence that can be measured and the range of RNA concentrations that can bind to a specific array feature. These two components of the dynamic range reflect the two types of saturation that can occur on a microarray: photomultiplier tube (PMT) saturation and biological saturation. A linear relationship between fluorescence and RNA concentration can only occur if the cDNA on the microarray captures proportional amounts of RNA and if the PMT is not saturated. The PMT measures the number of photons from the fluorescently labeled RNA that are excited by the lasers. PMT was adjusted to obtain a visualized equal intensity of positive control spots across arrays.

All fluorescence intensities data were processed first by Robust Multichip Average (RMA; Irizarry *et al.*, 2003) using NimbleScan Software version 2.6 to obtain normalized gene expression value for each gene (RMA.calls files). Normalized data were processed using the Limma package of R statistical software in order to display differentially expressed genes. *p*-values adjusted lower than 0.05 and induction or repression ratios higher than Log 2-fold change (Log2FC>1) criteria were applied to identify statistically significant differentially expressed genes.

The normalized log FC was used as input for cluster analysis. Significantly expressed genes in at least 1 time of sampling were then hierarchically clustered, with average linkage clustering and Euclidean distance as a measurement of similarity, using TMeV 4.7.1 (Multi Experiment Viewer software, http://www.tm4.org/mev/). Clustering was performed by choosing four clusters of genes using K-means algorithm command.

3.5 qRT-PCR expression analysis

Microarray gene expression results were validated by using qRT-PCR analysis. Eight representative differentially expressed genes, with different behaviour of regulation in the three times of sampling (T0.37, T0.25 and T0.15) in IS19453 genotype, were selected for the validation. The cDNA was synthesized from total RNA following the High-Capacity cDNA Reverse Transcription Kit protocol (Applied Biosystem). Single strand cDNA was determined with fluorimetric assay (Qubit, Invitrogen).

By the software Primer3 http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi, primers designe was carried out for 8 genes to amplify regions not longer than 250 bp (Tab. 2). To have a good amplification of the gene regions, amplicons for real-time qRT-PCR are tipically short 70-250 bp.

The amplification reaction was optimized in a final volume of 25 µl containing 20 ng cDNA, 2X iQ SYBR Green Supermix (Biorad) and 4 µM of each primer. The amplification was carried out in a MiniOpticon device (Biorad) according to the following thermal protocol: 95° C for 3 minutes (1 cycle), 95° C for 10 seconds, 58° C for 20 seconds (35 cycles), melting curve analysis, with an increase in temperature of 0.5° C per second from 60 to 95° C. Three replicates were carried out for each sample tested and Actin1 was used as reference gene to normalize relative quantification. Expression ratio and fold change (FC) were calculated using the $2^{-\Delta\Delta Ct}$ method.

Tab. 2: Primer used for the amplification for Actin 1 (reference gene) and eight differentially expressed genes.

Annotation	Primers	Amplicon (bp)
Sb01g010030 : similar to Actin-1	F 5'-GGCTAACCGTGAGAAGATGAC-3' R 5'-AGGGCGTATCCCTCGTAGAT-3'	129
Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	F 5'-GGAGGAGAAGACGGACACCT-3' R 5'-CCGGAAATCTGGATAGACTTGA-3'	185
Sb09g018420 : similar to Dehydrin DHN1	F 5'-CGAGGGAGGAGCATAAGACC-3' R 5'-GTGCTGGTTGTCCTTGTGG-3'	154
Sb09g022290 : similar to Delta-1-pyrroline-5-carboxylate synthetase (P5CS)	F 5'-AGGCTCCATATGAGGATTCATCT-3' R 5'-CACGTCACTGAGTAGAACAAGAA-3'	109
Sb01g025970 : similar to Proline-rich protein precursor	F 5'-ATGGTAATAGCCGTCGCAAG-3' R 5'-TACTCGCCTTTGCTGTTCCT-3'	158
Sb03g030050 : similar to ABRE-binding factor BZ-1	F 5'-ATTCAGTAAAAGCACCGTCACC-3' R 5'-TGCAATTCCAGGGTTAAAGAAT-3'	128
Sb01g001200 : similar to Cytochrome P450 79A1	F 5'-ATGTGCTCATCACTCTCAAGGA-3' R 5'-GGGTTGTTCACCATCTCTGC-3'	148
Sb01g001180 : similar to Cytochrome P450 71E1	F 5'-ATCCGCAACTACCACTTGAGAT-3' R 5'-GTGTGAGCTGCTTCTAGCTCTG-3'	175
Sb01g001230: similar to Glutathione S-transferase	F 5'-GCCGAGTACCAGATCGTACCTA-3' R 5'-GTACGTACCCCATAGGTGGAGA-3'	129

3.6 MicroRNAs array construction and analysis

All the available sorghum and maize miRNAs sequences were downloaded from miRBase database (http://www.mirbase.org/cgi-bin/sequence_get.pl) and used to realize 12K arrays using Combimatrix technology. Each chip was divided in four chambers of about 2K spots. Each chamber contained 61 mature miRNAs sequences of sorghum and 36 of maize (all redundant sequences were excluded). For each miRNA probe two control probes were designed (2mut|a and 2mut|b) whose sequences were the same of the reference miRNA but presented two mismatches, in order to evaluate specific hybridization. miRNA and mismatch probes were present in seven replicates. Furthermore were spotted 19 degradation controls (to exclude presence of mRNA), 19 smallRNAs positive controls (tRNAs and U6), 7 negative controls. All the controls were replicated two times. The total amount of spots for each chamber was 2127.

The small RNA fraction containing the miRNAs was isolated using *mir*Vana miRNA Isolation Kit (Ambion) from 50µg of total RNA, obtained as described above. Quality of integrity (absence of degradation) of small RNAs was checked by electrophoretic assay using Bioanalyzer 2100 (Agilent Technologies), while quantity was measured using NanoDrop spectrophotometer (Thermo Fisher Scientific). 2µg of small RNAs fraction were labelled with Cy5-ULS dye using ULS microRNA Labelling Kit (Kreatech Diagnostics, Amsterdam, The Netherlands). Hybridization was performed on the same biological replicates of IS19453 genotype used for the gene expression analysis, following Combimatrix instructions (MicroRNA 4x2k Microarray: Hybridization and Imaging Protocol). Hybridized arrays were scanned with GenePix 4000B scanner (Axon Instruments, Union City, CA, USA). Scanning was carried out at 5µm resolution and PMT was adjusted to obtain a visualized equal intensity of positive control spots across arrays. Data were extracted from TIFF images using software GenePix Pro 6.0 (Axon Instruments, Union City, CA, USA). Hybridization data were normalized and processed using the Limma package of R statistical software. Hybridization was considered specific if ratio between each probe and its control probes (mutant probes 2mut|a and 2mut|b) was >1.2. Induction or repression ratios higher than 1.5-fold change (FC) between stressed and control samples (p-value< 0.05) for each time of sampling were Log2 transformed. Log2FC >0.58 and <-0.58 values displayed differentially expressed miRNAs.

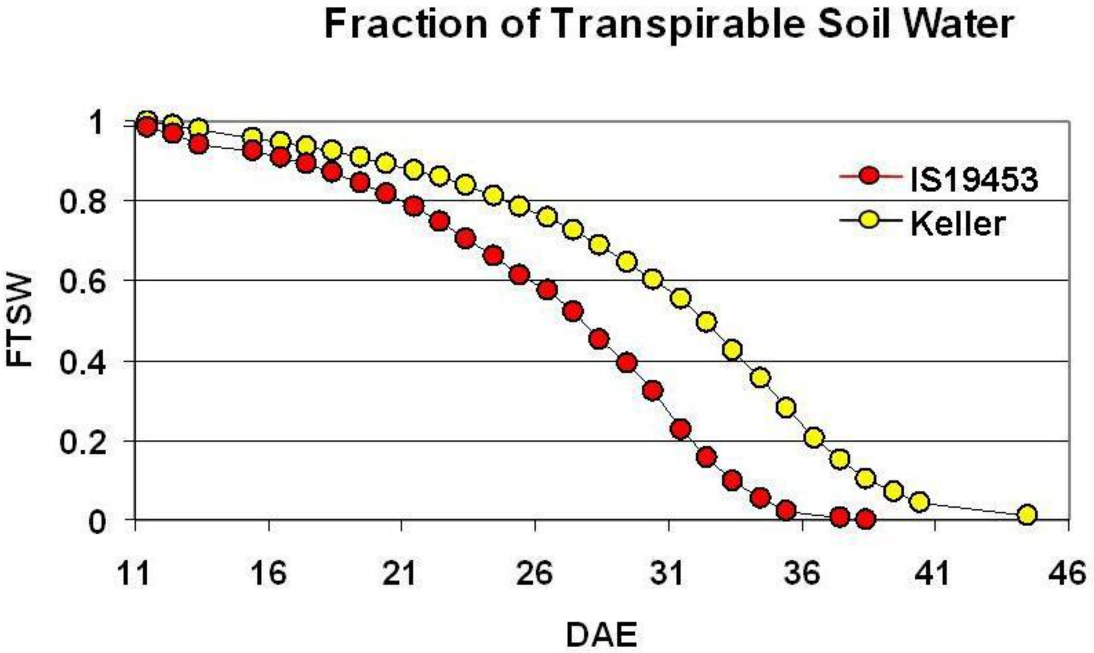
4. Results and Discussion

4.1 Dynamic of water stress and developmental traits in dry down experiments

4.1.1 Growth chamber experiment

In two previous dry-down experiments performed in growth chamber, trend of FTSW from field capacity (FTSW=1) to wilting point (FTSW=0) was calculated in Keller and IS19453 genotypes (Fig2).

Fig. 2: Fraction of Transpirable Soil Water (FTSW) dynamics during dry-down experiments, in relation to Day After Emergence (DAE).

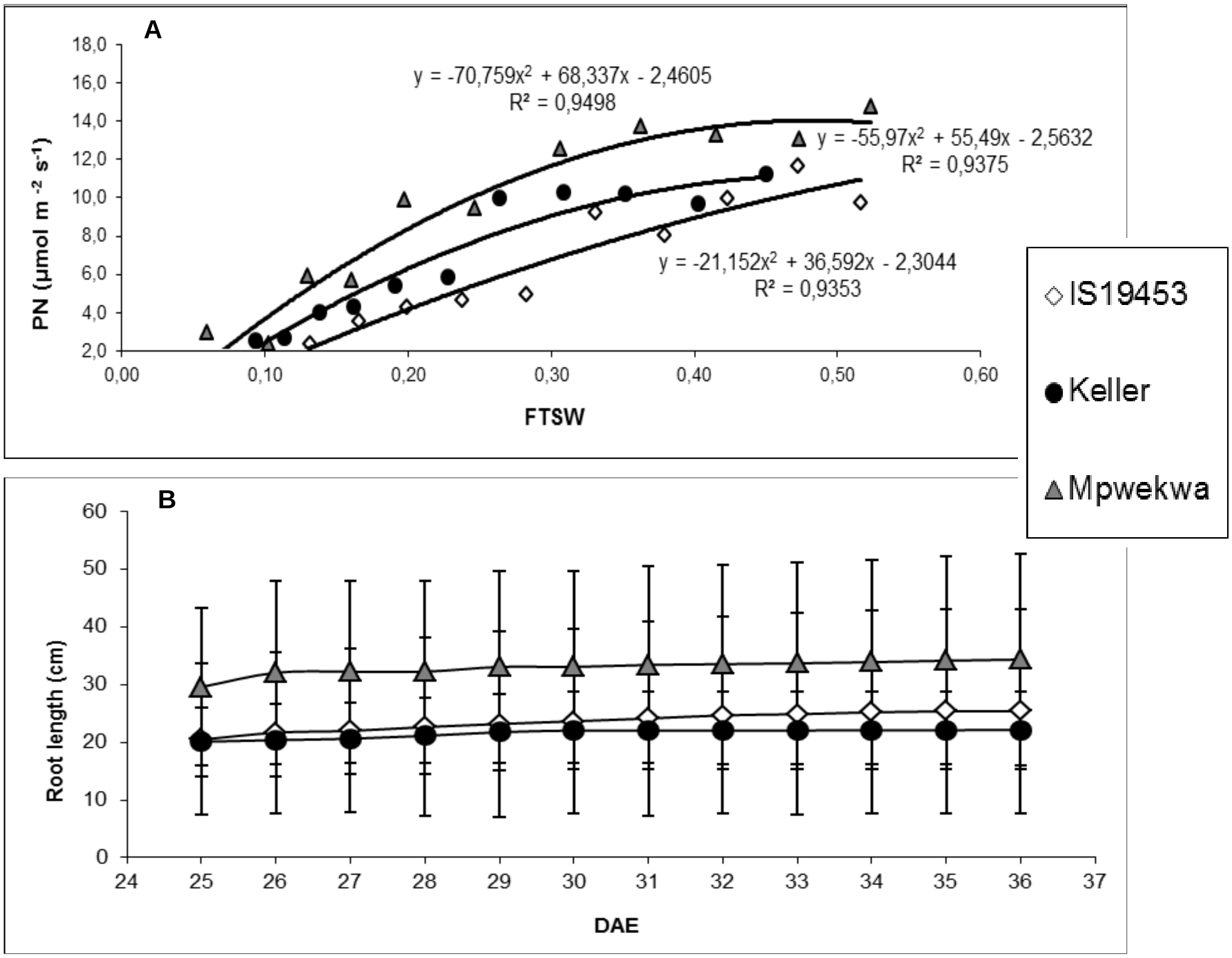


On the basis of previous data, plants from the sorghum genotype IS 19453 and Keller did not show any evident sign of stress until water remained above 0.5 FTSW. Based on data derived from physiological parameters we determined that stress starts when the plant reached the threshold of 50% of available water in the pots, corresponding to about 34 days after emergence (DAE).

In other previous growth chamber dry-down experiment IS19453, Keller, Mpwekwa genotypes were compared on the basis of trend of net photosynthesis in relation to FTSW and root growth during dry down experiment (Fig. 3 A-B, respectively). These data suggests that no significant difference relative to drought tolerance occurred between the

IS19453 and Keller genotypes. Mpwekwa genotype seemed to have the best performance under drought conditions because it presents higher values of net photosynthesis and root growth compared to genotypes IS19453 and Keller.

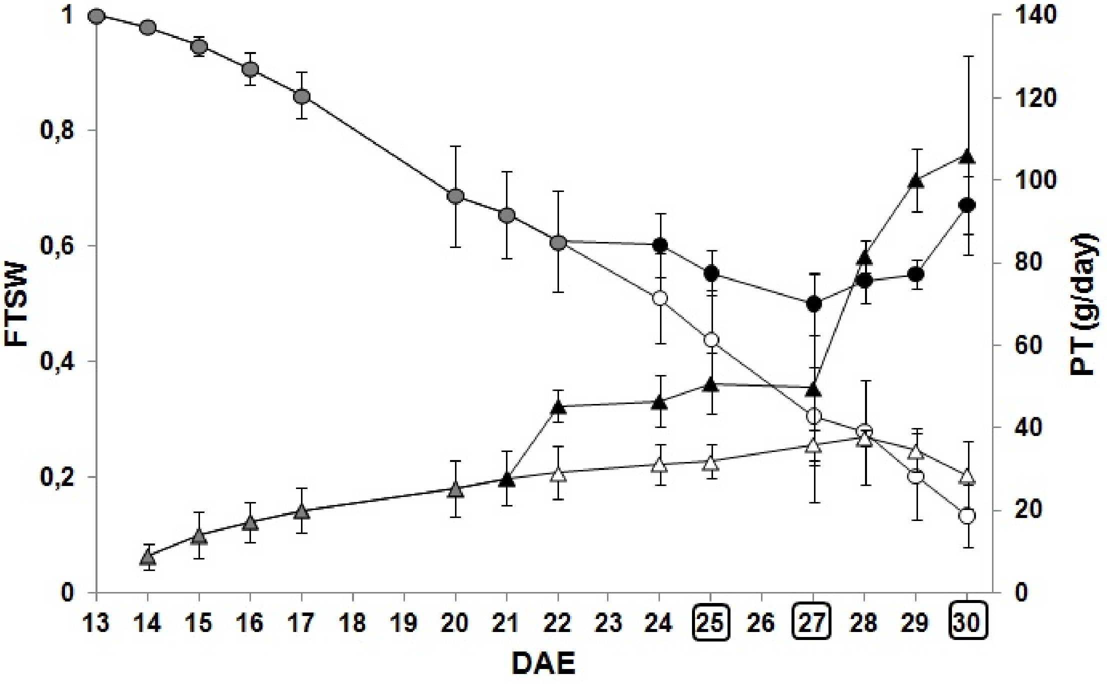
Fig. 3: Trend of net photosynthesis in relation to FTSW (A). For each genotype, trend curve is showed. Root growth during dry down experiment of three genotypes (B).



In growth chamber IS19453 experiment, well watered plants were therefore irrigated to keep transpirable water above the level of 50% of available water, while stressed plants were left to "dry-down". Irrigated plants were maintained at transpirable water content major of 50% (Fig.4, dark points). In order to capture the full range of responses encountered at increasing water stress levels, a set of plants was sampled after FTSW had fallen under 0.5 (on DAE 24) on 25, 27 and 30 DAE when FTSW was on average 0.37, 0.25 and 0.15.

The decreasing of FTSW and the increasing of transpiration rate (PT) in relation to DAE was due to the increment of transpiration leaf surface of the growing plant. PT intersected FTSW at a point, corresponding to 27.8% of transpirable water, which indicated the critical point below which water stress occurred. Observing the dynamics of FTSW in relation to daily PT (Fig. 4), drought stress appeared when plants had consumed 72.2% of transpirable soil water (FTSW=0.28), that corresponded to a subsequent decrease of daily PT. In order to evaluate the effect of water stress, destructive sampling was performed at three different values of FTSW. The mean FTSW values of stressed plant were 0.37, 0.25 and 0.15 sampled at 25, 27 and 30 DAE, respectively.

Fig. 4: Fraction of transpirable soil water (FTSW, left axis) and plant transpiration rate (PT, right axis) dynamics in plants of sweet sorghum IS19453 during a dry-down experiment. Enhanced DAE indicate the sampling points. Gray, black and white circles represent FTSW of plants before stress incoming, of irrigated plants and of stressed plants, respectively. Gray, black and white triangles indicate PT trend of plants before stress incoming, of irrigated plants and of stressed plants, respectively.



Statistical analysis confirmed that FTSW in the stressed plants decreased significantly at increasing DAE and of course it was always lower than in well watered plants. Daily PT significantly increased with increasing DAE in watered plants, indicating that water availability was not limiting leaf expansion and transpiration, while it did not show a significant increase in the stressed plants (Tab.3).

Statistical analysis revealed significant differences for FTSW both between DAE and stressed and well watered samples (Tab.3). The interactions between water treatment and DAE showed high differences among stressed plants in terms of FTSW. The PT rate significantly increased with DAE in watered plants while it remained at about 34 g/day in the stressed ones.

Significant differences in LA were identified at each sampling time between irrigated and stressed plants, the former showing a progressive increase in leaf area and leaves number, while the latter had a significant slowdown in LA increase from DAE 25 and 27 and a LA decrease from DAE 27 to 30 due to leaf senescence. Particularly evident is the onset of stress considering the transpiration per unit of leaf surface that drops by almost 30% from DAE 25 to 27 while it stays constant in well watered plants in the same period (Tab. 3). Similar behaviour was observed for root dry mass (RDM). RDM of IS19453 was significantly ($P<0.01$) reduced at high drought stress level (Tab. 3).

Significant differences in LA were identified at each time of sampling between irrigated and stressed plants (Tab. 3). It can be noted that LA increased in not watered samples at 27 DAE. At this point the transpiration rate was still increasing, showing that plant could grow also in presence of limited water amounts (FTSW 0.25). Stressed samples at 30 DAE showed evident decreasing of LA and PT. This could be explained by the presence of senescent leaves due to the severe water stress conditions.

The number of emerged leaves (EL) did not show significant differences between irrigated and stressed plants until 27 DAE. After, EL number progressively increased in watered samples, while it remained about the same in stressed samples, indicating a reduced growth at high level of stress.

Tab. 3: FTSW, LA, RDM, PT, EL, of IS19453 under wet and dry soil conditions at T 0.37, T 0.25, T 0.15 in growth chamber conditions. All parameters were determined at 25-27-30 DAE, respectively. Means ± SE, n=4. Values within a column followed by the same letter are not significantly different according to LSD test at the 1% probability level.

	FTSW	**LA**	**RDM**	**PT**	**EL**
25 DAE - stressed	0.368 ± 0.016^{C}	212.150 ± 31.399^{D}	1.007 ± 0.207^{C}	34.375 ± 5.542^{C}	9.750 ± 0.228^{C}
25 DAE - watered	0.548 ± 0.016 ^{B}	357.875 ± 31.399^{BC}	1.110 ± 0.207^{BC}	50.950 ± 5.542^{BC}	10.250 ± 0.228^{BC}
27 DAE - stressed	0.250 ± 0.016^{D}	318.400 ± 31.399^{CD}	1.887 ± 0.207^{B}	36.158 ± 5.542^{C}	10,000 ± 0.228^{BC}
27 DAE - watered	0.530 ± 0.016^{B}	477.700 ± 31.399^{B}	1.723 ± 0.207^{BC}	69.162 ± 5.542^{B}	10.750 ± 0.228^{B}
30 DAE - stressed	0.155 ± 0.016^{E}	276.225 ± 31.399^{CD}	1.262 ± 0.207 ^{BC}	31.800 ± 5.542^{C}	10.250 ± 0.228^{BC}
30 DAE - watered	0.673 ± 0.016^{A}	770.050 ± 31.399^{A}	2.870 ± 0.207^{A}	92.490 ± 5.542^{A}	12.250 ± 0.228^{A}

4.1.2 Green house experiment

In order to capture the full range of responses encountered at increasing water stress levels, 3 stressed plants per genotype was sampled after FTSW had fallen under 0.5 on 23, 24 and 26 DAE when FTSW was on average 0.30.

Up to the 41st DAE, in all genotypes is observed a direct proportionality between the percentage of available water and transpiration rate. A decreasing of FTSW corresponds to a decrease in the rate of transpiration; FTSW decreases progressively and reached steady values of 0.3.

In early stage of stress BR505, Mpwekwa, BR501 genotypes showed increased values of transpiration (Fig. 5 B-C-D), while IS33350 and SDS19483 showed a decrease in transpiration rate (Fig. 5 A-E).

The maintenance of water stress to a value of FTSW equal to 0.3 showed different defense strategies in the analyzed genotypes.

IS33350 genotype transpires an average of 603 gr of water per day during the phase of prolonged stress and the 42-43nd day transpiration rate appears stable.

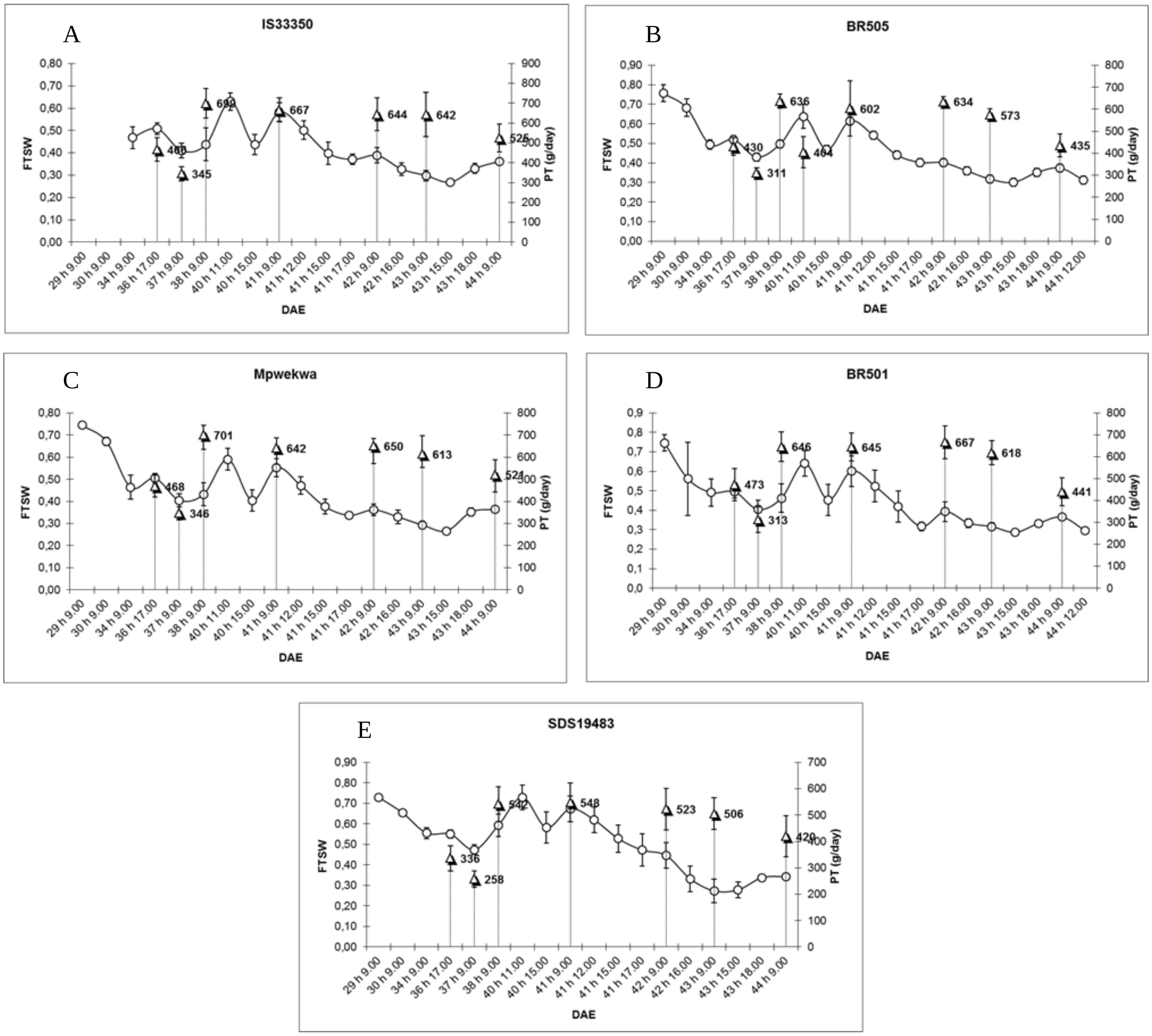
BR505, Mpwekwa, BR501, SDS19483 transpire an average of 547, 596, 575, 483 g per day, respectively. In theese genotypes, transpiration rate decreases progressively during the period of stress.

SDS19483 presents values of transpiration lower when compared with the values of the other 4 genotypes, this could be explained because its tillering failure and consequently to a lower breathables surface.

Commonality observed in plant transpiration profiles among different genotypes indicated that the response of sorghum to water stress started when FTSW value corresponding to 30% of transpirable water. This behavior was observed in genotype IS19453 and later found in the greenhouse experiment where plants reached a phenological stage advanced.

In all analyzed genotypes it has been found a good tolerance to water stress. In fact, in relation to the progressive decrease of the available water, it has not been found a sudden decrease in transpiration rate.

Fig. 5: Fraction of transpirable soil water (FTSW, left axis) and plant transpiration rate (PT, right axis) dynamics in plants of sweet sorghum IS33350, BR505, Mpwekwa, BR501, SDS19483 during a green house dry-down experiment. White circles represent FTSW of plants in stress condition. White triangles indicate PT trend of plants during stress.



4.2 Identification of differentially expressed genes under drought stress in growth chamber

The number of differentially expressed genes between stressed and control plants increased with the intensity of the water stress: 113, 418 and 1205 genes resulted differentially expressed with p-value <0.05 in 0.37, 0.25 and 0.15, respectively (Tab. 4).

Tab. 4: Functional categories and number of up and down-regulated genes in sweet sorghum IS19453 leaves sampled at three different FTSW values during a dry-down experiment.

Functional Category	**0.37**			**0.25**			**0.15**		
	up	**down**	**total**	**up**	**down**	**total**	**up**	**down**	**total**
Cell rescue, defence and virulence	16	10	26	31	32	63	130	46	176
Lipid metabolism	2	/	2	7	6	13	42	7	49
Aminoacid metabolism	/	4	4	5	13	18	31	2	33
Sugar metabolism	1	12	13	8	29	37	53	47	100
Transport	3	2	5	9	21	30	78	20	98
Protein fate	2	3	5	7	12	19	45	15	60
Nucleotide binding	/	1	1	6	14	20	17	12	29
Nucleic acid binding	1	14	15	19	26	45	66	34	100
Communication and signalling	1	6	7	9	28	37	69	35	104
Cell cycle-DNA processing	/	4	4	2	3	5	17	2	19
Interaction with the environment	1	2	3	5	3	8	15	4	19
Systemic interaction with the environment	1	1	2	/	1	1	7	2	9
Others	/	1	1	2	7	9	21	11	32
Unknown	9	16	25	44	69	113	299	78	377
Total	37	76	113	154	264	418	890	315	1205

The number of down-regulated genes was greater respect the up-regulated ones at the beginning of stress (76 against 37 and 264 against 154 at 0.37 and 0.25 FTSW, respectively) while at FTSW 0.15 the up-regulated genes were 890 against 315 down-regulated (Tab. 4).

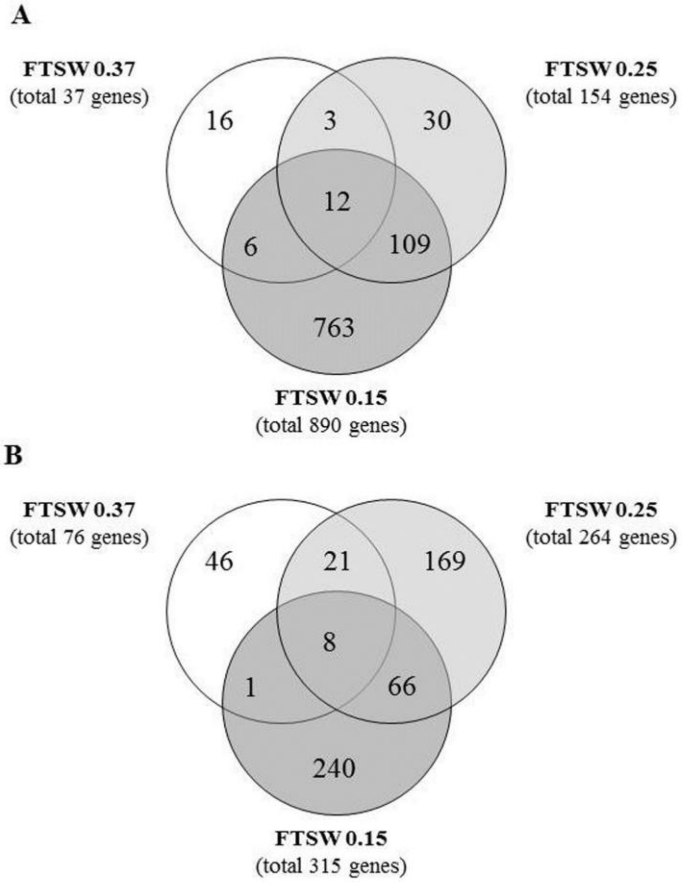
The differentially expressed sequences were classified into 12 functional categories according to the Munich Information Center for Protein Sequences (MIPS) FunCat database. “Others”, which included genes involved in the protein synthesis, cell fate, energy, subcellular localization and storage protein.

About 28% in average of differentially expressed genes were unknown or not annotated.

Genes represented by the 12 functional categories cited above increased their number as the water stress level increased. In early stages of water deprivation (FTSW 0.37) sorghum plants started to activate genes regulation mechanisms that showed an elevate grade of complexity when water stress reached higher levels (FTSW 0.15) near to wilting point. The functional class “Cell rescue, defence and virulence” was the most represented in all phases of stress but also genes involved in other pathways, such as lipid and sugar metabolism, communication and signalling, regulation of transcription became important in the plant response to water deprivation.

As shown by the Venn diagram, 37, 154, and 890 genes were induced at the stress level of FTSW 0.37, 0.25, 0.15, respectively (Fig. 6A; Tab. 4). Twelve genes were induced at all three times of sampling, including genes involved in defence, transport, signalling and interaction with environment (Fig. 6A; Tab. 4). Only three genes were in common between FTSW 0.37 and 0.25, 109 genes were common at FTSW 0.25 and 0.15, and 6 genes were shared between FTSW 0.37 and 0.15, respectively (Fig. 6A). Likewise, 76, 264, and 315 genes were down-regulated at FTSW of 0.37, 0.25, 0.15, respectively (Fig. 6B). Eight genes encoding for nucleic acid binding-related proteins, transporters, aminoacid metabolism enzymes and signalling and defence proteins were silenced during the time course of experiment (Fig. 6B). Commonality observed in gene expression profiles among different levels of water stress indicated that the response of IS19453 genotype to water stress started to be intense between FTSW 0.25 and FTSW 0.15.

Fig. 6: Venn diagrams showing numbers of overlapping and unique genes induced (A) and repressed (B) at 0.37, 0.25 and 0.15 FTSW in leaves of sweet sorghum IS19453. Results based on mean of four biological replicates considering 2-fold or higher fold change (Log_2FC).



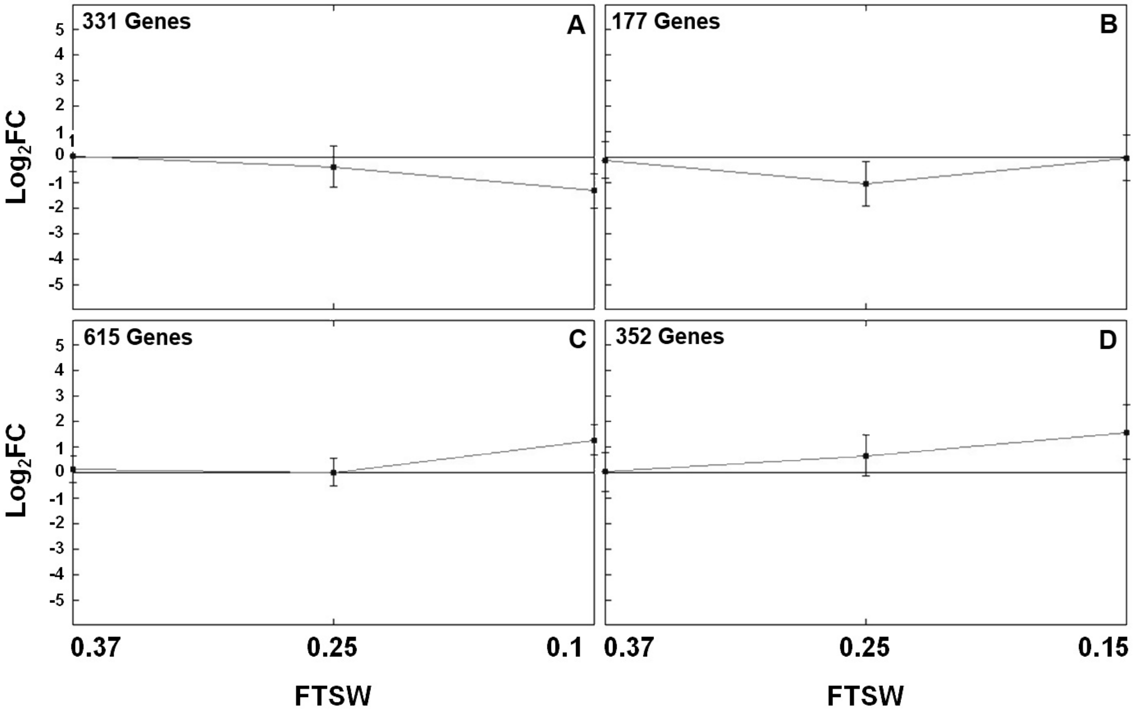
Among the 22 genes always differentially regulated at T0.37, T0.25, T0.15, 4 probes identified two genes, Sb01g040000 and Sb03g003530, encoding for some smHSPs that were up- regulated at the beginning of the drought stress and under-regulated thereafter (Tab. 5).

Tab. 5: Differentially regulated genes in sorghum IS19453 at three different level of FTSW 0.37, 0.25 and 0.15.

ID	**Annotation**	**Log_2 FC 0.37**	**Log_2FC 0.25**	**Log_2FC 0.15**	**Functional class**
BG837255	Sb01g040000.1: similar to 17.4 kDa class I heat shock protein 3	2.02	-1.29	-3.35	Cell rescue defence and virulence
TC466646	Sb03g003530: similar to 17.8 kDa class II heat shock protein	1.86	-1.49	-1.47	Cell rescue defence and virulence
EB705607	Sb03g003530: similar to 17.8 kDa class II heat shock protein	1.84	-1.35	-1.69	Cell rescue defence and virulence
TC134071	Sb01g001230: similar to Glutathione S transferase 1	1.46	1.27	2.14	Cell rescue defence and virulence
TC114851	Sb10g000890: hydrolase activity	1.44	1.64	1.75	Cell rescue defence and virulence
TC481803	Sb01g040000.1: similar to 17.4 kDa class I heat shock protein 3	1.35	-1.17	-1.98	Cell rescue defence and virulence
TC113948	Sb01g016900: Belongs to the cytochrome P450 family	1.30	1.70	2.56	Cell rescue defence and virulence
TC129311	Sb01g001230: Glutathione S transferase 1	1.29	1.09	1.79	Cell rescue defence and virulence
TC111396	Sb01g001200: similar to Cytochrome P450 79A1	1.24	1.73	2.99	Cell rescue defence and virulence
TC128001	Sb01g036770: Chaperone - similar to Cluster: DnaJ domain containing protein; Oryza sativa	-1.15	-2.43	-1.42	Cell rescue defence and virulence
TC116451	Sb02g003530: similar to LOC_Os02g20360 : tyrosine aminotransferase	-1.39	-1.22	-1.19	Aminoacid metabolism
TC132971	Sb08g023000: similar to Secretory acid phosphatase - Pfam Calcineurin-like phosphoesterase	1.04	1.05	3.56	Communication and signalling
TC123792	Sb06g029810: similar to LOC_Os04g54190: cysteine-rich receptor-like protein kinase 8 precursor	-1.36	-1.28	-1.09	Communication and signalling
TC123989	Sb03g024480: Contains 1 SPX domain (protein involved in vacuolar phosphate accumulation)	1.39	1.91	1.86	Interaction with the environment
TC133171	Sb01g025970: similar to Proline-rich protein precursor	-2.18	-4.16	-3.84	Interaction with the environment
TC131066	Sb01g045060: similar to AP2 domain containing protein	-1.50	-3.31	-2.01	Nucleic acid binding
TC125997	Sb01g045060: similar to AP2 domain containing protein	-1.62	-3.17	-1.90	Nucleic acid binding
TC123919	Sb09g001020: response to wounding - serine-type endopeptidase inhibitor activity	1.73	1.46	1.87	Protein fate
TC115469	Sb01g044010: similar to Mannitol transporter	1.29	1.11	1.26	Transport
TC126132	Sb02g036370: similar to Putative organic cation transporter	-1.88	-2.82	-1.18	Transport
TC131680	Sb05g016820: similar to Expressed protein	1.41	1.57	1.76	Unknown
TC121323	Sb02g004630: Putative uncharacterized protein; Oryza sativa Indica Group	1.29	1.02	1.86	Unknown
TC120484	Sb06g015140: similiar to Os04g0403600 protein	1.17	2.14	2.13	Unknown
TC123048	Similar to Cluster: Putative uncharacterized protein; Oryza sativa	-1.07	-1.32	-1.13	Unknown

All differentially expressed genes identified in IS19453 at three different levels of drought stress (FTSW 0.37, 0.25 and 0.15) were clustered. 1475 genes resulting differentially expressed at least in one of the considered stress level, were processed. These genes were grouped in four different clusters, each showing a specific trend at the increasing of water stress (Fig.7). In detail, 331 probes exhibited a progressive decreasing expression level with a marked down regulation at FTSW 0.15 (cluster 1 in Fig.7A), 177 probes showed pronounced down-regulation at FTSW 0.25 (cluster 2 Fig. 7B), 615 probes yielded no specific profiles until FTSW 0.25 while an increasing of expression is observed at maximum level of stress (cluster 3 in Fig. 7C), 352 genes showed a progressive increment in expression level at the increasing of stress (cluster 4 in Fig. 7D).

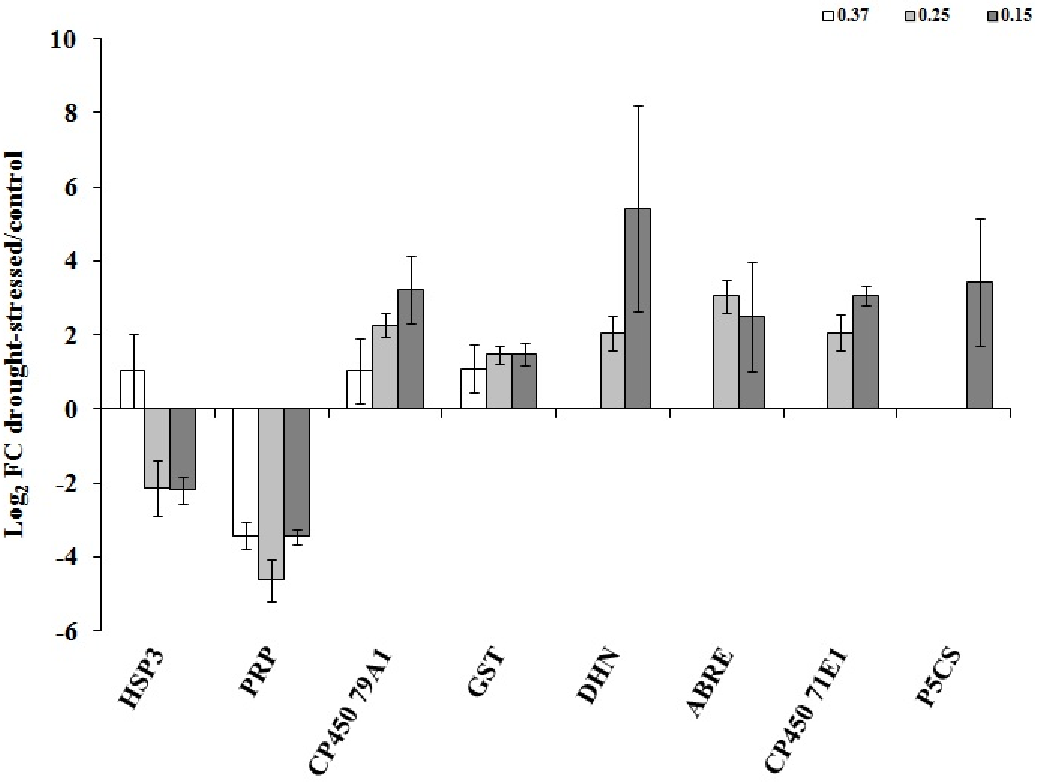
Fig. 7: Cluster analysis of gene expression profiles of sweet sorghum 19453, based on the mean Log_2FC, at three water stress levels (0.37, 0.25, 0.15 of FTSW). Four different clusters were detected: A) 331 genes decreased progressively their expression level; B) 177 genes exhibited high down-regulation at FTSW 0.25; C) 615 genes showed significant increasing of their expression at maximum level of stress; D) 352 genes progressively incremented their expression level at the increasing of water stress. Vertical bars represent standard deviation.



4.2.1 Validation of representative drought-regulated genes

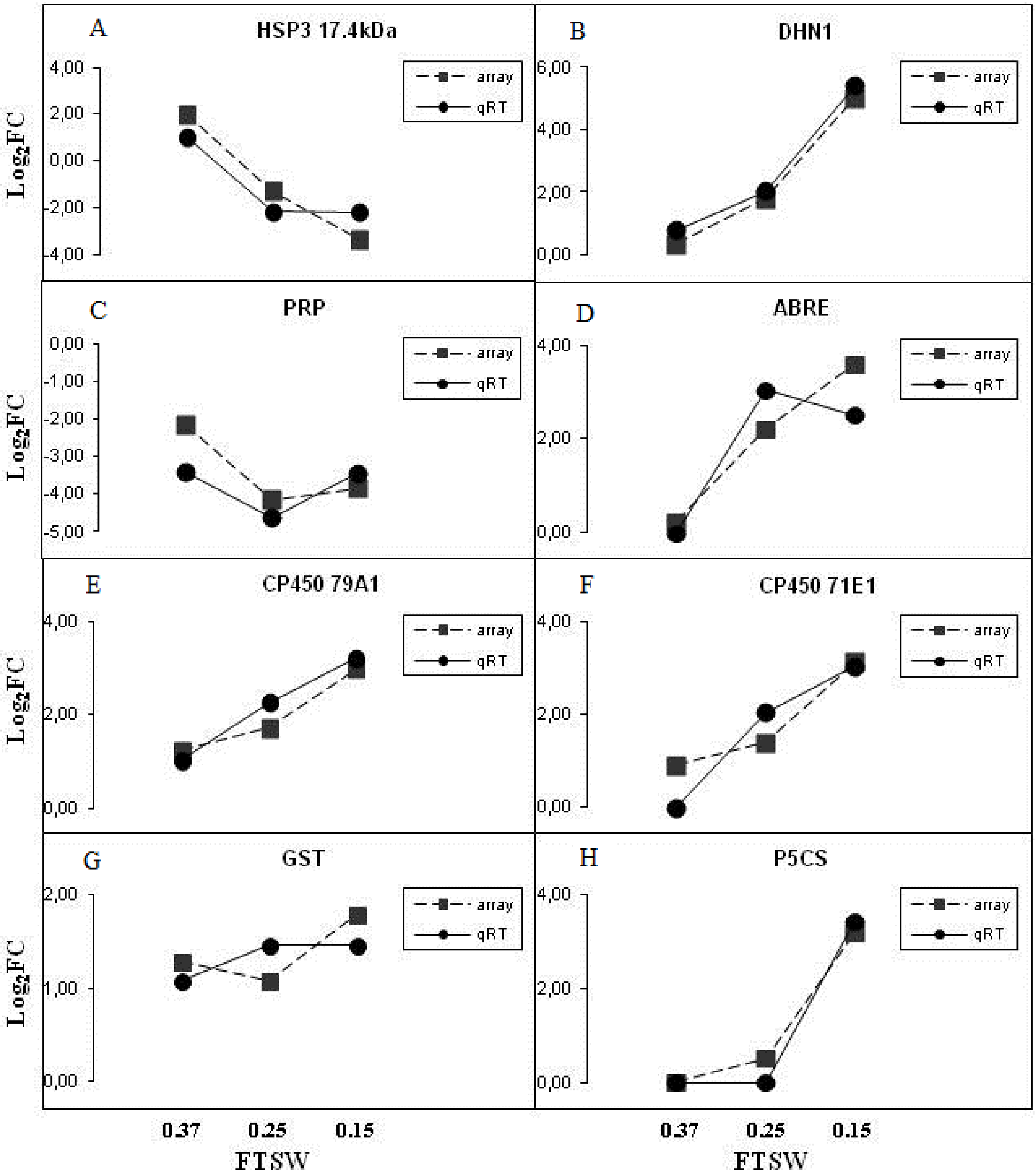
Differentially expressed genes isolated by the microarray analysis were validated using qRT-PCR (Fig. 8). The analysis was carried out with leaves collected at three drought stress levels, FTSW 0.37, 0.25 and 0.15, for the following eight genes: HSP3 (Heat Shock Protein 3), PRP (Prolin Reach Protein), Cytochrome P450 (CP450) 79A1 and 71E1, GST (Glutathione S Transferase), ABRE (ABA- Responsive Element), DHN1 (Dehydrin 1), P5CS (delta 1-Pirrolyne-5-Carboxylate Synthetase; Fig. 8). HSP3 was selected for its peculiar behavior in the three stress conditions: up-regulated at the beginning of stress and down-regulated when the stress became severe; PRP precursor was down-regulated at all the three stages of stress; cytochrome P450 79A1 and GST1 were always up-regulated in all phases of stress samples. Cytochrome P450 71E1, DHN1 and ABRE started to be expressed at FTSW 0.25 and increased their Log$_2$FC at T0.15; P5CS resulted strongly up-regulated only at FTSW 0.15.

Fig. 8: Logarithm of fold change (Log$_2$FC) ratios for eight differentially expressed drought-related genes at three levels of stress (0.37 ☐, 0.25 ▨and 0.15 ■FTSW) in leaves of sweet sorghum IS19453.



In general the qRT-PCR analysis confirmed the results obtained with the microarray analysis, with similar Log_2FC values, indicating the high sensitivity of the high density microarray technology (Fig.9).

Fig. 9: Expression profile of eight differential drought-related genes at three different value of FTSW. Log_2 FC values were obtained by using both microarray (dotted lines with square) and qRT-PCR (line with circles) techniques.



Differentially expressed genes isolated by the microarray analysis were validated using the qRT-PCR on the IS19453 genotype during drought stress treatment (Fig. 9, A-H).

Microarray hybridization revealed an up-regulation of the Heat Shock Protein 17.4 KDa at FTSW 0.37 with respect to FTSW 0.25 and FTSW 0.15. Fig. 9A shows the up-regulation of HSP during first stress phase by qRT-PCR, also confirming a similar degree of change to that obtained by microarray analyses. The expression profile of DHN1 (Fig. 9B) showed an increase starting from FTSW 0.37 to the full drought stress in both analyses. PRP was also down-regulated in all stress levels. In particular, Fig. 9C shows an increasing PRP trend with the same degree of change for both qRT-PCR and microarray experiments.

For the expression profiles of ABRE (Fig.9D), an almost regular increase was noted during drought treatment based on both the microarray and qRT-PCR analyses. In Fig 9 E-F Array and qRT-PCR data showed a progressive induction of two CP450 families.

The expression profiles of GST (Fig. 9G) were similar; an almost increase was noted during stress treatment based on both the microarray and qRT-PCR analyses. Finally, the expression profile of P5CS (Fig. 9H) showed an increase starting from stage FTSW0.37 to the full stress in both analyses.

4.2.2 Functional categories of genes differentially regulated at different drought stress levels

Genes modulated at FTWS level 0.37.

During the earlier phase of water stress 113 differentially expressed genes were detected and included in four functional classes: cell rescue, defence and virulence (26 genes), sugar metabolism (13), nucleic acid binding (15) and communication and signalling (7). In non irrigated plants, genes involved in cell defence represented the most abundant part of the up-regulated (16 out of 37), while genes classified in the others functional categories were prevalently down-regulated (Tab. 4).

The up-regulated cell defence genes encoded for the following enzymes and proteins: small heat shock protein family (smHSPs), glutathione S-transferase 1, terpene synthase

2, cytochrome P450 family, aldo/keto reductase family proteins and others oxidoreductase enzymes (Tab. 6).

Two sorghum genes, (Sb01g040000, Sb03g003530) encoding for smHSPs of 17.4 and 17.8 kDa, respectively, were identified. The up-regulation of smHSPs in response to water stress and the effect of smHSPs over-expression on stress tolerance were reported for several species (Sun *et al*., 2001; Torres *et al*., 2007; Sato *et al*., 2008). Five separate gene families encode mitochondrion, plastid, peroxisomal, nuclear and endoplasmic reticulum-localized smHsps, each with appropriate organelle targeting signals (Waters *et al*., 1996, Scharf *et al*., 2001). smHSPs are divided into several classes and they are implyied in biotic/abiotic stress. The evolutionary expansion of the plant smHsp family may be the result of selection pressure for tolerance to the many types of stresses encountered by plants, including drought and water stresses. Jordano and colleagues (Almoguera *et al.,* 1993; Coca *et al.,* 1994) have reported that certain smHSPs in sunflower are also regulated in response to water/desiccation stress in leaves: mRNA and protein of specific cytosolic smHsp tipe I-II accumulated in specific tissues of water stressed sunflower plants in the absence of heat stress.

Enzymatic antioxidant systems such as glutathione S-transferase (GST) and its metabolites provide protection against the toxic effects of ROS (reactive oxygen species). Modulation in the activities of this enzyme may be an important factor in tolerance of various plants to environmental stress (Smirnoff, 2005). The Sb01g001230 gene, encoding for GST1, included in the Phi class of GSTs, resulted up-regulated at FTSW 0.37 (Log_2FC 1.4) and the following stages (Log_2FC of 1.2 and 2.1 at T0.25 and 0.15, respectively). A recent genome-wide study identified and characterized all GST genes encoded by sorghum and other nine plant genomes, and confirmed the functions of Tau and Phi classes of GSTs in biotic and abiotic stress-related developmental processes, demonstrating also that plant GSTs may be involved in the sugar-related signalling pathway (Chi *et al*., 2011).

Increased expression of Sb01g044010 gene, encoding for a mannitol transporter, could indicate presence of mannitol accumulation mechanisms. Mannitol accumulation in both the cytosol and the vacuole might be necessary to balance the external water potential; mannitol might accumulate in the vacuole and be redistributed to the cytosol in response to stress. In addition, studies with plasma membrane vesicles have provided evidence for the existence of an active proton-cotransporting mannitol carrier in the plasma membrane (Salmon *et al*, 1995).

This metabolite is known to enhance tolerance to water deficit in some higher plants even if it's not well clear in which manner (Ashraf, 2010). Furthermore, the expression level of this transporter remained high also in more stressed samples at FTSW 0.25 and 0.15. Further research is necessary to gain more insight into the mechanisms of intracellular mannitol transport and the function of mannitol as a compatible solute in response to stress.

Sugar metabolism-related enzymes like isocitrate lyase, malate synthase (glioxysomal) and alpha-amylase, transcription factors such as AP2 containing proteins, MYBs and others nucleic acid binding proteins, protein kinases and others factors involved in signalling and cell communications, appeared repressed in not watered samples (Tab.6).

Four down-regulated genes presented Log_2FC values >2 and included Sb01g25970, encoding for a proline-rich protein (PRP) precursor (Tab.6). This gene was down-regulated at higher stress levels (T0.25, T0.15; Tab. 5, Tab. 7 and 8). Synthesis of PRPs, structural proteins of cell wall in plants, is sensitive to external stimuli and is modulated during development (Gothandam *et al*., 2010). In general these proteins are induced by stress although in our study PRP precursors accumulated in control plants (Gothandam *et al*., 2010; Lee *et al*., 2005; Peng *et al*., 2011). It was reported that loss of function of a PRP induced resistance to fungal disease in rice (Fukuoka *et al*., 2009).

In Soybean, high ABA levels may indirectly down-regulate expression of GPRP1, 3 and 4 genes by repressing the production or activity of transcription activators of them. Alternatively, ABA signal could directly down-regulate expression of these genes by increasing the production or activity of transcription repressors of them, since putative

ABRE exist in promoters of GPRP1, 3 and 4 genes (Peng *et al*., 2011). It is well known that the plant response to abiotic stresses involves both ABA-dependent and ABA-independent signalling pathways (Yamaguchi- Shinozaki and Shinozaki, 2006). Down regulation of the gene encoding for PRP was the result of an ABA independent pathway: ABA signals were not detected.

Tab. 6: Up and Down-regulated genes in sorghum IS19453 genotype at FTSW 0.37.

	UP-REGULATED		
ID	**Annotation**	**Log_2FC**	**Functional class**
TC115664	Sb03g003530: similar to 17.8 kDa class II heat shock protein	2.15	Cell rescue defence and virulence
BG837255	Sb01g040000.1: similar to 17.4 kDa class I heat shock protein 3	2.02	
TC466646	Sb03g003530: similar to 17.8 kDa class II heat shock protein	1.86	
EB705607	Sb03g003530: similar to 17.8 kDa class II heat shock protein	1.84	
TC134071	Sb01g001230: similar to Glutathione S transferase 1	1.46	
TC120398	Sb04g001810: similar to Terpene synthase 2	1.42	
TC111696	Sb04g001810: similar to Terpene synthase 2	1.39	
TC481803	Sb01g040000.1: similar to 17.4 kDa class I heat shock protein 3	1.35	
TC113948	Sb01g016900: Belongs to the cytochrome P450 family	1.30	
TC129311	Sb01g001230: Glutathione S transferase 1	1.29	
TC111396	Sb01g001200: similar to Cytochrome P450 79A1	1.24	
TC124003	Sb02g025850: similar to Putative uncharacterized protein; flavonoid 3 monooxygenase (Zea mays)	1.20	
TC117441	Sb03g038880: similar to protein LOC_Os01g61610.3: flavonol synthase/flavanone 3 hydroxylase	1.09	
TC124601	Sb02g002750: similar to Os07g0143000 protein - Aldo/keto reductase family	1.06	
TC112977	Sb03g041600: similar to Os01g0878800 protein - oxidation reduction	1.04	
TC114217	Sb06g021980: similar to Aldo/keto reductase family like protein	1.03	
TC130582	Sb05g026990: weakly similar to Patatin like protein	1.35	Lipid metabolism
TC119732	Sb02g029200: similar to Acyl-[acyl-carrier protein] thioesterase	1.00	
TC127341	Sb02g007740: similar to Beta -glucosidase aggregating factor	2.00	Sugar metabolism
TC115469	Sb01g044010: similar to Mannitol transporter	1.29	Transport
TC112064	Sb01g044010: similar to Mannitol transporter	1.07	
TC131052	Sb04g026650: similar to Aquaporin PIP2-3	1.03	
TC123919	Sb09g001020: response to wounding serine-type endopeptidase inhibitor activity	1.73	Protein fate
TC116483	Sb10g012160: similar to Putative protease	1.30	
TC130886	similar to Cluster: SPLAYED splice variant, Arabidopsis thaliana - regulation of transcription, DNA dependent	1.31	Nucleic acid binding
TC132971	Sb08g023000: similar to Secretory acid phosphatase - Pfam Calcineurin-like phosphoesterase	1.04	Communication and signalling
TC123989	Sb03g024480: Contains 1 SPX domain	1.39	Interaction with the environment
TC127990	Sb01g032020: similar to Probable indole 3 acetic acid amido synthetase GH3.8 auxin responsive promoter	1.34	Systemic interaction with the environment

TC121086	Sb01g009370: similar to Putative uncharacterized protein - peptidase, M50 family mRNA maize 96%	1.98	Unknown
TC114851	Sb10g000890: hydrolase activity	1.44	
TC131680	Sb05g016820: similar to Expressed protein	1.41	
TC121323	Sb02g004630: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.29	
TC119370	Sb03g043420: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.19	
TC120605	Sb03g025455.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.19	
TC120484	Sb06g015140 similiar to Os04g0403600 protein	1.17	
TC119888	Sb04g029940 : similar to Putative uncharacterized protein	1.08	
TC124775	similar to Cluster: O-methyltransferase family protein; Oryza sativa Japonica Group|Rep:, partial (65%)	1.03	

DOWN-REGULATED

ID	Annotation	Log_2 FC	Functional class
TC127266	Sb02g031940 : similar to Flavin containing monooxygenase 3-like	2.76	Cell rescue, defence and virulence
TC130533	Sb03g027330 : similar to Heat shock-like protein	2.10	
TC111971	Sb09g002260: similar to Putative isovaleryl-CoA dehydrogenase	2.00	
TC128623	Sb10g028700 : similar to CMO protein - oxidation-reduction process - 2 iron, 2 sulfur cluster binding	1.63	
TC120734	Sb05g019180: Terpene synthase family, metal binding domain containing protein	1.58	
TC119678	Sb0183s002030 Pfam: cytochrome P450	1.51	
TC124920	Sb05g019190 : similar to Terpene synthase family, metal binding domain containing protein	1.47	
TC118335	Sb02g007240 - 2OG-Fe(II) oxygenase superfamily	1.28	
TC128001	Sb01g036770 Chaperone - similar to Cluster: DnaJ domain containing protein; Oryza sativa	1.15	
TC131009	Sb01g007230 : similar to Putative uncharacterized protein - peroxidase activity	1.07	
TC117207	Sb01g042040 : similar to Branched-chain-amino-acid aminotransferase 5, chloroplast	1.48	Aminoacid metabolism
TC116451	Sb02g003530 - LOC_Os02g20360 : tyrosine aminotransferase, putative, expressed 86%	1.39	
TC112172	Sb01g012050 : similar to Thiamine biosynthesis protein thiC, putative, expressed	1.18	
TC125231	similar to Cluster: Branched-chain-amino-acid aminotransferase 5 chloroplast ; Oryza	1.02	
TC119454	Sb02g035150 : similar to Isocitrate lyase	1.84	Sugar metabolism
TC115845	Sb06g020720 : similar to Malate synthase, glyoxysomal	1.68	
TC116837	Sb04g034140 : similar to Alpha-amylase precursor	1.64	
TC117320	Sb06g002180 : similar to OSIGBa0147O06.8 protein - Belongs to the UDP-glycosyltransferase family	1.43	
TC118685	Sb05g001260 : similar to Putative uncharacterized protein - carbohydrate metabolic process, rice 90%	1.35	

TC126642	similar to Cluster: Enolase; Oryza sativa Indica, partial (3%)	1.30	
TC116796	Sb04g034140 : similar to Alpha-amylase precursor	1.22	
TC120343	Sb03g027310 : similar to Putative 1,4-alpha-glucan branching enzyme	1.21	
TC122288	Sb10g022160 myoinosiitol oxygenase	1.20	
TC130749	Sb03g027310 : similar to Putative 1,4-alpha-glucan branching enzyme	1.19	
TC120536	Sb04g034140 : similar to Alpha-amylase precursor	1.14	
TC130421	Sb05g008550 : similar to Putative xylanase inhibitor protein precursor	1.03	
TC126132	Sb02g036370 : similar to Putative organic cation transporter	1.88	Transport
TC122699	Sb01g042860 : similar to Os02g0154600 protein - KOG1444: Nucleotide-sugar transporter VRG4/SQV-7	1.12	
TC116258	Sb02g021080 Ubiquitin-conjugating enzyme E2	1.46	Protein fate
TC122862	Sb03g007180 : similar to uncharacterized protein - Prot. Family: Bowman-Birk serine protease inhibitor family	1.25	
TC133899	Sb02g025920 : similar to Cysteine proteinase 2 precursor	1.02	
TC123082	Sb03g034790 : similar to Putative GTPase	1.12	Nucleotide binding
TC125997	Sb01g045060 : similar to AP2 domain containing protein	1.62	Nucleic acid binding
TC131066	Sb01g045060 : similar to AP2 domain containing protein	1.50	
TC133159	Sb03g003120 : similar to Typical P-type R2R3 Myb protein	1.43	
TC121091	Sb07g028070 : similar to Parathymosin-like - KOG1946: RNA polymerase I transcription factor UAF	1.39	
TC122585	Sb01g048240 : similar to PolyA polymerase family protein, expressed	1.32	
TC130685	Sb04g002100 : similar to Putative tRNA (Guanine-N2-)-Methyltransferase	1.25	
TC129736	Sb03g047330 : similar to SbPCL1 protein Myb-like DNA-binding domain	1.21	
TC129858	Sb03g003120 : similar to Typical P-type R2R3 Myb protein	1.18	
TC133095	Sb02g032260 - Pfam RNA polymerases M/15 Kd subunit	1.16	
TC124493	Sb01g038230 : similar to Eukaryotic translation initiation factor 2 alpha subunit family protein,	1.13	
TC112527	Sb01g045060.2 AP2 domain containing protein	1.07	
TC122476	Sb06g024100 - RNA recognition motif - homologue to Cluster: Water-stress protein; Zea mays	1.04	
TC131799	Sb08g020800 : similar to Tesmin/TSO1-like CXC domain containing protein, expressed	1.04	
TC129292	Sb01g013820 : similar to Zinc finger protein	1.01	
TC123792	Sb06g029810 : similar to LOC_Os04g54190 : cysteine-rich receptor-like protein kinase 8 precursor	1.36	Communication and signalling
TC131478	Sb10g017820 - kinase activity - Pfam01326: Pyruvate phosphate dikinase, PEP/pyruvate binding domain	1.22	
TC124972	weakly similar to Cluster: Sensor histidine kinase; Plesiocystis pacifica	1.13	
TC126299	Sb01g005880 : weakly similar to Putative uncharacterized protein - KOG0472: Leucine-rich repeat protein	1.11	
TC133386	Sb06g021790 Protein kinase domain	1.04	
TC123460	Sb03g030110 : similar to Pyruvate kinase	1.02	
TC127530	Sb09g028970 : similar to protein OJ1008_D08.8 - KOG1974: DNA topoisomerase I-interacting protein	1.36	Cell cycle-DNA processing

TC119922	Sb10g005130 : similar to Helicase associated domain family protein,	1.27	
TC115071	Sb06g016980 : similar to OSJNBa0084A10.14 protein: ATP-dependent helicase activity	1.20	
TC133554	Sb06g016980 : similar to OSJNBa0084A10.14 protein - ATP-DEPENDENT RNA HELICASE	1.00	
TC133171	Sb01g025970 : similar to Proline-rich protein precursor	2.18	Interaction with the environment
TC123265	Sb01g025970 : similar to Proline-rich protein precursor	1.10	
TC128517	Sb01g002720: similar to Expressed protein - Sorghum bicolor clone BAC IS_91D5 ABA-induced gene	1.23	Systemic interaction with the environment
TC118698	Sb01g020210 electron carrier activity	1.17	Others
TC124327	Sb06g002220 similar to CBS domain containing protein-like - catalytic activity	1.67	Unknown
TC132610	Sb02g028600.1 catalytic activity	1.54	
TC126207	weakly similar to Cluster: MMPL domain protein; Caldicellulosiruptor saccharolyticus	1.37	
TC116103	Sb03g001650 : similar to Putative esterase	1.34	
TC120928	Sb09g026920 - Os05g0539800 protein	1.26	
TC129498	Sb06g014870 : Predicted protein	1.25	
TC126199	Sb09g004330 : similar to Putative uncharacterized protein	1.24	
TC129686	Sb04g028820 : similar to uncharacterized protein - 94% with Zea mays clone 218087 APO2 mRNA, complete cds	1.21	
TC133746	homologue to Cluster: Putative uncharacterized protein; Oryza sativa Japonica	1.17	
TC123545	similar to Cluster: Enabled homolog; Rattus norvegicus (Rat)	1.11	
TC125459	Sb03g001990 : weakly similar to Os01g0212500 protein	1.11	
TC115632	Sb02g035960 : weakly similar to OSJNBa0086B14.7 protein	1.07	
TC123048	similar to Cluster: Putative uncharacterized protein; Oryza sativa|Rep: Putative uncharacterized protein	1.07	
TC134592	weakly similar to Cluster: Os04g0461400 protein; Oryza sativa Japonica	1.05	
TC128980	Sb06g014870 : Predicted protein	1.04	
TC129050	homologue to Cluster: Putative uncharacterized protein F9F13.120; Arabidopsis thaliana	1.01	

Genes modulated at FTWS level 0.25.

At 25% of FTSW water deficiency induced affected the expression of 418 genes. (Tab. 4). The number of genes involved in cell defence was consistent (63 genes) equally divided between up-regulated (31) and down-regulated (32). The two groups of genes differed qualitatively for the molecular process in which they were involved (Tab. 7). The up-regulated genes with Log_2FC values between 1.4 and 1.8, were represented by dehydrin DHN1, aldo/keto reductase (AKR) family protein, three cytochrome P450 and a uncharacterized oxidoreductase enzyme (Tab. 7).

Dehydrins are a class of hydrophilic globular proteins also named late embryogenesis abundant (LEA) proteins. These proteins accumulate in response to drought stress in plants and play a vital role in plant protection against adverse effects caused by water deficit stress (Hong *et al*., 2005; Gosal *et al*., 2009).

These proteins may protect other macromolecules or cellular structures and help in maintaining the integrity of cell membranes (Close *et al*., 1993; Bray, 1997; Beck *et al*., 2007). The known physical properties of dehydrins suggest roles as stabilizers of nuclear or cytoplasmic macromolecules under water stress conditions (Campbell and Close, 1997). Dehydrin proteins have been most extensively studied in relation to drought stress (Labhilili *et al*., 1995; Close, 1996; Borovskii *et al*., 2002; Jiang and Huang, 2002). Dehydrin accumulation has been reported to be positively correlated with dehydration tolerance in annual crops (Close *et al*., 1993; Mohammadkhani and Heidari, 2008).

Annual and perennial plants exhibit contrasting responses of dehydrin expression to cope with severe drought stress. Dehydrin accumulation in annual plants appears to be more closely associated with plant tolerance to moderate water deficit rather than for survival of severe drought as found in perennial grasses (Volaire, 2003). The metabolic basis of perennial grass survival of drought stress are likely to be different from those of annual crops in which drought tolerance is evaluated for seed or fruit yield production while drought survival is more important for perennial grasses, particularly under severe stress conditions (Hartung *et al*., 1998).

Members of the aldo-keto reductases (AKRs) family are involved in a variety of cellular processes, such as the antioxidant defence against the harmful effects of lipid peroxidation. Plant AKRs, among other enzymes, have been shown to be effective in the detoxification of lipid peroxidation-derived reactive aldehydes (Hideg *et al*. 2003; Mundree *et al*. 2000; Oberschall *et al*. 2000). The first isolated aldose reductase homologue protein from barley showed osmoprotective function during embryo development and was linked to the acquisition of desiccation tolerance (Bartels *et al*. 1991; Roncarati *et al*. 1995). The recently characterized AKR4C9 (At2g37770) from Arabidopsis thaliana (Simpson *et al*., 2009) and OsAKR1-2-3 (Os01g0847600, Os01g0847700, Os01g0847800, respectively) from Oryza sativa (Turoczy *et al*., 2011) supports the importance of the enzymatic action directed towards oxidative stress-linked aldehydes, such as malondialdehyde (MDA) and methylglyoxal (MG). Turoczy and colleagues, revealed the positive role of rice AKR1 in abiotic stress-related reactive aldehyde detoxification pathways and its use for improvement of stress tolerance in plants.

In our experiment, the gene Sb06g021980, encoding for an AKR protein, resulted effectively up-regulated over a time course of five days at T0.25 (Tab. 6 and Tab. 7).

Out of 31 up-regulated probes, 17 represented 11 genes, encoding for cytochrome P450 proteins (Tab. 7). Cytochrome P450 family CYP709C1 (gene Sb02g040490) was differentially up-regulated at FTSW 0.25. Sb01g001180 (Log_2FC 1.4) and Sb01g001200 (Log_2FC 1.7) encoded for two specific cytochrome CYP71E1 and CYP79A1, respectively. The latter was detected also up-regulated at FTSW 0.37 (Tab. 6). These enzymes are known to be involved in the biosynthetic pathway of the cyanogenic glucoside dhurrin (Bak *et al*., 2000). Degradation of cyanogenic glucosides is catalyzed by β-glucosidases and α-hydroxynitrilases and results in release of hydrogen cyanide. Accordingly, cyanogenic glucosides are classified as phytoanticipins that may play a role in plant defence (Nielsen *et al*., 2008). The other enzymes involved in dhurrin biosynthesis, UDP-glucose glucosyltransferase (gene Sb01g001220), and degradation, cyanogenic beta-glucosidase dhurrinase (geneSb08g007610) and P-(S)-

hydroxymandelonitrile lyase (Sb04g036350), resulted up-regulated at FTSW 0.25 (Tab. 6) and their expression increased at FTSW 0.15 (Tab. 7).

Others up-regulated genes, with expression levels up to Log_2FC 1.4, were represented by different classes of cytochrome P450 enzymes, GST1 and enzymes involved in oxidation-reduction processes (Tab. 7).

At the levels of water stress ranging from 0.25 and 0.15 FTSW smHSPs resulted strongly down-regulated in not irrigated plants (Tab. 7 and 8). Small HSPs have molecular mass from 15 to 30 kDa (Waters *et al.* 1996, Smykal *et al.* 2000). Higher plants synthesize predominantly (up to 1 % of the total proteins) and ubiquitously smHSPs in response to stress, which function as molecular chaperone (Vierling 1991, Miernyk 1999, Smykal *et al.* 2000, Wang *et al.* 2004).

In contrast to HSP70 and HSP60, which are present in plant tissues both constitutively and under adverse environmental impacts, smHSPs are synthesized basically in response to stresses and some of them are also expressed during certain developmental stages (Waters *et al*. 1996, Miernyk 1999, Smykal *et al*. 2000, Wang *et al*. 2004, Kotak *et al*. 2007). It should be borne in mind that low molecular weight HSP were detected not only under stress conditions but also in the tissues of control monocots plants. By biochemical approach, Mansfield and colleagues (Mansfield and Key, 1987) characterized more precisely the number of low molecular weight HSP accumulated in a variety of crop plants using two-dimensional SDS-PAGE electrophoresis and silver staining. In their experiment seedlings were grown until 3 days after germination. In leaves of young wheat, rice, maize, pearl millet and Panicum miliaceum control samples, smHSP of molecular weight of 17-18 KDa were identified. In a similar study, by SDS-PAGE electrophoresis and immunoblot analyses of HSPs, Grigorova and colleagues (Grigorova *et al*., 2011) showed that smHSPs were highly represented in leaves of young wheat control samples. In their experiment, plants were grown in a growth chamber, drought stress was applied to 8-days-old plants with fully developed first and expanding second leaf. Control plants were irrigated daily to maintain 70 % soil moisture (Grigorova *et al*., 2011). Other experimental data also showed that smHSPs were highly represented in control plants. Their accumulation could be provoked either by adverse environmental conditions, or by developmental factors (Vierling 1991, Waters *et al.* 1996, Smykal *et al.*

2000). This probably could explain the increased contents of these proteins in the leaves of the experimental plants at an early vegetation stage (8 - 14 d after germination).
Expression of small HSPs in control samples suggests that they are involved in essential physiological functions, although their functions at normal conditions may be different from those during stress.

Among the up-regulated genes at FTSW 0.25 in not watered plants, the following functional classes were detected: lipid metabolism, transport, nucleic acid binding (Tab.7).
More in details, 2 genes involved in lipid metabolism, Sb06g014320 and Sb04g021010, were highlighted: a glycerophosphodiester phosphodiesterase and a glycerophosphoryl diester phosphodiesterase, with Log_2FC values of 2.2 and 1.9, respectively. Two uncharacterized lipid transporters were up-regulated (Sb06g016160 and Sb03g039880) together with two generic sugar transporters (Sb04g021000 and Sb01g050580).

Water stress at FTSW 0.25 activated several transcription factors in a greater number compared to FTSW 0.37 level (Tab. 4). The higher expression levels was measured for an ABRE-binding factor BZ-1 (ABA-Responsive Element basic-leucine zipper (bZIP), encoded by Sb03g030050, with Log_2FC value of 2.2, Tab. 7); a homeobox-leucine zipper protein HOX24 (Log_2FC 2.0) and two elements containing basic Helix-Loop-Helix (bHLH) domain (Log_2FC 2.3 and 1.5, respectively). ABRE is a major cis-acting element that controls expression of drought-induced genes in ABA-dependent drought stress response (Hirayama *et al*., 2007). The expression level of Sb03g030050 was also high at FTSW 0.15 (Tab. 8) as confirmed by qRT-PCR analysis (Fig. 8).
The phytohormone ABA plays an important role in cellular signaling in abiotic stresses, such as drought and salinity. ABA synthesis is induced under conditions of water stress and the increased level of ABA signals for stomatal closure in guard cells and induces expression of drought stress-related genes that encode proteins contributing to dehydration tolerance. Promoters of many ABA-responsive genes bear cis-acting elements known as ABRE (Uno *et al*., 2000). ABA-inducible transcription typically requires the existence of more than two ABREs or the combination of an ABRE with a

coupling element at appropriate positions in the promoter regions (Uno *et al*., 2000). Many of the Ca^{2+}-regulated genes have these elements, suggesting that ABA may regulate ABA-responsive genes through cellular Ca^{2+} changes (Kaplan *et al*., 2006). Additionally, MYC and MYB recognition sites mediate ABA signaling for some stress-inducible genes (Urao *et al*., 1993; Abe *et al*., 1997). Besides ABA dependent pathways, drought responses are also mediated by ABA-independent signaling pathways, such as those mediated by the DREB proteins (Agarwal *et al*., 2006; Yamaguchi-Shinozaki and Shinozaki, 2006; Seki *et al*., 2007). Here, differential expressed genes involved in ABA biosynthesis pathway (zeaxanthin epoxidase (ZEP), 9-*cis*-epoxycarotenoid dioxygenase (NCED), short-chain dehydrogenases/reductases (SDR), aldehyde oxidase (AO), were not detected. Viceversa, ABRE-binding factor BZ-1 (Sb03g030050) was up regulated in T0.25 and T0.15.

Two genes, Sb01g023270 (Log_2FC 1.9) and Sb02g024480 (Log_2FC 2.9), included in the functional category interaction with environment and encoding for proteins up-regulated during phosphate starvation and accumulation, respectively, resulted over-expressed.

The down-regulated genes in not irrigated plants belonged to the functional classes, sugar metabolism, communication and signalling, nucleic acid binding and transport and included 29, 28, 26 and 21 genes, respectively (Tab. 4). Among them were: alkaline alpha galactosidase 3, glycosyl hydrolase and cell wall invertases (Sb07g028620, Sb01g025310 and Sb04g021810 genes with Log_2FC 2.8, 2.4 and 2.1, respectively); cation transporters (mean Log_2FC 3.0); several kinases and phosphatases proteins, genes encoding AP2 domain containing proteins (Sb01g045060 gene with Log_2FC 3.3); transcriptional regulators, mainly MADS-box proteins, P-type R2R3 Myb proteins (Tab. 7). The gene Sb01g25970, encoding for proline-rich protein precursor, already mentioned above in FTSW 0.37 section, resulted among the most down-regulated at FTSW 0.25 (Log_2FC 4.1).

Tab. 7 - Up and Down-regulated genes in sorghum IS19453 genotype at FTSW 0.25.

	UP-REGULATED		
ID	**Annotation**	**Log_2 FC**	**Functional class**
TC118527	Sb09g018420: similar to Dehydrin DHN1	1.83	Cell rescue defence and virulence
TC129730	Sb06g021980: similar to Aldo/keto reductase family-like protein (Betaine aldehyde dehydrogenase, Zea mays)	1.82	
TC129062	Sb09g018420: similar to Dehydrin DHN1	1.80	
TC120709	Sb03g036980: similar to Os01g0794400 protein - Probable nucleoredoxin 2 - Oxidoreductase	1.78	
TC131575	Sb09g018420: similar to Dehydrin DHN1	1.76	
TC111396	Sb01g001200: similar to Cytochrome P450 79A1	1.73	
TC113948	Sb01g016900: Belongs to the cytochrome P450 family	1.70	
TC118856	Sb01g001180: similar to Cytochrome P450 71E1	1.40	
TC126325	Sb10g025880: similar to Putative GDP-L-fucose synthase 2	1.38	
TC112519	Sb01g016900: Belongs to the cytochrome P450 family	1.36	
TC130246	Sb03g039530: similar to Putative laccase LAC5-6 - Multicopper oxidase	1.34	
TC120066	Sb01g001180: similar to Cytochrome P450 71E1	1.33	
TC128324	Sb01g001180: similar to Cytochrome P450 71E1	1.33	
TC130181	Sb02g040490: similar to Cytochrome P450 CYP709C1	1.29	
TC127185	Sb08g018780: Belongs to the cytochrome P450 family	1.28	
TC134071	Sb01g001230.2: similar to Glutathione S-transferase 1	1.27	
TC129358	Sb01g001180: similar to Cytochrome P450 71E1	1.26	
TC112149	Sb08g003110: similar to Cytochrome P450 family protein	1.24	
TC115148	Sb02g040510: similar to Putative cytochrome P450 (Triticum aestivum: Cytochrome P450 CYP709C1)	1.18	
TC130297	Sb02g009410: Belongs to the cytochrome P450 family	1.18	
TC123297	Sb01g014550: similar to Putative uncharacterized protein - Belongs to the iron/ascorbate-dependent oxidoreductase family.	1.15	
TC121277	Sb01g034730.1: monooxygenase activity Belongs to the cytochrome P450 family	1.14	
TC133561	Sb01g001180: similar to Cytochrome P450 71E1	1.14	
TC131239	Sb06g021980: similar to Aldo/keto reductase family-like protein	1.13	
TC112605	Sb06g014550: similar to Os03g0439500 protein - Belongs to the iron/ascorbate-dependent oxidoreductase family	1.10	
TC129311	Sb01g001230: similar to Glutathione S-transferase 1	1.09	
TC112299	Sb07g000500: similar to Os08g0106000 protein -Belongs to the cytochrome P450 family - monooxygenase activity	1.07	
TC120422	Sb04g028750.1: similar to MutT/nudix-like - hydrolase activity	1.07	
TC134185	Sb02g040500: similar to Cytochrome P450 CYP709C1	1.07	

TC124500	Sb08g016390: similar to Iron-deficiency specific clone No.3 - 2'-deoxymugineic-acid 2'-dioxygenase activity	1.04	
TC116336	Sb07g000500: Belongs to the cytochrome P450 family	1.03	
TC115037	Sb06g014320: similar to H0718E12.3 protein - glycerophosphodiester phosphodiesterase activity - lipid metabolic process	2.25	Lipid metabolism
TC118289	Sb06g014320: similar to H0718E12.3 protein - glycerophosphodiester phosphodiesterase activity - lipid metabolic process	2.23	
TC124597	Sb04g021010: similar to Glycerophosphoryl diester phosphodisterase	1.91	
TC128600	Sb07g027910: Monogalactosyldiacylglycerol (MGDG) synthase	1.91	
TC117579	Sb04g021010: similar to Glycerophosphoryl diester phosphodisterase	1.61	
TC128265	Sb09g019100: similar to Putative UDP-sulfoquinovose synthase	1.06	
TC130582	Sb05g026990: weakly similar to Patatin-like protein - lipid metabolism	1.03	
TC117845	Sb03g039820.1: similar to Delta-1-pyrroline-5-carboxylate synthetase (P5CS)	1.40	Aminoacid metabolism
TC115006	Sb01g011750: similar to Glutamine amidotransferase class-I family protein - glutamine metabolic process	1.23	
TC124705	Sb08g002620: similar to Chalcone-flavanone isomerase family protein, expressed	1.20	
TC128125	Sb05g014470: similar to Adenosylhomocysteinase 1 - one-carbon metabolic process - aa biosynthesis	1.02	
TC124267	Sb04g005350: KOG:3179 Predicted glutamine synthetase	1.01	
TC117813	Sb01g001220: similar to UDP-glucose glucosyltransferase	1.51	Sugar metabolism
TC111669	Sb08g007610: similar to Cyanogenic beta-glucosidase dhurrinase-2	1.36	
TC129891	Sb02g006320: similar to Alkaline alpha galactosidase 1 - catalytic activity	1.21	
FL274235	Sb04g032830: similar to Expansin-B11 precursor	1.18	
TC118366	Sb01g004130: similar to Putative raffinose synthase or seed imbibition protein - catalytic activity	1.17	
TC130541	Sb02g006320: similar to Alkaline alpha galactosidase 1 - catalytic activity	1.16	
TC125970	Sb01g019850: similar to Beta-amylase	1.09	
TC115239	Sb09g029610: Glycan biosynthesis; starch biosynthesis	1.05	
TC123141	Sb06g016160: LTP family - lipid transport	2.15	Transport
TC124266	Sb04g021000: similar to Putative uncharacterized protein - KOG:1623 Multitransmembrane protein -sugar transport	1.97	
TC115722	Sb04g021000: Pfam: nodulin MtN3 family protein - Sugar transport	1.54	
TC131223	Sb01g050580: Pfam:00083 Sugar (and other) transporter	1.27	
TC129606	Sb03g039880: similar to Non-specific lipid-transfer protein	1.27	
TC115469	Sb01g044010: similar to Mannitol transporter	1.11	
TC113409	Sb01g010670: similar to Integral membrane protein DUF6 containing protein	1.01	
TC111472	Sb08g002670: similar to Non-specific lipid-transfer protein	1.00	
TC127710	Sb03g001113: Pfam:03595 C4-dicarboxylate transporter/malic acid transport protein	1.00	
TC121260	Sb04g036350: similar to P-(S)-hydroxymandelonitrile lyase precursor (HNL) - proteolysis	1.74	Protein fate
TC115284	Sb09g027590: Protease inhibitor/seed storage/LTP fam (58%)	1.46	
TC123919	Sb09g001020: response to wounding serine-type endopeptidase inhibitor activity	1.46	

TC111575	Sb06g000780.1: cysteine-type endopeptidase activity	1.38	
TC129656	Sb04g036350: P-(S)-hydroxymandelonitrile lyase Involved in cyanogenesis	1.31	
TC133798	Sb04g036350: P-(S)-hydroxymandelonitrile lyase Involved in cyanogenesis	1.09	
TC117884	Sb10g012160: serine-type peptidase activity	1.03	
TC129572	Sb01g016970: similar to Os10g0519300 protein - ATPase, AAA family protein, expressed	1.38	Nucleotide binding
TC129545	Sb06g033410: catalytic activity - Pfam AMP-binding enzyme	1.26	
TC128124	Sb04g032280: similar to Rho-GTPase-activating protein-like	1.26	
TC116020	Sb10g023370: signal transduction GTP-binding	1.16	
TC123152	Sb03g032540: similar to Glycylpeptide N-tetradecanoyltransferase	1.15	
TC131597	weakly similar to Cluster: PREDICTED: adenylosuccinate synthase; Pan troglodytes - GTP binding	1.08	
TC111526	Sb07g004190: similar to OSJNBb0018A10.7 protein - Contains 1 basic helix-loop-helix (bHLH) domain	2.32	Nucleic acid binding
TC112240	Sb03g030050: similar to ABRE-binding factor BZ-1	2.24	
TC124880	Sb04g033380: transcription regulator activityHomeobox-leucine zipper protein HOX24	2.08	
TC122897	Sb03g030050.1: similar to ABRE-binding factor BZ-1	1.73	
TC117463	Sb02g031170: Belongs to the RNase T2 family	1.58	
TC129178	Sb01g008570: similar to Putative helix-loop-helix DNA-binding protein	1.56	
TC131520	Sb02g031170: similar to S-like Rnase	1.46	
TC130886	similar to Cluster: SPLAYED splice variant; Arabidopsis thaliana - regulation of transcription, DNA-dependent	1.42	
TC114720	Sb01g023330: similar to ZIM motif family protein	1.41	
TC128961	Sb09g006060: regulation of transcription	1.40	
TC129578	Sb02g031170: similar to S-like Rnase	1.32	
TC127222	Sb10g012110: similar to Putative glycine rich protein	1.30	
TC124669	Sb03g032420.1: transcription regulator activity	1.27	
TC111682	Sb03g008560.1: transcription regulator activity	1.26	
TC118771	Sb08g003180: similar to Myb family transcription factor-like	1.21	
TC125312	Sb09g006060: regulation of transcription - Myb-like DNA-binding domain	1.17	
TC132590	Sb04g000500: sequence-specific DNA binding transcription factor activity MADS-box domains	1.13	
TC128338	Sb09g021850: zinc ion binding	1.12	
TC469670	Sb09g021265: weakly similar to Os12g0175400 protein - MYB10	1.04	
TC117700	Sb03g033100: similar to Acid phosphatase	1.61	Communication and signalling
TC134541	Sb01g001350: similar to Ser/Thr protein phosphatase family protein	1.36	
TC123314	Sb02g012400.1: protein serine/threonine kinase activity	1.33	
TC131697	Sb08g023000: similar to Secretory acid phosphatase	1.29	
TC116626	Sb01g039890: Pfam Protein phosphatase 2C	1.29	

TC124248	Sb09g022410: KOG:0698 Serine/threonine protein phosphatase	1.23	
TC124710	Sb03g036210: similar to Purple acid phosphatase precursor	1.21	
TC114934	Sb08g019090: similar to Os12g0576600 protein - Calcineurin-like phosphoesterase	1.13	
TC132971	Sb08g023000.2: similar to Secretory acid phosphatase - Pfam Calcineurin-like phosphoesterase	1.05	
TC123181	Sb03g007330.1: similar to Putative nuclease I	1.24	Cell cycle-DNA processing
TC116566	Sb03g000850: nucleoside metabolic process	1.07	
TC125651	Sb02g024480: Contains 1 SPX domain - Protein involved in vacuolar polyphosphate accumulation	2.91	Interaction with the environment
TC132562	Sb02g024480: Contains 1 SPX domain - Protein involved in vacuolar polyphosphate accumulation	2.59	
TC117413	Sb01g023270.2: Up-regulated under phosphate starvation. Up-regulated during cold stress - SPX domain containing	1.96	
TC123989	Sb03g024480: Contains 1 SPX domain - Protein involved in vacuolar polyphosphate accumulation	1.91	
TC115207	Sb04g030640: similar to 9-cis-epoxycarotenoid dioxygenase 2	1.01	
TC128498	Sb07g024090: weakly similar to chr7 scaffold_31, whole genome shotgun sequence - Chlorophyllase family	1.39	Others
TC120016	Sb01g035960.1: transaminase activity - class-III pyridoxal-phosphate-dependent aminotransferase family	1.02	
TC119387	Sb07g021840: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	3.00	Unknown
TC120984	Sb01g046120: similar to TPR Domain containing protein	2.42	
TC121587	Sb04g009640: similar to Cluster: Putative uncharacterized protein B1178F07.4; Oryza sativa Japonica Group	2.34	
TC118178	Sb03g005490: similar to Cluster: Putative uncharacterized protein P0011G08.23; Oryza sativa Japonica Group	2.29	
TC125719	Sb03g005490: similar to Cluster: Os05g0119300 protein; Oryza sativa	2.28	
TC122544	Sb03g043410: similar to Cluster: Putative uncharacterized protein P0470A12.6; Oryza sativa Japonica	2.23	
TC122692	Sb03g005490: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group|	2.19	
TC120484	Sb06g015140: similiar to Os04g0403600 protein	2.14	
TC127109	Sb07g021840: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2.00	
TC117176	Sb03g031950: methyltransferase activity	1.98	
TC120556	Sb03g006480: biosynthetic process	1.96	
TC129786	weakly similar to Cluster: RIKEN cDNA 1700021P22 gene; Mus musculus	1.92	
TC125693	Sb06g004280: similar to OSIGBa0139I12.3 protein - transketolase activity	1.73	
TC119370	Sb03g043420.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.67	
TC114851	Sb10g000890: similar to Os09g0407700 protein - catalytic activity	1.64	
TC127367	Sb10g008730: similar to Os06g0242000 protein - GO:0008168 methyltransferase activity	1.60	
TC132787	Sb05g022680: similar to Cluster: PREDICTED: similar to Protein C14orf155; Macaca mulatta	1.58	
TC131680	Sb05g016820: similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1.57	
TC126602	Sb01g017440: similar to Cluster: Os10g0509600 protein; Oryza sativa Japonica Group	1.52	
TC121394	homologue to Cluster: Putative uncharacterized protein; Sorangium cellulosum 'So ce 56'	1.42	
TC111603	Sb10g021910: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.42	

TC125123	Sb01g046120: similar to TPR Domain containing protein	1.42	
TC120909	Sb08g009120: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.41	
TC124794	Sb01g017440: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.39	
TC116356	Sb01g040410: similar to Expressed protein	1.31	
TC133029	Sb04g000250: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.30	
TC123034	Sb10g027660: similar to Cluster: Os06g0683700 protein; Oryza sativa	1.30	
TC122705	Sb09g030780: weakly similar to Putative uncharacterized protein	1.29	
TC119766	Sb10g021910: similar to Putative uncharacterized protein	1.28	
TC123657	Sb06g029880.1: similar to Cluster: H0315F07.12 protein; Oryza sativa	1.28	
TC118790	Sb09g022700: similar to Putative uncharacterized protein	1.22	
TC119711	Sb03g031940: methyltransferase activity	1.20	
TC118156	Sb05g024940: Cluster: Putative uncharacterized protein S126P21.2; Sorghum bicolor	1.18	
TC126440	Sb03g033000: similar to Cluster: Os01g0718500 protein; Oryza sativa	1.18	
TC127239	Sb02g013190: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.16	
TC125663	Sb04g023200.1: homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.12	
TC125319	Sb01g045180.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.11	
TC114267	Sb02g040450.1: catalytic activity	1.10	
TC121028	Sb02g025470: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.07	
TC118310	Sb09g030780: similar to Cluster: LigA precursor; Halorubrum lacusprofundi ATCC 49239	1.05	
TC111878	Sb06g001870.1: transferase activity, transferring acyl groups other than amino-acyl groups	1.03	
TC130020	similar to Cluster: Putative uncharacterized protein; Aspergillus terreus NIH2624	1.03	
TC121323	Sb02g004630: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.02	
TC129491	Sb04g016960: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.00	

DOWN-REGULATED

ID	**Annotation**	**Log$_2$ FC**	**Functional class**
TC132654	Sb03g006890: similar to 16.9 kDa class I heat shock protein 1	2.70	Cell rescue, defence and virulence
TC128001	Sb01g036770: similar to DnaJ domain containing protein - DNAJ/HSP40	2.43	
TC124920	Sb05g019190: similar to Terpene synthase family, metal binding domain containing protein	2.00	
TC120734	Sb05g019180: terpene synthase	1.99	
TC127266	Sb02g031940: similar to Flavin containing monooxygenase 3-like	1.82	

TC133460	Sb02g042850: similar to Peroxidase precursor	1.70	
TC122862	Sb03g007170: similar to Wound induced protein homolog	1.67	
TC123805	Sb01g041280: similar to SCUTL1 - Thaumatin family	1.60	
TC131009	Sb01g007230: peroxidase	1.59	
TC115670	Sb03g039360: similar to Heat shock 70 kDa protein	1.58	
TC466646	Sb03g003530: similar to 17.8 kDa class II heat shock protein	1.49	
TC125246	Sb03g012940: similar to Os01g0314800 protein - Late embryogenesis abundant protein	1.45	
TC134689	Sb09g002260: similar to Putative isovaleryl-CoA dehydrogenase	1.41	
TC490440	Sb01g040000: similar to 17.4 kDa class I heat shock protein 3	1.40	
FL292626	Sb01g040000: similar to 17.4 kDa class I heat shock protein 3	1.39	
EB705607	Sb03g003530: similar to 17.8 kDa class II heat shock protein	1.35	
BG837255	Sb01g040000.1: similar to 17.4 kDa class I heat shock protein 3	1.29	
TC117744	Sb05g007030: similar to Putative uncharacterized protein - Molecular chaperone (small heat-shock protein Hsp26/Hsp42)	1.29	
TC126915	Sb01g018855: similar to Cytochrome P450 family protein, expressed	1.28	
TC128648	Sb06g032460: similar to OSJNBb0004A17.1 protein - Amine oxidase	1.25	
CO452191	Sb01g040000.1: similar to 17.4 kDa class I heat shock protein 3	1.24	
TC119576	Sb02g004910: oxidation-reduction process	1.22	
TC111726	Sb07g001360: Belongs to the cytochrome P450 family	1.20	
FL050942	Sb03g039360: similar to Heat shock 70 kDa protein	1.19	
TC114515	Sb06g032460: oxidation-reduction process	1.17	
TC481803	Sb01g040000: similar to 17.4 kDa class I heat shock protein 3	1.17	
TC118189	Sb02g004300: DIMETHYLANILINE MONOOXYGENASE	1.15	
TC115190	Sb02g004910: oxidation-reduction process	1.14	
TC122884	Sb02g040870: similar to 10 kDa chaperonin	1.13	
TC119014	Sb06g001970: similar to Probable L-ascorbate peroxidase 3	1.12	
TC132833	Sb06g032460: similar to OSJNBb0004A17.1 protein - oxidation-reduction process	1.09	
TC116363	Sb10g003890: similar to Putative lipase	1.30	Lipid metabolism
TC117343	Sb05g023970: similar to Acyl-coenzyme A oxidase 2, peroxisomal, putative, expressed - fatty acid beta-oxidation	1.27	
TC126807	Sb05g023970: similar to Acyl-coenzyme A oxidase 2, peroxisomal	1.19	
TC119902	Sb07g028050.1: similar to Putative glycerophosphoryl diester phosphodiesterase 2	1.02	
TC112381	Sb02g029200: similar to Acyl-[acyl-carrier protein] thioesterase	1.01	
TC126901	Sb01g000970: similar to Pantoate--beta-alanine ligase	1.01	
TC117207	Sb01g042040: similar to Branched-chain-amino-acid aminotransferase 5, chloroplast - Amino-acid biosynthesis	1.56	Aminoacid metabolism
TC132264	Sb01g029660: similar to Proline dehydrogenase family protein	1.55	

TC131220	Sb08g020810: similar to Methylcrotonoyl-CoA carboxylase subunit alpha - Amino-acid degradation	1.24	
TC128420	Sb08g020810: similar to Methylcrotonoyl-CoA carboxylase subunit alpha - amino-acid degradation	1.23	
TC116451	Sb02g003530: LOC_Os02g20360 : tyrosine aminotransferase, putative, expressed 86%	1.22	
TC112154	Sb01g045680: similar to Alanine-glyoxylate aminotransferase 2, mitochondrial	1.20	
TC122863	Sb01g029660: similar to Proline dehydrogenase family protein	1.16	
TC133420	Sb01g045680: similar to Alanine-glyoxylate aminotransferase 2	1.12	
TC127595	Sb06g028690: similar to Imidazoleglycerol-phosphate dehydratase	1.05	
TC113802	Sb01g012050: similar to Thiamine biosynthesis protein thiC	1.04	
TC115992	Sb10g009590: similar to Asparagine synthetase [glutamine-hydrolyzing]	1.04	
TC132856	Sb02g005200: similar to Methylmalonate semi-aldehyde dehydrogenase - valine metabolic process	1.01	
TC113071	Sb04g008910: similar to Chalcone isomerase-like - amino acid biosynthetic process - flavonoid biosynthetic process	1.01	
TC125745	Sb07g028620: similar to Alkaline alpha galactosidase 3	2.88	Sugar metabolism
TC128911	Sb01g025310: similar to Glycosyl hydrolases family 17 protein, expressed	2.47	
TC122288	Sb10g022160: myoinosiitol oxygenase	2.24	
TC120410	Sb04g021810.3: similar to Cell wall invertase	2.12	
TC123728	Sb04g021810: similar to Cell wall invertase	2.00	
TC128904	Sb04g021810: similar to Cell wall invertase - carbohydrate metabolic process	1.95	
TC114269	Sb06g020280: similar to H0510A06.3 protein - Pfam Raffinose synthase or seed inhibition protein Sip1	1.91	
TC130749	Sb03g027310: similar to Putative 1,4-alpha-glucan branching enzyme	1.71	
TC130040	Sb06g020280: similar to H0510A06.3 protein - Pfam Raffinose synthase or seed inhibition protein Sip1	1.69	
TC131519	Sb03g023520: 1,3-beta-glucan synthase complex	1.46	
TC125887	Sb10g004950: similar to Alkaline alpha galactosidase 2	1.44	
TC127506	Sb10g031040: Pfam sucrose synthase	1.41	
TC128419	Sb09g021620: Pfam Fructose-1 6-bisphosphatase	1.40	
TC120343	Sb03g027310: similar to Putative 1,4-alpha-glucan branching enzyme	1.32	
TC126201	Sb02g035490: carbohydrate metabolism	1.32	
TC117320	Sb06g002180: similar to OSIGBa0147O06.8 protein - Belongs to the UDP-glycosyltransferase family	1.27	
TC117345	Sb09g021620: Fructose-1-6-bisphosphatase	1.25	
TC126754	Sb01g024390: hydrolase activity, hydrolyzing O-glycosyl compounds - glucan 1,3-beta-glucosidase	1.20	
TC120012	Sb06g023450: similar to B1011H02.4 protein - carbohydrate metabolism	1.18	
TC120805	Sb01g040750: similar to Beta-galactosidase 6 precursor	1.18	
TC116933	Sb03g012910: DIHYDROLIPOAMIDE ACETYL/SUCCINYL-TRANSFERASE-RELATED	1.10	
TC115572	Sb03g012910: DIHYDROLIPOAMIDE ACETYL/SUCCINYL-TRANSFERASE-RELATED	1.10	
TC121378	Sb01g024390.1: hydrolase activity, hydrolyzing O-glycosyl compounds - carbohydrate metabolic process	1.06	

TC114936	Sb09g027150: Pfam - Glycosyl hydrolases family 28 - carbohydrate metabolic process	1.06	
TC121077	Sb09g016230: similar to Os05g0346500 protein - Pfam Glycosyl hydrolase family 85 - carbohydrate metabolic process	1.05	
TC129707	Sb05g007100: similar to Alpha-L-arabinofuranosidase C-terminus family protein	1.03	
TC133104	Sb06g001540: similar to Pullulanase-type starch debranching enzyme	1.03	
TC134022	Sb07g025220: similar to Sorbitol dehydrogenase	1.03	
TC131749	Sb09g028320: similar to UDP-glucosyltransferase BX8	1.01	
TC117050	Sb02g028200: sodium ion transport	3.37	Transport
TC126132	Sb02g036370: similar to Putative organic cation transporter - Major Facilitator Superfamily	2.82	
TC117799	Sb01g011360.1: transporter activity	2.20	
TC127467	Sb06g018350: Plastocyanin-like domain putative	2.02	
TC111431	Sb01g029470: similar to Nitrate/chlorate transporter,	1.30	
TC114157	Sb09g028810: Sugar (and other) transporter	1.21	
TC131953	Sb02g006930: similar to Os07g0231400 protein - Amino acid transporters	1.15	
TC124283	Sb01g047140: similar to Aquaporin TIP1-1	1.15	
TC112797	Sb02g027260: Pfam Plastocyanin-like domain - electron carrier activity	1.13	
TC116204	Sb05g004250: similar to Putative auxin transporter-like protein 4	1.13	
TC111549	Sb01g042260: iron ion binding - oxygen transport	1.11	
TC119366	Sb08g016530: similar to Sugar transporter family protein	1.11	
TC119643	Sb09g028810: sugar transporter	1.10	
TC116158	Sb01g047690.1: oligopeptide transport	1.09	
TC122101	Sb05g018520.1: transmembrane transport	1.08	
TC114129	Sb02g031140: nodulin-like protein - transmembrane transport	1.07	
TC121736	Sb03g043020: Major Facilitator Superfamily	1.05	
TC121634	Sb02g028210: sodium ion transport	1.04	
TC115931	Sb03g043020: Major Facilitator Superfamily	1.03	
TC117259	Sb05g018520.1: transmembrane transport	1.02	
TC123586	Sb02g028520.1: transmembrane transport	1.00	
TC130470	Sb10g012970: similar to FK506-binding protein	2.22	Protein fate
TC117083	Sb10g012970: similar to FK506-binding protein - protein folding	2.05	
TC114235	Sb01g006420: similar to DNAJ protein - chaperonine	2.00	
TC125262	Sb04g001030: similar to Serine carboxypeptidase 3 precursor	1.36	
TC119571	Sb04g001030: similar to Serine carboxypeptidase 3 precursor	1.36	
TC122004	Sb03g034110: similar to Profilin-3 - actin cytoskeleton organization	1.35	
TC131345	Sb06g023100: similar to OSIGBa0153E02-OSIGBa0093I20.21 protein - proteolysis and peptidolysis	1.30	

TC130646	Sb01g027570: similar to Serine carboxypeptidase family protein	1.18	
TC126261	Sb01g027570: similar to Serine carboxypeptidase family protein	1.17	
TC133899	Sb02g025920: similar to Cysteine proteinase 2 precursor	1.16	
TC120718	Sb05g022990: similar to Targeting protein for Xklp2 containing protein	1.09	
TC124622	Sb01g027570: similar to Serine carboxypeptidase family protein	1.08	
TC128736	Sb01g005580: Peptidase family M50	1.02	
TC126193	Sb09g029900: ATP binding	1.90	Nucleotide binding
TC129177	Sb01g037600: similar to AMP-binding enzyme family protein	1.62	
TC129723	Sb01g037600: similar to AMP-binding enzyme family protein	1.52	
TC114660	Sb03g031270: AMP-binding enzyme	1.37	
TC116260	Sb03g031270: similar to Os01g0681200 protein - AMP binding enzyme	1.29	
TC124736	Sb03g029790: CTP synthase N-terminus	1.23	
TC130641	Sb08g000320: GTPase of unknown function	1.22	
TC115187	Sb03g029790: CTP synthase	1.22	
TC124557	Sb03g031270: similar to Os01g0681200 protein - AMP-binding enzyme	1.20	
TC134667	Sb09g026670: Putative GTPase activating protein for Arf	1.20	
TC126997	Sb03g027960: similar to Os01g0616900 protein - CTP SYNTHASE	1.19	
TC134051	Sb03g031270: AMP binding enzyme	1.17	
TC128523	Sb03g029790: CTP synthase N-terminus	1.04	
TC132868	Sb06g002070: similar to OSJNBb0022P19.1 protein - ATPase activity, coupled to transmembrane movement of substances	1.03	
TC131066	Sb01g045060: similar to AP2 domain containing protein (Root abundant factor - Hordeum vulgare)	3.31	Nucleic acid binding
TC125997	Sb01g045060: similar to AP2 domain containing protein (Root abundant factor - Hordeum vulgare)	3.17	
TC133146	Sb06g020810: Contains 1 basic helix-loop-helix (bHLH) domain transcription regulator activity	2.60	
TC133159	Sb03g003120: similar to Typical P-type R2R3 Myb protein	1.85	
TC119236	Cluster: Sigma-54 dependent DNA-binding transcriptional regulator; Burkholderia mallei SAVP1	1.70	
TC116354	Sb01g030570: similar to MADS-box transcription factor TaAGL7	1.62	
TC125406	Sb10g026450.1: sequence-specific DNA binding transcription factor activity (RISBZ5 - Oryza sativa)	1.55	
TC129765	Sb01g030570: similar to MADS-box transcription factor TaAGL7	1.49	
TC133745	Sb10g006780: regulation of transcription	1.43	
TC122564	Sb04g027280: Helix-loop-helix DNA-binding domain	1.38	
TC129858	Sb03g003120: similar to Typical P-type R2R3 Myb protein	1.36	
TC133385	Sb07g027050: similar to Putative oligouridylate binding protein -RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain)	1.33	
TC126373	Sb03g037940.1: regulation of transcription	1.30	
TC124386	Sb02g030330: similar to SbCBF6 regulation of transcription - AP2 domain	1.18	

TC118744	Sb05g003660: similar to CCT motif family protein - regulation of transcription, DNA-dependent	1.17	
TC131879	Sb02g026820: nucleic acid binding	1.14	
TC128178	Sb07g006195: similar to Ethylene responsive transcription factor	1.14	
TC132760	Sb08g020800: similar to Tesmin/TSO1-like CXC domain containing protein, expressed	1.13	
TC118314	Sb09g026670: Putative GTPase activating protein for Arf	1.09	
TC127476	Sb04g028960: MADS BOX PROTEIN	1.08	
TC112208	Sb02g040260: similar to Chloroplast 29 kDa ribonucleoprotein	1.07	
TC113954	Sb08g020800: similar to Tesmin/TSO1-like CXC domain containing protein	1.07	
TC134430	Sb01g007790: similar to M4 protein - MADS box transcription factor	1.04	
TC130682	Sb01g034120: similar to RNA recognition motif family protein	1.04	
TC129393	Sb09g001590: regulation of transcription Myb superfamily	1.03	
TC124341	Sb03g047230: similar to Transcription initiation factor IIA gamma chain	1.02	
TC117665	Sb07g000890: similar to Putative pherophorin-dz1 protein – serine-threonine protein kinase, plant type	2.65	Communication and signalling
TC124273	Sb09g026180: similar to Putative receptor like protein kinase	2.33	
TC131290	Sb05g021320: similar to Ser/Thr protein phosphatase family protein	2.21	
TC130846	Sb02g022436: serine-threonine protein kinase, plant type	2.04	
TC133215	Sb05g021320: similar to Ser/Thr protein phosphatase family protein	1.89	
TC120353	Sb05g024100: serine-threonine protein kinase	1.88	
TC119449	Sb06g028760.1: protein serine/threonine kinase activity	1.85	
TC133704	Sb05g024100: serine-threonine protein kinase	1.84	
TC121390	Sb05g027230.1: protein kinase activity	1.49	
TC117407	Sb10g006780: Pantothenate kinase	1.48	
TC121855	Sb09g005840: similar to Hexokinase-7 glycolysis	1.45	
TC114652	Sb04g032740: Trehalose-phosphatase	1.44	
TC116353	Sb02g035560.1: protein serine/threonine kinase activity	1.44	
TC133178	Sb09g005840: similar to Hexokinase-7	1.36	
TC131478	Sb10g017820: kinase activity - Pfam01326: Pyruvate phosphate dikinase, PEP/pyruvate binding domain	1.33	
TC123792	Sb06g029810: similar to LOC_Os04g54190 : cysteine-rich receptor-like protein kinase 8 precursor	1.28	
TC123226	Sb01g007340: similar to BTH-induced protein phosphatase 2C 2 K2 form	1.28	
TC121361	weakly similar to Cluster: Extracellular signal-regulated kinase 2; Dictyostelium discoideum	1.20	
TC121124	Sb10g017820: Pyruvate phosphate dikinase, PEP/pyruvate binding domain	1.12	
TC127960	Sb06g028570: serine-threonine protein kinase, plant type	1.12	
TC111925	Sb02g035550.1: Serine/threonine-protein kinase	1.09	
TC129720	Sb06g018760: similar to SERK1 protein precursor - Serine/threonine-protein kinase	1.07	

TC132947	Sb10g025280: similar to Vacuolar H+-pyrophosphatase	1.06	
TC134274	Sb09g007250: similar to OSIGBa0148I18.4 protein - protein serine/threonine kinase activity	1.04	
TC121758	Sb03g025930: similar to Calcineurin B-like protein 9	1.04	
TC118763	Sb01g048570: similar to Calcium-dependent protein kinase isoform 11	1.01	
TC114526	Sb05g001050: similar to Protein kinase	1.01	
TC119586	Sb01g036020.1: protein serine/threonine kinase activity	1.01	
TC124280	Sb09g023360: similar to DNA replication licensing factor MCM3 homolog 1	1.12	Cell cycle-DNA processing
TC127530	Sb09g028970: similar to protein OJ1008_D08.8 - KOG1974: DNA topoisomerase I-interacting protein	1.05	
TC125926	Sb04g023680: similar to Cryptochrome 1a	1.03	
TC133171	Sb01g025970: similar to Proline-rich protein precursor	4.16	Interaction with the environment
TC121800	Sb01g025970: similar to Proline-rich protein precursor	3.33	
TC123265	Sb01g025970: similar to Proline-rich protein precursor	1.67	
TC125048	Sb03g036770: similar to Cluster: Putative ethylene-induced esterase; Oryza sativa Japonica	1.14	Interaction with the environment
TC132003	Sb05g008030: similar to NB-ARC domain, putative - apoptosis	1.20	Others
TC130137	Sb05g008030: similar to NB-ARC domain, putative - defense response	1.04	
TC111994	Sb01g006100: similar to Ferredoxin--NADP reductase, root isozyme, chloroplast precursor	1.27	
TC112143	Sb09g021810: similar to Ferredoxin-6	1.22	
TC121475	Sb05g000530: similar to Ferritin-1	1.15	
TC111495	Sb03g045980.1: nutrient reservoir activity	2.00	
TC123116	Sb09g004970.1: nutrient reservoir activity	1.32	
TC111670	Sb02g038450: similar to Early nodulin 75-like protein	1.86	Unknown
TC117749	Sb09g022260: weakly similar to Cluster: Putative uncharacterized protein; Neosartorya fischeri NRRL 181	1.83	
TC124262	similar to Cluster: Drosophila melanogaster GH13383p; Plasmodium yoelii yoelii	1.70	
TC119450	Sb03g027790: similar to Os01g0613300 protein	1.70	
TC120053	Sb02g003600: similar to Os07g0162700 protein	1.69	
TC119530	Sb02g038440: similar to Early nodulin 75-like protein	1.68	
TC127409	similar to Cluster: SJCHGC06399 protein; Schistosoma japonicum	1.62	
TC115192	Sb10g010810.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.53	
TC121465	Sb07g003730: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.53	
TC112125	Sb02g002720.1: similar to Early nodulin 75-like protein	1.50	
TC115421	Sb05g027150: similar to Cluster: Os08g0413100 protein; Oryza sativa Japonica Group	1.50	
TC132400	Sb04g006510: homologue to Cluster: Putative uncharacterized protein OJ1225_F07.7; Oryza sativa Japonica Group	1.48	
TC131851	Sb02g002710: weakly similar to Early nodulin 75-like protein	1.48	
TC111492	Sb09g005270.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.47	

TC124426	Sb09g030280.1: similar to Cluster: Putative sigma54 specific transcriptional regulator; Anaeromyxobacter sp. K	1.45
TC119943	Sb01g038150.1: similar to Cluster: Streptomyces cyclase/dehydrase family protein; Oryza sativa Japonica Group	1.41
TC133388	Sb02g033300: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.38
TC132362	Sb02g006130: homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1.33
TC123048	similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.32
TC125410	weakly similar to Cluster: Predicted protein; Nematostella vectensis	1.32
TC130975	Sb01g007870: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.31
TC126840	Sb02g023110: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.30
TC120928	Sb09g026920: Cluster: PREDICTED: hypothetical protein; Gallus gallus	1.28
TC120814	Sb10g010810.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.28
TC122043	Sb05g020820: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.28
TC118029	Sb01g001520 : similar to ARTEMIS protein - integral to membrane	1.25
TC122020	Sb04g026020.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.25
TC113224	Sb03g003830 : similar to 3'-N-debenzoyltaxol N-benzoyltransferase-like	1.25
TC112186	Sb09g022260 : similar to Putative uncharacterized protein OJ1651_D06.1	1.24
TC114114	Sb04g025690: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.24
TC116364	Sb01g001520: integral to membrane	1.22
TC127814	Sb03g027790: homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.22
TC119031	Sb03g034430: similar to Cluster: Putative uncharacterized protein P0481E12.8-1; Oryza sativa Japonica Group	1.22
TC124743	Sb04g030470.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.22
TC117021	Sb01g041580.2: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.22
TC124008	Sb09g020040 : similar to Os05g0406200 protein - Inosine-uridine preferring nucleoside hydrolase	1.18
TC133428	Sb06g033160 : similar to Os04g0680400 protein - hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	1.18
TC131366	Sb06g033430: similar to Cluster: Putative uncharacterized protein P0481E12.8-1; Oryza sativa Japonica Group	1.17
TC126207	weakly similar to Cluster: MMPL domain protein; Caldicellulosiruptor saccharolyticus DSM 8903	1.12
TC131072	Sb06g023180: similar to Cluster: OSIGBa0115K01-H0319F09.9 protein; Oryza sativa	1.11
TC120119	Sb02g035720: similar to Cluster: Putative uncharacterized protein OJ1612_A04.103; Oryza sativa Japonica Group	1.10
TC111355	Cluster: Maturase K; Hyparrhenia hirta	1.09
TC113122	Sb02g020830.1: similar to Cluster: Os09g0307300 protein; Oryza sativa	1.09
TC112021	Sb09g028630: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.08
TC132113	Sb05g006423.1: similar to Cluster: Chr. undetermined SCAF1938 whole genome shotgun seq.;Tetraodon	1.08
TC133373	similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.07
TC132610	Sb02g028600.1: catalytic activity	1.07
TC118353	Sb06g023190.2: homologue to Cluster: OSIGBa0115K01-H0319F09.10 protein; Oryza sativa	1.06

TC122602	similar to Cluster: Putative uncharacterized protein; Caenorhabditis elegans	1.06
TC126199	Sb09g004330: similar to Cluster: Putative uncharacterized protein; Oryza sativa	1.06
TC130450	Sb02g003530 - Aminotransferase class I and II	1.06
TC120455	similar to Cluster: Putative acetoacetyl-coenzyme A thiolase; Oryza sativa Japonica Group - acyltransferase activity	1.06
TC112352	Sb07g027360: weakly similar to Cluster: Ternary complex factor-like; Oryza sativa Japonica Group	1.06
TC126275	Sb02g036133.1: similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1.06
TC131542	Sb04g018940: homologue to Cluster: Putative uncharacterized protein P0419C03.10-2; Oryza sativa Japonica Group	1.05
TC130268	Sb06g021860: weakly similar to OSJNBa0014K14.20 protein	1.05
TC115328	Sb06g021860: weakly similar to OSJNBa0014K14.20 protein	1.05
TC129736	Sb03g040420: WD domain, G-beta repeat	1.05
TC128969	Sb06g026710: similar to Cluster: H0307D04.13 protein; Oryza sativa	1.04
TC124992	Sb09g005330: similar to Cluster: Putative uncharacterized protein OSJNBa0029B02.7; Oryza sativa	1.03
TC122695	Sb04g025680: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.02
TC129836	Sb04g010240: Aminotransferase class IV	1.02
TC132597	Sb09g029040: similar to Cluster: Putative uncharacterized protein OJ1735_C10.3; Oryza sativa Japonica Group	1.02
TC129945	Sb10g026970: homologue to Cluster: OSJNBa0088H09.19 protein; Oryza sativa Japonica Group	1.02
TC121489	Sb03g036300: homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1.01
TC112844	Sb02g007460: hydrolase activity	1.01
TC119278	Sb06g026686: similar to Cluster: H0307D04.13 protein; Oryza sativa	1.00
TC130857	Sb09g005330: similar to Cluster: Putative uncharacterized protein OSJNBa0029B02.7; Oryza sativa	1.00
TC120682	Sb06g002220: similar to CBS domain containing protein-like - catalytic activity	1.00

Genes modulated at FTWS level 0.15.

Sorghum leaves sampled at FTSW 0.15 displayed 1205 differentially expressed gene were up-regulated (Tab. 4).

Cell rescue, defence and virulence was the most representative functional category with 130 up and 46 down-regulated genes (Tab. 4).

The genes with highest Log_2FC values were Sb09g018420, encoding for dehydrin DHN1 (mean Log_2FC 3.6) and genes encoding for different LEA proteins (mean Log_2FC 2.6; Tab. 8). It has been suggested that the role of LEA proteins in plant drought tolerance is due to their involvement in maintenance of cell membrane structure and ion balance, binding water, acting as molecular chaperones (Browne *et al*., 2002; Babu *et al*., 2004. Xiao et colleagues showed enhanced drought tolerance in natural field conditions of rice transformed with a LEA protein gene (Xiao *et al*., 2007).

Twenty one probes representing 14 different cytochrome P450 genes (Log_2FC ranging from 1 to 3) were detected, indicating an increment in abundance and expression level compared to the previous level of stress FTSW 0.25 (Tab. 8). The sorghum genome contains 326 cytochrome P450 sequences that help the plants to respond to stress (Paterson *et al*., 2009).

The level of induction of the GST1 gene Sb01g001230 increased to Log_2FC 2.1 compared to the two previous (T0.37, T0.25) stress levels (Tab. 5). Two additional genes, Sb02g043250 and Sb09g002800, both encoding for different kind of GSTs, appeared up-regulated at FTSW 0.15 with Log_2FC values of 1.8 and 1.2, respectively. Elevated level of expression was showed for genes involved in detoxification processes such as thioredoxins (mean Log_2FC 2.6) and a putative laccase LAC5-6 (multicopper oxidase) (Log_2FC 3.3; Tab. 8).

The genes involved in lipid metabolism, such as glycerophosphodiester phosphodiesterase reported before, increased their expression levels up to Log_2FC 3.1. Lypoxygenases genes Sb06g031350 and Sb03g042440, probably induced by oxidative stress related to drought, reached Log_2FC values > 2. High Log_2FC values were associated to 2 GDSL-like lipase/acylhydrolase family proteins (Log_2FC 2.1 and 2.0), a phosphatidylinositol phosphatase and to genes encoding for proteins involved in fatty acid biosynthesis.

The aminoacids metabolism was affected by the increased water depletion. The most over-expressed genes encoded for delta-1-pyrroline-5-carboxylate synthetase (P5CS) and glutamine-dependent asparagine synthetase (AS; Tab. 8). P5CS is the key enzyme for proline biosynthesis: under the glutamate pathway, proline is synthesized from glutamic acid via the intermediate γ-glutamic semialdehyde (GSA) and Δ1-pyrroline-5- carboxylate (P5C). Δ1-pyrroline-5-carboxylate synthetase (P5CS), which catalyzes the first two reactions of proline biosynthesis, is a bifunctional enzyme containing both the γ-glutamyl kinase and the glutamic-γ-semialdehyde dehydrogenase activities. The γ- glutamyl kinase activity of P5CS represents the rate-limiting step in this pathway and also features feedback inhibition by proline. Proline is an important compatible organic osmolyte and its accumulation plays an important protective role in plants exposed to abiotic stress that alter water status (Xiao *et al*., 2007; Verslues *et al*., 2006; Ashraf, 2010). Several transgenic plants transformed with P5CS (Ronde *et al*., 2004; Yamada *et al*., 2005; Gubis *et al*., 2007) showed enhanced accumulation of proline as well as high drought tolerance. More in detail, two different genes with P5CS activity, Sb09g022290 and Sb03g039820, were detected. In accordance with our results, in a recent study on bioenergy sorghum, the two genes, called SbP5CS1 and SbP5CS2, located on chromosomes 3 and 9, respectively, were isolated and was demonstrated the induction of these genes by drought and salt stresses in a ABA-dependent manner (Su *et al*., 2011). The induction of AS and the consequent asparagine accumulation was demonstrated during water stress in sugarcane (Iskandar *et al*., 2011).

Genes involved in sugar metabolism play an important role in the response of sorghum IS 19453 to drought (Tab. 5). One hundred differentially expressed genes were detected, 53 of which were up-regulated. The gene Sb03g034280, encoding for NADP-malic enzyme (NADP-ME), presented the highest Log_2FC value (5.1; Tab. 8). NADP-ME is widely distributed in plant, which mainly appear in mitochondria, chloroplast and cytoplasm Detarsio *et al*., 2003). It can catalyze the oxidative decarboxylation of malate to produce pyruvate, CO2 and NADPH under metallic ion (Mg2+, Mn2+ etc.) (Edwards and Andreo, 1992). Therefore, it is one of the critical enzymes in malate metabolism, which play an important role in plant development. It can keep osmotic potential of cell, stabilize pH of cytoplasm and keep balance the ion absorption (Detarsio *et al*., 2003; Martinoia and Rentsch, 1994; Drincovich *et al*., 2001). Drought stress suppresses the extention of leaf, causes the close of stoma, reduces the absorption of CO2, raises the resistance of mesophyll cell and decreases the activity of some enzymes. Water deprivation affects CO2 fixation, destroys the structure of chloroplast and reduces the content of chlorophyll. The last result is suppressing the photosynthesis and lowering the photosynthetic rate in wheat under drought stress (Fu *et al*., 2009; Sun *et al*., 2004)

Because of the function of NADP-ME in malate metabolism and photosynthesis, the NADP-ME plays an important role in anti-drought. The expression of NADP-ME gene is induced by drought to resist drought (Wu *et al*., 2008). The leaf stoma is closed because of drought stress, which would decrease the content of malate in cell and raise the NADP-ME activity.

The mechanisms of NADP-malic enzyme resist drought stress. In fact, the function of NADP-ME is to catalyze the delivery of CO2 in photosynthesis II (Zhang, 2003). In order to keep the reaction of photosynthesis II, plant can increase the content of NADP-ME to reinforce the delivery and fixing of CO2 when the absorption of CO2 is decreased because of the shut of stoma under drought stress. The goal is to compensate the deficiency of CO2 in this situation. This theory can explain the reinforcement of C4 enzyme system in C3 plant under drought stress (Wu *et al*., 2008). At the same time, the protein of NADP-ME is hydrophilicity, which can protect cells through increasing the osmotic pressure of cell and decreasing the water loss (Schroeder *et al*., 2001; Li *et al*., 2009). NADP-ME is involved in C_4 cycles and has an important role in the production of NADPH, which meets Calvin cycles requirement for reducing power. Nevertheless experiments on drought-tolerant C3 species, like barley, revealed a role for NADP-ME in control of stomatal closure, indicating that its up-regulation could be a tool for drought avoidance and water conservation (Doubnerová *et al*., 2011).

Drought induced major expression of the phosphoenolpyruvate carboxylase (PEPC) enzyme involved in C_4 cycle, even if with low Log_2FC values (1.3; Tab. 8). Aconitate hydratase enzyme (Sb02g034590) was strongly up-regulated (mean Log_2FC 4.1). Aconitate hydratase catalyzes the formation of isocitrate from citrate. This one serves as critical control point in the tricarboxylic acid cycle of respiration process (Zhao *et al.*, 2011). Several citrate cycle- related proteins, including aconitate hydratase, exhibited increases in abundance under salt or osmotic stress in Arabidopsis (Ndimba *et al.*, 2005). The increases in the abundance of aconitate hydratase in perennial bermudagrass genotypes may be reflective of respiratory adjustment for adaptation to water deficit (Zhao *et al.*, 2011).

Several genes implied in sugar metabolism like glycosyl hydrolase family protein (Sb04g000620), alkaline alpha galactosidase 1 (Sb02g006320) and other genes involved in starch biosynthesis and degradation such as glucose-1-phosphate adenylyltransferase large subunit 1 (Sb09g029610) and β-amylase (Sb01g047500) showed high up-regulation (Tab. 8), with Log_2FC values ranging from 1.8 to 3.3. Drought slightly promoted sucrose synthesis, as indicated by the induction of sucrose synthase 3 gene (Sb01g035890, Log_2FC 1.6). As showed in Tab. 7 and 8, β-amylase was up regulated in stressed plants indicating that the source of sucrose during drought stress may occur also from starch. The induction of β-amylase has been demonstrated under water and salinity stress (Todaka *et al.*, 2000; Yang *et al.*, 2001; Seki *et al.*, 2001; Rizhsky *et al.*, 2004). In contrast, cell wall invertase (CWI), encoded by the Sb04g021810 gene, was down-regulated in stressed plants (mean Log_2FC 2.5).

Invertases catalyze the irreversible hydrolysis of sucrose into its constituent monosaccarides fructose and glucose and have been classified into three isoforms based on optimal pH requirements and subcellular localization (reviewed by Roitsch and Gonzalez, 2004). Soluble acid invertases are present in vacuoles; whereas the insoluble acid invertases are localized to the apoplast, usually ionically bound to the cell wall. A major role of CWIs in apoplastic cleavage of sucrose, and establishment and maintenance of sink metabolism has been illustrated across various plant species, including tomato (Godt and Roitsch, 1997), fava bean (Weber et al., 1996), barley (Weschke *et al.*, 2003;

Sreenivasulu *et al*, 2004), rice (Hirose *et al*., 2002; Cho *et al*., 2005; Ishimaru *et al*., 2005) and Arabidopsis (Sherson *et al*., 2003).

The induction of β-amylase has also been demonstrated under a number of abiotic stresses, such as water stress and salinity stress (Yang *et al*., 2001; Todaka *et al*., 2000; Seki *et al*., 2001).

The response of Arabidopsis to a combination of drought and heat stress revealed the expression of β-amylase. (Rizhsky *et al*., 2004). In Tab.7-8, β-amylase was up regulated in stressed plants. Thus, the synthesis of sucrose during a combination of drought and heat stress may occur from starch. In this experiment, it was excluded that CWI could catalyze the hydrolysis of sucrose because their down regulation in stress conditions. Additional studies are, however, required to examine this possibility.

In addition, two genes involved in glycolisis pathway (Sb07g012720, Sb09g006030) were up regulated in T0.15. The activity of 6- phosphofructokinase rises when the ATP becomes scarce in the cell, or when ATP is crushed in AMP.

ABA concentration was negatively correlated with β-amylase activity during barley grain filling (Wei *et al*., 2009). In this work, the up regulation of β-amylase and the absence of gene regulation of ABA in stressed samples (Tab. 8), support the results of Wei et colleagues.

Transport category of genes was represented by 98 differentially regulated sequences, 78 of which were up-regulated. Water deficit influenced the expression of several sugar, lipid, aminoacid and salt transporters, and aquaporins (Tab. 8).

The gene Sb03g001130, encoding for an AAA ATPase family protein, included in the nucleotide binding functional class, presented the highest values of up-regulation at FTSW 0.15 (Log_2FC 5.98; Tab. 8). AAA family proteins are a class of chaperone-like ATPases associated with a variety of cellular activities such as assembly, and disassembly of protein complexes in stressed conditions (Neuwald *et al.*, 1999). The AAA-type ATPases activity was determined crucial for Rubisco activity in Arabidopsis under cold stress (An *et al.*, 2011). Other activities associated with AAA modules include peroxisome biogenesis, assembly of mitochondrial membrane proteins, cell-cycle control, mitotic spindle formation, cytoskeleton interactions, vesicle secretion, signal transduction, and transcription (Beyer *et al*., 1997; Waterham *et al*., 1997; Subramani *et al*., 1998). Another gene with chaperone-like ATPases function, Sb01g016970, was up-regulated (Log$_2$FC 1.38) in stressed leaves of sorghum at FTSW 0.25 (Tab. 7). On the other hand, Sb08g023150 and Sb02g005630 genes, involved in cell division and ATPase activity, respectively, were down regulated.

The nucleic acid functional class resulted strongly induced by drought at FTSW 0.15, with 100 genes involved in stress response, 66% of them up-regulated (Tab. 4). The ABRE-binding factor BZ-1, a member of bZIP transcription factors (bZIP TFs) family, increased its expression level respect to the FTSW 0.25 (Tab. 8). bZIP TFs are involved in various biological processes under normal and stressed growth conditions. Accumulated data show that bZIP TFs have been regarded as important regulators in response to various biotic/abiotic stresses, signaling, such as hormone and sugar signaling (Finkelstein *et al*., 2000; Niggeweg *et al*., 2000; Uno *et al*., 2000; Nieva *et al*., 2005) and osmotic stresses (Satoh *et al*., 2004; Weltmeier *et al*., 2006). Some memebers of the bZIP family are induced by drought, respond to ABA and activate expression of genes containing ABRE elements (Jakoby *et al*., 2002).

Drought stress affected several genes involved in communication and signaling: 69 genes were up-regulated while 35 were down-regulated at FTSW 0.15 (Tab. 4). Sb08g023000, encoding for a calcineurin-like phosphoesterase, resulted up-regulated over the time course of 5 days of water stress (Tab. 5). Calcineurin-like phosphoesterases are members of hydrolases family proteins. These are likely to be involved in the decomposition of damage products in bacteria under stress conditions (Makarova *et al*., 2001). In a CDNA-AFLP profile of cotton genes in response to drought stress, calcineurin-like phosphoesterase gene was up regulated in stressed samples (Feng *et al*., 2003). Some other expanded families are even more directly related to stress-response systems, for example, proteins involved in tellurium resistance (TerE) and homologs of plant pathogenesis-related proteins (PR1) (Makarova *et al*., 2001).

In the Pfam databases, Sb08g023000 matches calcineurin-like phosphoesterase (Pfam: PF12146). This family includes a diverse range of phosphoesterases, including protein phosphoserine phosphatases, nucleotidases, sphingomyelin phosphodiesterases, and 2’3’-

cAMP phosphodiesterases. This family also shows remote homology to the C-terminus of a lariat debranching enzyme. The most conserved regions in this superfamily are centered on the metal chelating residues.

Another conserved domain, found in the small subunits of archaeal DNA polymerase II and eukaryotic DNA polymerases α and δ, is shown to belong to the superfamily of calcineurin-like phosphoesterases, which unites a variety of phosphatases and nucleases (Aravind *et al*.,1998).

Experimental elucidations of the role of calcineurin-like phosphoesterase are needed in order to understand the pathway of regulation in which they are involved.

Phosphatase 2C proteins, acid phosphatases and serine/threonine-protein kinases increased their amount and their expression level at FTSW 0.15, indicating a strong induction of signal transduction pathway by water stress.

Tab. 8 - Up and Down-regulated genes in sorghum IS19453 genotype at FTSW 0.15.

UP-REGULATED			
ID	**Annotation**	**Log$_2$FC**	**Functional class**
TC118527	Sb09g018420: similar to Dehydrin DHN1	5,02	Cell rescue, defence and virulence
TC131575	Sb09g018420: similar to Dehydrin DHN1	4,87	
TC129062	Sb09g018420: similar to Dehydrin DHN1	4,84	
TC123196	Sb01g046000.1 - Late embryogenesis abundant protein	3,56	
TC130246	Sb03g039530 : similar to Putative laccase LAC5-6 - Multicopper oxidase	3,37	
TC128324	Sb01g001180.1 : similar to Cytochrome P450 71E1	3,12	
TC127817	Sb09g027110 : similar to Late embryogenesis abundant protein, group 3	3,04	
TC120066	Sb01g001180 : similar to Cytochrome P450 71E1	3,04	
TC111396	Sb01g001200 : similar to Cytochrome P450 79A1	2,99	
TC118856	Sb01g001180 : similar to Cytochrome P450 71E1	2,95	
TC129358	Sb01g001180: similar to Cytochrome P450 71E1	2,94	
TC130394	Sb06g000660 : similar to Heat shock protein 82	2,93	
TC114753	Sb01g001180 : similar to Cytochrome P450 71E1	2,87	
TC120709	Sb03g036980 : similar to Os01g0794400 protein - THIOREDOXIN - Oxidoreductase	2,85	
TC121909	Sb06g027090 : similar to OSJNBa0006A01.5 protein - Pfam: Polyketide cyclase / dehydrase and lipid transport	2,71	
TC133561	Sb01g001180 : similar to Cytochrome P450 71E1	2,65	
TC116336	Sb07g000500 Belongs to the cytochrome P450 family	2,60	
TC113948	Sb01g016900 - Belongs to the cytochrome P450 family	2,56	
TC112299	Sb07g000500 : similar to Os08g0106000 protein -Belongs to the cytochrome P450 family - monooxygenase activity	2,53	
TC115879	Sb03g036980 THIOREDOXIN - cell redox homeostasis Belongs to the nucleoredoxin family	2,47	
TC113222	Sb08g018720 -Thaumatin-like cytokinin-binding protein	2,33	
TC121822	Sb09g030570 cysteamine dioxygenase activity - oxidation-reduction process	2,29	
TC115054	Sb06g008990 oxidoreductase activity	2,27	
TC114294	Sb01g008730 : similar to Alcohol dehydrogenase 1 - oxidation-reduction process	2,14	
TC134071	Sb01g001230.2: similar to Glutathione S-transferase 1	2,14	
TC127185	Sb08g018780 Belongs to the cytochrome P450 family	2,13	
TC119059	Sb03g033900 -Pfam Late embryogenesis abundant protein	2,12	
TC129533	Sb07g000500.1 : similar to Os08g0106000 protein -Belongs to the cytochrome P450 family - monooxygenase	2,10	
TC130570	Sb06g028970 oxidoreductase activity	2,08	

TC115199	Sb01g015040 Belongs to the cytochrome P450 family	1,96
TC112519	Sb01g016900 Belongs to the cytochrome P450 family	1,88
TC131830	Sb02g043250.2 - Pfam Glutathione S-transferase	1,84
TC133752	Sb02g009120.2: similar to Probable aldehyde oxidase 4 - metal ion binding	1,83
TC119558	Sb01g036790 : similar to Late embryogenesis abundant protein 1	1,82
TC112605	Sb06g014550 : similar to Os03g0439500 protein - Belongs to the iron/ascorbate-dependent oxidoreductase family	1,80
TC129311	Sb01g001230.2: similar to Glutathione S-transferase 1	1,79
TC116069	Sb06g002820 - Terpene synthase - lyase activity	1,78
TC115619	Sb05g016850 similar to OSJNBa0081C01.20 protein - short chain dehydrogenase - oxidoreductase activity	1,77
TC131239	Sb06g021980: similar to Aldo/keto reductase family-like protein	1,75
TC129109	Sb03g032720 cytidine deaminase activity zinc ion binding	1,73
TC121326	Sb09g026970 Belongs to the chaperonin (HSP60) family	1,72
TC112953	Sb07g006090 oxidoreductase activity	1,72
TC122100	Sb10g005170 oxidoreductase activity	1,72
TC133789	Sb04g004850 Belongs to the cytochrome P450 family	1,71
TC126117	Sb07g022250.1 oxidation reduction	1,70
TC122313	Sb06g029870 : similar to H0315F07.12 protein - Wound-induced protein	1,69
TC129730	Sb06g021980 : similar to Aldo/keto reductase family-like protein (Betaine aldehyde dehydrogenase, Zea mays)	1,69
TC115678	Sb10g003700 : weakly similar to Dehydrin	1,68
TC125045	Sb02g009120.2: similar to Probable aldehyde oxidase 4 -metal ion binding	1,66
TC133679	Sb01g019000 cytokinin metabolic process	1,64
TC112345	Sb01g032640 (homologue to Cluster: Dehydrin; Zea mays)	1,64
TC111493	Sb01g042270: similar to Allene oxide synthase	1,62
TC133270	Sb01g013080.1 Belongs to the short-chain dehydrogenases/reductases (SDR) family	1,62
TC124367	Sb01g038760 : similar to L-ascorbate peroxidase 1, cytosolic	1,60
TC119258	Sb09g018420 : similar to Dehydrin DHN1	1,57
TC120399	Sb06g001960 oxidation reduction flavin-containing monooxygenase activity	1,55
TC114797	Sb01g015110 : similar to DSBA-like thioredoxin domain containing protein, expressed	1,54
TC130097	homologue to Cluster: Cytochrome B; Neisseria meningitidis serogroup A	1,53
TC133005	Sb06g002820 - Terpene synthase - lyase activity	1,51
TC118294	Sb07g003070.1 - oxidoreductase activity	1,51
TC130297	Sb02g009410 - Belongs to the cytochrome P450 family	1,48
TC130444	Sb06g017080 : similar to Probable L-ascorbate peroxidase 7, chloroplast precursor	1,47

TC118989	Sb03g003550 - 2OG-Fe(II) oxygenase superfamily	1,46
TC118698	Sb01g020210 Zinc-finger protein binding	1,45
TC112140	Sb07g003070 - oxidoreductase activity	1,45
TC124287	Sb09g028890 iron-sulfur cluster assembly protein binding	1,43
TC119563	Sb01g031410 oxidoreductase activity	1,43
TC114186	Sb02g010910 : similar to Cytochrome P450	1,42
TC124500	Sb08g016390 : similar to Iron-deficiency specific clone No.3 - 2'-deoxymugineic-acid 2'-dioxygenase activity	1,41
TC115670	Sb03g039360 : similar to Heat shock 70 kDa protein	1,40
TC111419	Sb03g041600 - oxidation-reduction process	1,39
TC112150	Sb02g030830 : similar to Senescence-inducible chloroplast stay-green protein	1,38
TC111486	Sb01g044230 : similar to Flavin containing polyamine oxidase precursor	1,36
TC131489	Sb04g005570.1 - Chaperone	1,36
TC114217	Sb06g021980 : similar to Aldo/keto reductase family-like protein	1,36
TC122211	Sb02g036870 - copper ion binding	1,35
TC130644	Sb01g034460 Belongs to the cytochrome P450 family	1,35
TC112788	Sb09g026810 ALDEHYDE DEHYDROGENASE-RELATED;1-pyrroline-5-carboxylate dehydrogenase	1,34
TC127560	Sb01g001660 - Pfam Late embryogenesis abundant protein	1,33
TC127862	Sb02g036870 copper ion binding	1,32
TC118534	Sb01g020210 : similar to CHY zinc finger family protein	1,32
TC125456	Sb10g022220 Belongs to the TCP-1 chaperonin family	1,31
TC129764	Sb04g035130 - SMALL HEAT-SHOCK PROTEIN (HSP20) FAMILY	1,31
TC111776	Sb04g000830 : similar to Hydroperoxide lyase	1,30
TC111979	Sb09g026810 ALDEHYDE DEHYDROGENASE-RELATED; 1-pyrroline-5-carboxylate dehydrogenase	1,30
TC111756	Sb01g020210 : similar to CHY zinc finger family protein	1,29
TC123608	Sb03g016720 metal ion binding	1,28
TC123297	Sb01g014550 : similar to Putative uncharacterized protein - iron/ascorbate-dependent oxidoreductase family.	1,28
TC111696	Sb04g001810 : similar to Terpene synthase 2 - lyase activity	1,27
TC132797	Sb01g008730 : similar to Alcohol dehydrogenase 1	1,26
TC118311	Sb06g025970 Copper/zinc superoxide dismutase (SODC) - oxidation-reduction process	1,25
TC127377	Sb04g033350 Belongs to the cytochrome b5 family heme binding	1,25
TC118219	Sb06g028970 oxidoreductase activity	1,24
TC114257	Sb06g025990 Belongs to the cytochrome P450 family	1,24
TC124161	Sb04g002330 Belongs to the small heat shock protein (HSP20) family	1,24

TC120398	Sb04g001810 : similar to Terpene synthase 2 - lyase activity	1,23
TC119878	Sb02g032640 oxidation reduction	1,22
TC116962	Sb06g033680 zinc ion binding	1,22
TC126391	Sb02g041880 cell redox homeostasis	1,22
TC122527	Sb01g042680 Belongs to the heat shock protein 70 family	1,22
TC111995	Sb03g028560 : similar to Cytochrome P450-like	1,19
TC128899	Sb03g045760 : similar to Phytochromobilin synthase precursor - oxidation-reduction process	1,19
TC129441	Sb09g002800.1 - Glutathione S-transferase	1,18
TC119199	Sb07g022750 : similar to Nitrate reductase [NADH] 1	1,17
TC111626	Sb09g000980 - Terpene synthase family - lyase activity	1,17
TC131815	Sb01g003960 : similar to OSJNBa0081C01.23 protein - short-chain dehydrogenases/reductases (SDR) family	1,17
TC121089	Sb09g019740 oxidoreductase activity	1,16
TC111762	Sb04g005950 : similar to Cinnamyl alcohol dehydrogenase	1,15
TC118488	Sb09g002800 - Glutathione S-transferase	1,15
TC133311	Sb01g032910 cell redox homeostasis	1,13
TC113517	Sb06g025990 Belongs to the cytochrome P450 family	1,13
TC121747	Sb01g003960 : similar to OSJNBa0081C01.23 protein - short-chain dehydrogenases/reductases (SDR) family	1,13
TC112516	Sb01g006990 thioredoxin	1,13
TC122358	Sb06g029440 : similar to H0323C08.3 protein - zinc ion binding	1,12
TC117537	Sb06g000790 - similar to Cluster: Triticum aestivum sulfur-rich/thionin-like protein	1,11
TC120638	Sb10g021690 oxidoreductase activity	1,09
TC120341	Sb06g025970 - Copper/zinc superoxide dismutase (SODC) - superoxide metabolic process metal ion binding	1,09
TC128696	Sb10g021620 : similar to Peroxidase precursor	1,09
TC112902	Sb04g016970 Belongs to the cytochrome P450 family	1,08
TC113388	Sb01g017050 Belongs to the heat shock protein 70 family	1,06
TC115834	Sb04g035220 : similar to Lysine-ketoglutarate reductase/saccharopine dehydrogenase bifunctional enzyme	1,06
TC115530	Sb07g021070 - dihydrolipoyllysine-residue acetyltransferase activity - 2-oxoacid dehydrogenases acyltransferase	1,06
TC119912	Sb01g047120 : similar to Lethal leaf-spot 1 - oxidoreductase activity	1,05
TC131819	Sb09g019740 oxidoreductase activity	1,04
TC130182	Sb03g006090.1 - metal ion binding (Metallothinein-like protein; Saccharum officinarum)	1,03
TC111897	Sb03g013210 : similar to Class III peroxidase 15 precursor	1,03
TC112508	Sb03g004270 - NADH-CYTOCHROME B5 REDUCTASE	1,03
TC114469	Sb10g021610 : similar to Peroxidase precursor	1,02

TC133383	similar to Cluster: Possible oxidoreductase subunit; Mycobacterium leprae	1,01	
TC115257	Sb08g004880 : similar to Probable L-ascorbate peroxidase 6, chloroplast precursor	1,00	
TC117579	Sb04g021010 : similar to Glycerophosphoryl diester phosphodisterase	3,16	Lipid metabolism
TC124597	Sb04g021010: similar to Glycerophosphoryl diester phosphodisterase	3,07	
TC115037	Sb06g014320 : similar to H0718E12.3 protein - glycerophosphodiester phosphodiesterase activity	3,04	
TC118289	Sb06g014320 : similar to H0718E12.3 protein - glycerophosphodiester phosphodiesterase activity	2,65	
TC130362	Sb06g031350 : similar to Lipoxygenase	2,30	
TC122071	Sb04g028415 - GDSL-like Lipase/Acylhydrolase	2,15	
TC131792	Sb03g042440 : similar to Lipoxygenase	2,14	
TC113646	Sb08g007150.2 - GDSL-like Lipase/Acylhydrolase	2,04	
TC134222	Sb07g020740 inositol or phosphatidylinositol phosphatase activity	1,96	
TC127672	Sb01g014230 inositol or phosphatidylinositol phosphatase activity	1,94	
TC114595	Sb01g019590: similar to CER1 protein - fatty acid biosynthesis	1,89	
TC132142	Sb06g012520 Belongs to the fatty acid desaturase family	1,86	
TC112926	Sb01g019590 : similar to CER1 protein - fatty acid biosynthesis	1,73	
TC111378	Sb03g042440 : similar to Lipoxygenase	1,72	
TC131525	Sb01g044290 Myo-inositol-1-phosphate synthase	1,63	
TC114184	Sb03g042440 : similar to Lipoxygenase	1,63	
TC125725	Sb04g005330 fatty acid biosynthetic process oxidoreductase activity	1,61	
TC126834	Sb09g005960 - HAD superfamily, subfamily IIIB (Acid phosphatase) fatty acid biosynthetic process	1,54	
TC127036	Sb02g042310 : similar to Putative triacylglycerol lipase	1,42	
TC126430	Sb02g029710 lipid metabolic process	1,38	
TC121627	Sb01g029480 steroid metabolic process	1,37	
TC119981	Sb01g029480 steroid metabolic process	1,36	
TC130460	Sb09g029260 fatty acid biosynthetic process - transferring acyl groups other than amino-acyl groups	1,32	
TC113746	Sb09g019100 : similar to Putative UDP-sulfoquinovose synthase	1,29	
TC113885	Sb01g044290 Myo-inositol-1-phosphate synthase	1,28	
TC112895	Sb01g019590 : similar to CER1 protein - fatty acid biosynthesis	1,24	
TC133780	Sb06g031350 : similar to Lipoxygenase	1,22	
TC126010	Sb03g026750 lipid metabolic process hydrolase activity, acting on ester bonds	1,21	
TC123077	Sb03g042440 : similar to Lipoxygenase	1,19	
TC130811	Sb07g027480 Catalyzes the synthesis of mevalonate. The specific precursor of all isoprenoid compounds.	1,19	
TC128265	Sb09g019100 : similar to Putative UDP-sulfoquinovose synthase	1,19	

TC129004	Sb06g031350 : similar to Lipoxygenase	1,17	
TC112344	Sb1306s002010 - triglyceride lipase activity	1,15	
TC113968	Sb06g031350 : similar to Lipoxygenase	1,14	
TC126968	Sb06g031350 : similar to Lipoxygenase	1,13	
TC111430	Sb05g024380 Patatin-like phospholipase	1,13	
TC127891	Sb07g020750.1 - inositol or phosphatidylinositol phosphatase activity	1,11	
TC129259	Sb10g022680 lipid biosynthetic process cyclopropane-fatty-acyl-phospholipid synthase activity	1,11	
TC123277	Sb01g030160 : similar to Putative C-4 sterol methyl oxidase - fatty acid biosynthetic process	1,09	
TC128673	Sb05g005330 : similar to Putative uncharacterized protein - Acyl-CoA reductase	1,06	
TC119076	Sb03g002740 : similar to Lecithin:cholesterol acyltransferase family protein - lipid metabolic process	1,05	
TC118535	Sb03g032940 : similar to Os01g0716800 protein - inositol or phosphatidylinositol phosphatase activity	1,03	
TC116171	Sb09g022290 : similar to Delta-1-pyrroline-5-carboxylate synthetase (P5CS)	3,21	Aminoacid metabolism
TC128105	Sb05g000440 asparagine synthase (glutamine-hydrolyzing) activity	2,15	
TC117845	Sb03g039820.1: similar to Delta-1-pyrroline-5-carboxylate synthetase (P5CS)	2,07	
TC116797	Sb05g000440 asparagine synthase (glutamine-hydrolyzing) activity	1,80	
TC113060	Sb09g022290 : similar to Delta-1-pyrroline-5-carboxylate synthetase (P5CS)	1,77	
TC128346	Sb09g018200 thiamin diphosphate biosynthetic process	1,54	
TC134263	Sb10g029510 tyrosine biosynthetic process	1,47	
TC124636	Sb01g023750 Aminotransferase class I and II (Alanine aminotransferase; n=1; Zea mays)	1,47	
TC117068	Sb03g031880 threonine biosynthetic process	1,44	
TC123220	Sb10g002070 : similar to Arginine decarboxylase	1,42	
TC128219	Sb03g041410.1 glycine hydroxymethyltransferase activity L-serine metabolic process	1,38	
TC115769	Sb06g022740 L-phenylalanine = trans-cinnamate + ammonia	1,24	
TC118846	Sb03g031230 histidine catabolic process	1,23	
TC133628	Sb04g034910 cellular amino acid derivative biosynthetic process intramolecular lyase activity	1,22	
TC111655	Sb07g022670 : similar to GAD1 - glutamate decarboxylase	1,21	
TC112089	Sb03g033120 cysteine biosynthetic process from serine	1,21	
TC119054	Sb10g002230 : similar to 3-phosphoshikimate 1-carboxyvinyltransferase - Amino-acid biosynthesis	1,21	
TC124267	Sb04g005350 - KOG:3179 Predicted glutamine synthetase	1,21	
TC115006	Sb01g011750 similar to Glutamine amidotransferase class-I family protein - glutamine metabolic process	1,19	
TC121240	Sb04g025560 branched chain family amino acid biosynthetic process	1,19	
TC122932	Sb03g041410 glycine hydroxymethyltransferase activity	1,18	
TC115337	Sb04g004720 : similar to Putative glycine decarboxylase complex H-protein	1,18	

TC126019	Sb01g038460 : similar to Asparagine synthetase	1,14	
TC131491	Sb04g034910.1 intramolecular lyase activity cellular amino acid derivative biosynthetic process	1,12	
TC113201	Sb01g010270 : similar to Glutamine synthetase root isozyme 2	1,10	
TC126208	Sb01g017060 Belongs to the trans-sulfuration enzymes family cellular amino acid metabolic process	1,08	
TC129194	Sb10g002230 : similar to 3-phosphoshikimate 1-carboxyvinyltransferase	1,06	
TC115690	Sb04g026510 : similar to Phenylalanine ammonia-lyase - drought recovery	1,06	
TC116351	Sb04g018290 tryptophan metabolic process	1,04	
TC124705	Sb08g002620 : similar to Chalcone-flavanone isomerase family protein, expressed	1,02	
TC122011	Sb06g019160 Glutamine amidotransferase class-I - glutamine metabolic process	1,02	
TC120631	Sb03g034280 : similar to Oxidoreductase precursor - NADP+-dependent malic enzyme	5,12	Sugar metabolism
TC119198	Sb02g034590 : similar to Putative aconitate hydratase, cytoplasmic	4,57	
TC126147	Sb02g034590 : similar to Putative aconitate hydratase, cytoplasmic	3,70	
TC117813	Sb01g001220: similar to UDP-glucose glucosyltransferase	3,29	
TC133184	Sb04g000620 Belongs to the glycosyl hydrolase 32 family - carbohydrate metabolic process	3,16	
TC116398	Sb03g034280 : similar to Oxidoreductase precursor - NADP+-dependent malic enzyme	3,06	
TC126325	Sb10g025880 - similar to GDP-L-fucose synthase 2	2,82	
TC130541	Sb02g006320: similar to Alkaline alpha galactosidase 1 - catalytic activity	2,67	
TC114929	Sb01g036420 similar to 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	2,56	
TC115239	Sb09g029610 Glycan biosynthesis; starch biosynthesis	2,54	
TC133661	Sb04g000620 Belongs to the glycosyl hydrolase 32 family	2,40	
TC118037	Sb10g008820 glycolysis pyruvate dehydrogenase (acetyl-transferring) activity - glycolysis	2,37	
TC113073	Sb09g029610 Glycan biosynthesis; starch biosynthesis	2,32	
TC125869	Sb02g006320: similar to Alkaline alpha galactosidase 1 - catalytic activity	2,25	
TC129193	Sb01g004130 - similar to Putative raffinose synthase or seed imbibition protein - catalytic activity	2,24	
TC129891	Sb02g006320: similar to Alkaline alpha galactosidase 1 - catalytic activity	2,17	
TC121521	Sb02g038770 cellular cell wall organization	1,95	
TC121618	Sb01g047500.1: similar to Beta-amylase	1,88	
TC133905	Sb01g047500.1: similar to Beta-amylase	1,82	
TC122545	Sb08g004400 : similar to Amidase family protein	1,76	
TC111669	Sb08g007610: similar to Cyanogenic beta-glucosidase dhurrinase-2	1,64	
TC127341	Sb02g007740 : similar to Beta-glucosidase aggregating factor	1,64	
TC127192	Sb01g035890 : similar to Sucrose synthase 3	1,63	
TC120740	Sb06g016310 : similar to Extensin precursor - Cell wall biogenesis/degradation	1,62	

TC127526	Sb10g027110.1 hydrolase activity, hydrolyzing O-glycosyl compounds carbohydrate metabolic process	1,59	
TC129914	Sb01g002630 hydrolase activity, hydrolyzing O-glycosyl compounds carbohydrate metabolic process	1,57	
TC112535	Sb09g026150 glycolysis 6-phosphofructokinase complex	1,50	
TC131280	Sb03g005240: similar to chr8 scaffold_115 - thiamine pyrophosphate enz. (Pyruvate decarboxylase isozyme 2, maize)	1,47	
TC130593	Sb01g047500.1: similar to Beta-amylase	1,47	
TC125970	Sb01g019850: similar to Beta-amylase	1,38	
TC130736	Sb03g035090.1: similar to Phosphoenolpyruvate carboxylase	1,36	
TC118331	Sb09g005810 : similar to Malic enzyme	1,34	
TC114796	Sb08g014630 : similar to L-galactose dehydrogenase	1,27	
TC119287	Sb03g035090.1: similar to Phosphoenolpyruvate carboxylase	1,27	
TC115845	Sb06g020720 : similar to Malate synthase, glyoxysomal	1,27	
TC128671	Sb07g027200 : similar to Su1p - Isoamylase type debranching enzyme; Sorghum bicolor	1,26	
TC112264	Sb04g008720 phosphoenolpyruvate carboxylase activity	1,23	
TC127441	Sb06g022490 Belongs to the glycosyl hydrolase 1 family	1,22	
TC122443	Sb01g049330 cell wall macromolecule catabolic process	1,21	
TC129443	Sb04g021540.1 complete carbohydrate metabolic process	1,20	
TC115351	Sb02g023480 : similar to Enolase	1,19	
TC128562	Sb01g018410 : similar to Putative alpha-galactosidase	1,18	
TC115864	Sb01g007560 : similar to Alpha 1,4-glucan phosphorylase L isozyme	1,14	
TC118361	Sb03g042690 Belongs to the glycosyl hydrolase 1 family	1,12	
TC133104	Sb06g001540 : similar to Pullulanase-type starch debranching enzyme	1,08	
TC133996	Sb09g019350 hydrolase activity, hydrolyzing O-glycosyl compounds carbohydrate metabolic process	1,08	
TC112189	Sb09g018160 Belongs to the glycosyl hydrolase 1 family carbohydrate metabolic process	1,05	
TC112055	Sb04g004750 - Putative glyceraldehyde-3-phosphate dehydrogenase	1,05	
TC111666	Sb03g042690 Belongs to the glycosyl hydrolase 1 family	1,04	
TC124419	Sb04g035020 - polygalacturonase activity	1,03	
TC122975	Sb04g005960: similar to Putative Limonoid UDP-glucosyltransferase	1,03	
TC115970	Sb04g004920 - Aldehyde dehydrogenase	1,02	
TC115686	Sb03g003220 : similar to NADP-dependent malic enzyme	1,01	
TC124266	Sb04g021000 : similar to uncharacterized protein - KOG:1623 Multitransmembrane protein	4,44	Transport
TC115722	Sb04g021000 - Pfam: nodulin MtN3 family protein - Sugar transport	3,87	
TC123141	Sb06g016160 - LTP family - lipid transport	3,28	
TC118711	Sb06g016160 - LTP family - lipid transport	3,15	

TC116757	Sb03g011240: similar to Major facilitator superfamily antiporter - transmembrane transport	3,09
TC117290	Sb03g038470 : similar to Os01g0825800 protein - Putative N system amino acids transporter NAT-1	3,06
TC113214	Sb01g010500 : similar to Plastocyanin-like domain - electron carrier activity	2,88
TC117699	Sb08g001710 : similar to MATE efflux family protein	2,63
TC119038	Sb01g037560.1- Tim17/Tim22/Tim23 family - Mitochondrial import inner membrane translocase subunit	2,16
TC121409	Sb01g010500 : similar to Plastocyanin-like domain	2,15
TC132649	Sb08g002690 Non-specific lipid-transfer protein 2 precursor	2,15
TC116826	Sb01g001240.2 - transmembrane transport	2,13
TC134462	Sb01g044090 : similar to Sulfate transporter ST1	2,09
TC127123	Sb08g002690 : similar to Non-specific lipid-transfer protein 1 precursor	2,04
TC118722	Sb10g008090 : similar to Aquaporin NIP2-3	2,03
TC113409	Sb01g010670 : similar to Integral membrane protein DUF6 containing protein	2,00
TC116704	Sb03g035270 transporter activity	1,98
TC132531	Sb04g032430 Belongs to the MIP/aquaporin family	1,94
TC129606	Sb03g039880: similar to Non-specific lipid-transfer protein	1,93
TC111472	Sb08g002670 : similar to Non-specific lipid-transfer protein	1,86
TC129682	Sb02g025930 - Similar to Os09g0554000 protein - Belongs to the mitochondrial carrier family	1,80
TC131223	Sb01g050580 - Pfam:00083 Sugar (and other) transporter	1,80
TC113356	Sb01g038660 : similar to Phosphatidylinositol transfer protein	1,79
TC130134	Sb05g001760.1 antiporter activity	1,74
TC115821	Sb02g036340.1 - microtubule motor activity	1,72
TC125538	Sb06g027060 - SNARE associated Golgi protein - transport	1,70
TC115350	Sb10g026090 voltage-gated chloride channel activity	1,65
TC114763	Sb08g001400 : similar to Transporter - transmembrane transport	1,65
TC111549	Sb01g042260 - iron ion binding - oxygen transport	1,59
TC132660	Sb10g008090 Belongs to the MIP/aquaporin family (NOD26-like major intrinsic protein)	1,51
TC111382	Sb04g028020: similar to Aquaporin NIP2-1 - Belongs to the MIP/aquaporin family	1,50
TC127710	Sb03g001113 - Pfam:03595 C4-dicarboxylate transporter/malic acid transport protein	1,49
TC129997	Sb08g002680 lipid transport	1,48
TC132008	Sb01g006820.1 transmembrane transport transporter activity	1,47
TC130514	Sb08g002680 lipid transport	1,43
TC121634	Sb02g028210 sodium ion transport	1,40
TC125115	Sb01g033830 lipid transport	1,36

TC131767	Sb01g013910.1 transporter activity transmembrane transport	1,34
TC124403	Sb07g003690 integral to membrane transporter activity	1,33
TC125938	Sb07g020120 transmembrane transport	1,32
TC133970	Sb04g003270.1: similar to Nucleoporin-like protein	1,28
TC115469	Sb01g044010 : similar to Mannitol transporter	1,26
TC122933	Sb02g041080 DNA metabolic process	1,25
TC122887	Sb09g021820 - Golgi vesicle transport	1,22
TC123879	Sb09g021160 : similar to Potassium channel protein ZMK2	1,21
TC112996	Sb10g026090 voltage-gated chloride channel activity	1,21
TC112833	Sb01g013910 transmembrane transport	1,19
TC120301	Sb03g011060 : similar to Mitochondrial import receptor subunit tom40-like	1,19
TC127694	Sb03g024250 : similar to Putative MtN3	1,18
TC118585	Sb07g000380: similar to Farnesylated protein 3 - metal ion transport	1,18
TC112064	Sb01g044010 : similar to Mannitol transporter	1,17
TC128670	Sb08g002690.1 lipid transport	1,15
TC125329	Sb04g032430 : similar to Aquaporin PIP1-3/PIP1-4	1,15
TC131765	Sb07g019510 ATP binding Belongs to the ABC transporter superfamily	1,15
TC126421	Sb03g024820 : similar to Ca2+/H+-exchanging protein	1,15
TC132865	Sb10g022390 zinc ion transmembrane transporter activity integral to membrane	1,15
TC118025	Sb10g002840 - EamA-like transporter family - transmembrane transport	1,14
TC123438	Sb04g009770 - BCR-ASSOCIATED PROTEIN - intracellular protein transport	1,14
TC132574	Sb10g026090.1 voltage-gated chloride channel activity	1,13
TC127616	Sb08g002690.1 lipid transport	1,13
TC120890	Sb02g023340.1 transmembrane transport	1,12
TC130902	Sb03g038420 - transmembrane transport	1,12
TC130562	Sb02g034720 - similar to Os07g0516600 - transmembrane transport	1,11
TC111759	Sb10g008090 : similar to Aquaporin NIP2-3 - NOD26-like major intrinsic protein	1,11
TC126385	Sb04g022105 - similar to Cluster: Arabidopsis t., partial (12%) Mitochondrion; Single-pass membrane protein	1,10
TC115662	Sb01g001120 : similar to Putative phosphatidylinositol/phosphatidylcholine transfer protein	1,09
TC112322	Sb05g007500 : similar to At5g50300 - Adenine/guanine permease AZG2	1,08
TC132167	Sb02g005900 lipid transport	1,08
TC116298	Sb04g010400 : similar to Putative sugar transporter	1,08
TC120905	Sb05g026100 transmembrane transport	1,08

TC115586	Sb09g027590 lipid transport	1,06	
TC117739	Sb03g028510 : similar to TOM7-like protein - intracellular protein transport	1,05	
TC112805	Sb02g020360 : similar to Phosphate/phosphoenolpyruvate translocator	1,03	
TC116859	Sb07g024860 : similar to Acyl carrier protein	1,03	
TC123009	Sb02g038210 vesicle-mediated transport	1,02	
TC123089	Sb06g025150 Belongs to the MIP/aquaporin family	1,02	
TC112797	Sb02g027260 - Pfam Plastocyanin-like domain - electron carrier activity	1,02	
TC131532	homologue to Cluster: Os03g0825400 protein - complete protein import into mitochondrial inner membrane	1,01	
TC123197	Sb01g003260 : similar to Actin-depolymerizing factor 3	3,60	Protein fate
TC115284	Sb09g027590 Protease inhibitor/seed storage/LTP fam (58%)	3,58	
TC121260	Sb04g036350 : similar to P-(S)-hydroxymandelonitrile lyase precursor (HNL) - proteolysis	2,64	
TC134481	Sb05g027350 proteolysis	2,59	
TC129656	Sb04g036350 P-(S)-hydroxymandelonitrile lyase Involved in cyanogenesis	2,33	
TC133599	Sb04g036350 P-(S)-hydroxymandelonitrile lyase Involved in cyanogenesis	2,13	
TC128479	Sb05g027350 proteolysis	2,07	
TC133798	Sb04g036350 : similar to P-(S)-hydroxymandelonitrile lyase precursor	1,96	
TC127425	Sb05g027350 proteolysis	1,95	
TC123919	Sb09g001020 response to wounding serine-type endopeptidase inhibitor activity	1,87	
TC130716	Sb05g022900: similar to Intracellular protease, PfpI family protein	1,80	
TC117979	Sb05g024160 protein domain specific binding	1,79	
TC111814	Sb03g039800 : similar to Putative Bowman-Birk serine protease inhibitor	1,69	
TC117884	Sb10g012160 serine-type peptidase activity	1,67	
TC127548	Sb02g039690 peptidase activity	1,62	
TC124719	Sb03g039800 Belongs to the Bowman-Birk serine protease inhibitor family	1,62	
TC129116	Sb03g039800 serine-type endopeptidase inhibitor activity	1,56	
TC123651	Sb09g001020 response to wounding serine-type endopeptidase inhibitor activity	1,53	
TC117183	Sb10g028870 Serine protease	1,41	
TC116390	Sb01g014730 : similar to Putative serine protease inhibitor	1,39	
TC116483	Sb10g012160.1 Contains 1 PDZ (DHR) domain proteolysis	1,37	
TC119322	Sb09g024940 Belongs to the peptidase C1 family	1,37	
TC111750	Sb04g036350 : similar to P-(S)-hydroxymandelonitrile lyase precursor (HNL)	1,35	
TC127713	Sb09g001030 serine-type endopeptidase inhibitor activity response to wounding	1,34	
TC131881	Sb04g036350 : similar to P-(S)-hydroxymandelonitrile lyase precursor (HNL) - serine-type carboxypeptidase activity	1,28	

TC127485	Sb09g024940.1 proteolysis cysteine-type endopeptidase activity	1,28	
TC118212	Sb10g026870 - Ubiquitin and ubiquitin-like proteins	1,26	
TC113029	Sb02g003160 : similar to Kelch repeat containing F-box protein family-like	1,24	
TC132067	Sb01g037800 microtubule motor activity	1,23	
TC117341	Sb10g007410 : similar to Peptidyl-prolyl cis-trans isomerase - protein folding	1,19	
TC128542	Sb03g037370 - Cysteine proteinase inhibitor activity	1,18	
TC118227	Sb03g030350 : similar to Os01g0663400 protein - Aspartyl protease	1,18	
TC116583	Sb10g027540: (2R)-phospho-3-sulfolactate synthase-like	1,14	
TC114987	Sb01g046380 protein binding	1,13	
TC116784	Sb09g021400 : similar to Pi starvation-induced protein - serine-type endopeptidase activity	1,11	
TC130169	Sb02g031460 Aspartyl protease	1,11	
TC111432	Sb02g040830 : similar to Pyrrolidone carboxyl peptidase-like protein	1,09	
TC124327	Sb06g002220 similar to CBS domain containing protein-like - protein binding	1,09	
TC134660	Sb10g028870 : similar to Putative meiotic serine proteinase	1,08	
TC129596	Sb01g014730.1: similar to Putative serine protease inhibitor	1,06	
TC122966	Sb01g045700 : similar to Kelch repeat-containing F-box family protein	1,05	
TC131351	Sb10g008170 protein glycosylation Golgi apparatus	1,05	
TC112734	Sb03g037370 - Cysteine proteinase inhibitor activity	1,04	
TC131704	Sb04g032000 Contains 1 F-box domain	1,02	
TC119116	Sb03g041500 Contains 1 F-box domain	1,02	
TC127198	Sb03g001130 - Belongs to the AAA ATPase family - nucleoside-triphosphatase activity	5,98	Nucleotide binding
TC134570	Sb03g001130 - Belongs to the AAA ATPase family - nucleoside-triphosphatase activity	5,33	
TC116020	Sb10g023370 signal transduction GTP-binding	1,92	
TC131268	Sb01g028540 - catalytic activity (Haloacid dehalogenase-like hydrolase family protein; Oryza)	1,77	
TC126174	Sb01g039680 - catalytic activity (Haloacid dehalogenase-like hydrolase family protein, Oryza)	1,76	
TC127492	Sb01g039680 - catalytic activity (Haloacid dehalogenase-like hydrolase family protein, Oryza)	1,75	
TC123152	Sb03g032540 : similar to Glycylpeptide N-tetradecanoyltransferase	1,70	
TC131597	weakly similar to Cluster: PREDICTED: adenylosuccinate synthase; Pan troglodytes - GTP binding	1,65	
TC129545	Sb06g033410 - catalytic activity - Pfam AMP-binding enzyme	1,50	
TC128124	Sb04g032280: similar to Rho-GTPase-activating protein-like	1,48	
TC111476	Sb03g029530 GTP-binding nuclear protein Ran	1,31	
TC123591	Sb01g008450 sulfate assimilation sulfate adenylyltransferase (ATP) activity (ATP sulfurylase; Zea mays)	1,20	
TC131509	Sb03g011170 ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism	1,18	

TC122720	Sb01g046790 : similar to Os03g0152400 protein - AMP-binding enzyme	1,17	
TC116549	Sb01g037000 : similar to CIPK-like protein 1	1,11	
TC133251	Sb09g007420 Belongs to the small GTPase superfamily. Rho family	1,08	
TC131450	Sb04g028420 nucleoside-triphosphatase activity	1,04	
TC112240	Sb03g030050 : similar to ABRE-binding factor BZ-1	3,59	Nucleic acid binding
TC127222	Sb10g012110 : similar to Putative glycine rich protein	3,37	
TC124880	Sb04g033380 - transcription regulator activity - Homeobox-leucine zipper protein HOX24	2,73	
TC125312	Sb09g006060 regulation of transcription - Myb-like DNA-binding domain	2,53	
TC122897	Sb03g030050.1-similar to ABRE-binding factor BZ-1	2,35	
TC123342	Sb01g020530 Zinc-finger	2,25	
TC115128	Sb09g018630 translation release factor activity, codon specific	2,23	
TC132590	Sb04g000500 sequence-specific DNA binding transcription factor activity MADS-box domains	2,15	
TC128961	Sb09g006060 regulation of transcription	2,07	
TC132910	Sb01g040100.4 similar to RNA recognition motif family protein	2,04	
TC117463	Sb02g031170 Belongs to the RNase T2 family	2,02	
TC118771	Sb08g003180 : similar to Myb family transcription factor-like	1,99	
TC131552	Sb01g045660 : similar to Leucine zipper protein	1,88	
TC126209	Sb06g024000 : similar to H0501D11.4 protein - Homeobox-leucine zipper protein HOX22	1,87	
TC117841	Sb09g018630 translation release factor activity, codon specific	1,81	
TC126645	Sb05g021640 RNA binding	1,76	
TC117814	Sb03g012890 : similar to Ethylene-responsive factor-like protein 1	1,76	
TC124414	Sb09g020750 - similar to Os05g0421600 - regulation of transcription	1,75	
TC114402	Sb08g003180 : similar to Myb family transcription factor-like	1,70	
TC115544	Sb02g023650 - Homeobox associated leucine zipper	1,68	
TC129578	Sb02g031170 : similar to S-like Rnase	1,67	
TC131520	Sb02g031170 : similar to S-like Rnase	1,67	
TC128812	Sb03g013013 - Sb03g013010 : similar to ASF/SF2-like pre-mRNA splicing factor SRP30	1,61	
TC117755	Sb03g002660 : similar to Class III HD-Zip protein 5	1,61	
TC116488	Sb02g029300 : similar to Squamosa promoter-binding-like protein 18 - Transcription regulation	1,60	
TC128427	Sb04g032940: sequence-specific DNA binding transcription factor activity (Putative AP2; Oryza sativa)	1,58	
TC128699	Sb01g022330.1 regulation of transcription	1,49	
TC125401	Sb01g040100 : similar to RNA recognition motif family protein	1,45	
TC121350	Sb03g030050 : similar to ABRE-binding factor BZ-1	1,42	

TC123917	Sb04g034190 regulation of transcription	1,41
TC129478	Sb10g026350 - Pfam B-box zinc finger	1,40
TC132057	Sb06g024000 sequence-specific DNA binding transcription factor activity	1,40
TC122617	Sb02g040350 nucleic acid binding	1,39
TC117254	Sb01g040100 : similar to RNA recognition motif family protein	1,32
TC117531	Sb02g031170 : similar to S-like Rnase	1,31
TC126912	Sb01g042030 Transcription regulation	1,29
TC131026	Sb02g043270 regulation of transcription	1,26
TC121056	Sb01g020820 sequence-specific DNA binding	1,26
TC120483	Sb09g020750 - similar to Os05g0421600 - regulation of transcription	1,25
TC120594	Sb04g005370 nucleotide-excision repair	1,24
TC116272	weakly similar to Cluster: Transcription factor bZIP63; Glycine max	1,23
TC122506	similar to Cluster: Transcription factor-like; Oryza sativa Japonica Group	1,22
TC119187	Sb09g005430 : similar to Auxin-responsive protein IAA15 -regulation of transcription	1,22
TC131626	Sb08g009100.1 - nucleic acid binding	1,21
TC128040	Sb07g015030 Belongs to the pirin family nucleus - Possible transcriptional coregulator	1,21
TC124204	Sb09g025750 RNA metabolic process cyclic-nucleotide phosphodiesterase activity	1,21
TC111439	Sb02g013010 : similar to Os09g0106700 protein - Myb-like DNA-binding domain	1,21
TC122759	Sb06g026500 regulation of transcription	1,20
TC116345	Sb10g030960 - Myb-like DNA-binding domain	1,19
TC115758	Sb01g020430 - weakly similar to Cluster: Transposon protein putative CACTA En/Spm sub-class; Oryza sativa	1,19
TC127247	Sb07g028710 : similar to Zinc finger protein family-like	1,16
TC117934	Sb04g029160 ribonucleoprotein complex	1,16
TC121091	Sb07g028070 : similar to Parathymosin-like - KOG1946: RNA polymerase I transcription factor UAF	1,14
TC127640	Sb02g030660 regulation of transcription (Homeodomain leucine zipper; Oryza sativa)	1,14
TC131159	Sb07g001550 regulation of transcription	1,13
TC115339	Sb09g029070 regulation of transcription, DNA-dependent	1,10
TC119968	Sb03g032530 regulation of transcription	1,08
TC115659	Sb06g024000 : similar to H0501D11.4 protein - Homeobox-leucine zipper protein HOX22	1,08
TC133770	Sb03g044160 DNA binding	1,08
TC120404	Sb02g023650 regulation of transcription	1,07
TC125350	Sb09g006060.1 regulation of transcription	1,07
TC130550	Sb07g005220 RNA binding	1,06

TC122677	Sb04g005370 nucleotide-excision repair	1,04	
TC126145	Sb04g021023 : weakly similar to H0718E12.7 protein - DNA-directed RNA polymerase activity	1,04	
TC134607	Sb02g035890 nucleotide binding	1,01	
TC113072	Sb03g002180 : similar to RNA-binding protein-like	1,01	
TC132971	Sb08g023000.2 - Pfam Calcineurin-like phosphoesterase	3,56	Communication and signalling
TC126448	Sb03g039630 Protein phosphatase 2C	2,86	
TC111992	Sb10g026710 - Phosphoglycerate kinase	2,52	
TC116626	Sb01g039890 - Pfam Protein phosphatase 2C	2,18	
TC117700	Sb03g033100: similar to Acid phosphatase	2,17	
TC111667	Sb04g027590 : similar to Annexin p35 - calcium ion binding	2,11	
TC118235	Sb01g010290 : similar to Atypical receptor-like kinase MARK	2,11	
TC128992	Sb07g012720 6-phosphofructokinase activity	2,01	
TC133293	Sb01g042480 : similar to Os03g0223000 protein - Serine/threonine protein kinase	1,98	
TC128357	Sb07g021660 Serine/threonine-protein kinase	1,92	
TC133325	Sb02g024770 Serine/threonine-protein kinase	1,88	
TC132723	Sb03g011880.1 Serine/threonine-protein kinase	1,88	
TC124710	Sb03g036210 : similar to Purple acid phosphatase precursor	1,83	
TC120378	Sb07g025980 : similar to Putative diphosphonucleotide phosphatase	1,80	
TC131697	Sb08g023000 : similar to Secretory acid phosphatase	1,78	
TC129454	Sb08g007130 : similar to Diacylglycerol kinase 1	1,75	
TC116518	Sb03g002340 Serine/threonine-protein kinase	1,75	
TC131813	Sb09g006030 6-phosphofructokinase complex glicolisi	1,75	
TC119767	Sb09g026860 : similar to Os05g0537400 protein - Protein phosphatase 2C	1,72	
TC129974	Sb04g027880 - Shikimate kinase 1	1,68	
TC118493	Sb03g026070 protein serine/threonine phosphatase complex	1,66	
TC116332	Sb01g028760 : similar to Serine/threonine-protein kinase SAPK3	1,66	
TC112869	Sb02g020400 : similar to Putative pyrophosphate-dependent phosphofructokinase alpha subunit	1,57	
TC126375	Sb01g039890 protein serine/threonine phosphatase complex	1,56	
TC124248	Sb09g022410 - KOG:0698 Serine/threonine protein phosphatase	1,54	
TC111527	Sb06g032940 : similar to Pyruvate kinase	1,49	
TC119296	Sb09g025420 Serine/threonine-protein kinase	1,49	
TC121201	Sb03g011880 Serine/threonine-protein kinase	1,47	
TC117992	Sb04g036570 : similar to Phosphoenolpyruvate carboxylase kinase 1	1,46	

TC115159	Sb09g026150 6-phosphofructokinase complex	1,44
TC128934	Sb03g026070 protein serine/threonine phosphatase activity	1,44
TC119655	Sb01g041570 hydrolase activity	1,42
TC129731	Sb01g042010 : similar to Inositol 1,3,4-trisphosphate 5/6-kinase	1,39
TC134541	Sb01g001350 : similar to Ser/Thr protein phosphatase family protein, expressed	1,39
TC122790	Sb06g022730 : similar to OSIGBa0145M07.10 protein - adenosine kinase activity purine ribonucleoside salvage	1,38
TC126512	Sb05g025240 sulfate assimilation ATP + adenylyl sulfate = ADP + 3'-phosphoadenylyl sulfate	1,36
TC118957	Sb05g001160 calcium ion binding	1,36
TC119092	Sb01g036190 integral to membrane receptor activity	1,36
TC114934	Sb08g019090 : similar to Os12g0576600 protein - Calcineurin-like phosphoesterase	1,34
TC129908	Sb01g048750 Serine/threonine-protein kinase	1,29
TC125779	Sb02g026740 : similar to Cyclin-dependent kinase inhibitor 6	1,29
TC120150	Sb05g005850 : similar to At5g63940 - Serine/threonine protein kinase - universal stress protein	1,24
TC113656	Sb01g015510 inositol or phosphatidylinositol phosphatase activity	1,22
TC120569	Sb01g037000 Serine/threonine-protein kinase	1,21
TC114126	Sb04g037180 : similar to ABC1-like - protein kinase activity	1,21
TC120591	Sb10g006850 : similar to Dual-specificity phosphatase protein	1,19
TC126485	Sb02g022090 : similar to Os09g0325700 protein - protein serine/threonine phosphatase complex	1,18
TC132527	Sb06g016790 Serine/threonine-protein kinase	1,18
TC126144	Sb04g037180 : similar to ABC1-like - protein kinase activity	1,17
TC131834	Sb07g021660 Serine/threonine-protein kinase	1,17
TC116769	Sb05g001160 calcium ion binding	1,16
TC128781	Sb04g006930 mitochondrial inner membrane calcium ion binding	1,15
TC129181	Sb09g023990 - similar to Cluster: Putative leucine-rich repeat transmembrane protein kinase; Oryza sativa Japonica	1,14
TC126081	Sb06g032940 : similar to Pyruvate kinase	1,13
TC123246	Sb02g039460 - CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE-RELATED	1,12
TC119993	Sb06g032940 : similar to Pyruvate kinase	1,11
TC114491	Sb08g014350 : similar to Protein kinase, putative, expressed	1,11
TC129391	Sb09g018200 thiamin diphosphate biosynthetic process	1,09
TC116297	Sb01g043540 : similar to Transposon protein, putative, Mutator sub-class - signal transducer activity	1,08
TC116473	Sb03g002340.1 Serine/threonine-protein kinase	1,08
TC122982	Sb01g048750 Serine/threonine-protein kinase	1,08
TC130987	Sb10g006340 SAC domain containing protein - phosphatase activities	1,06

TC130293	Sb06g022730 purine ribonucleoside salvage adenosine kinase activity	1,06	
TC134154	Sb09g030840.1: similar to Nucleoside diphosphate kinase	1,06	
TC111514	Sb04g026500 - Adenosine kinase-like protein	1,05	
TC118966	Sb08g002770 : similar to Dual-specificity protein-like phosphatase 3	1,04	
TC131186	Sb01g038280 : similar to Os03g0295600 protein - protein kinase activity	1,02	
TC117464	Sb09g022950 - protein serine/threonine kinase activity	1,00	
TC128583	Sb02g021050 catalytic activity - Contains 1 PP2C-like domain	1,00	
TC116566	Sb03g000850 nucleoside metabolic process	2,15	Cell cycle-DNA processing
TC123181	Sb03g007330 : similar to Putative nuclease I	1,96	
TC130081	Sb01g042880.1 nucleus binding	1,81	
TC125306	Sb01g049200.2 - Cell cycle control protein	1,66	
TC117312	Sb06g025740 : similar to OSIGBa0139P06.6 protein - DNA replication	1,54	
TC116431	Sb09g027223 : similar to DNA-directed RNA polymerase	1,51	
TC114350	Sb10g011790 - KOG:4302 - Microtubule-associated protein essential for anaphase spindle elongation	1,43	
TC123513	Sb03g040890 nucleosome assembly	1,41	
TC127624	homologue to Cluster: Putative DNA helicase INO80; Eremothecium gossypii	1,41	
TC562720	Sb03g004890 : similar to Histone H4	1,40	
TC127195	Sb06g028980.1 exonuclease activity	1,39	
TC125944	Sb06g028980 exonuclease activity nucleic acid binding	1,39	
TC119782	Sb01g012840 - Glyoxalase - similar to Cluster: Putative cell division control protein CDC91; O. sativa Japonica	1,16	
TC116412	Sb06g018910 : similar to H0219H12.3 protein - Belongs to the nucleosome assembly protein (NAP) family	1,09	
TC116374	Sb09g027223 Transcription Nucleotidyltransferase	1,09	
TC117220	Sb03g043355 : similar to Putative Endoribonuclease Dicer homolog	1,09	
TC127857	Sb06g031030 : similar to Nuclease I - DNA catabolic process	1,01	
TC117413	Sb01g023270.2 - Up-regulated under phosphate starvation. Up-regulated during cold stress	2,32	Interaction with the environment
TC123989	Sb03g024480 Contains 1 SPX domain - Protein involved in vacuolar polyphosphate accumulation	1,86	
TC115475	Sb03g008760 : similar to Isoflavone reductase homolog IRL - Stress response	1,84	
TC122716	Sb08g004190.2: similar to 22 kDa drought-inducible protein	1,74	
TC127842	Sb09g028710.1: similar to Cold acclimation protein COR413-TM1	1,45	
TC120951	Sb05g000940 : similar to Hypoxia induced protein conserved region containing protein	1,39	
TC133732	Sb04g024580 : similar to Hypoxia-responsive family protein-like	1,37	
TC117646	Sb10g023590 - Contains 1 SPX domain.	1,28	
TC120274	Sb03g005760: similar to Universal stress protein	1,26	

TC130549	Sb05g000940.1: similar to Hypoxia induced protein conserved region containing protein	1,26	
TC130995	Sb02g004390.1: similar to Early light-inducible protein ELIP	1,16	
TC121353	Sb03g008760 : similar to Isoflavone reductase homolog IRL - Stress response	1,12	
TC123691	Sb10g026760 : weakly similar to Zinc finger POZ domain protein-like - cellular response to water deprivation	1,03	
TC125747	Sb05g000940 : similar to Hypoxia induced protein conserved region containing protein	1,01	
TC116482	Sb03g012950: similar to Os01g0314800 protein - response to stress	1,00	
TC123561	Sb08g014070 : similar to ABA-responsive protein	1,84	Interaction with the environment
TC132967	Sb05g017940 : similar to HVA22-like protein e - response to abscisic acid stimulus	1,69	
TC114695	Sb06g016540: similar to OSIGBa0076I14.3 protein - ABA/WDS induced protein - response to stress	1,31	
TC128517	Sb01g002720 : similar to Expressed protein - Sorghum bicolor clone BAC IS_91D5 ABA-induced gene	1,30	
TC127517	Sb08g004190: similar to 22 kDa drought-inducible protein - ABA/WDS induced protein	1,24	
TC117109	Sb02g007720 : similar to Putative IAA amidohydrolase	1,12	
TC116207	Sb01g007360 : similar to Giberellin regulated family protein	1,10	
TC123042	Sb01g036330 - Ribosomal protein L16p/L10e - translation	1,48	Others
TC130965	Sb01g004430 : similar to Elongation factor P family protein - Protein biosynthesis	1,28	
TC128253	Cluster: Rps12 protein - Triticum aestivum (Wheat) - translation	1,08	
TC115804	Sb05g004130 : similar to 60S ribosomal protein L3	1,06	
TC124485	Sb01g006720 structural constituent of ribosome	1,06	
TC124755	Sb01g041800 structural constituent of ribosome	1,05	
TC131905	Sb10g008780.1 Belongs to the class-II aminoacyl-tRNA synthetase family.ATP + L-alanine + tRNA(Ala)	1,04	
TC120585	Sb08g022150 apoptosis	1,14	
TC113565	Sb03g025160 - Development and cell death domain - homologue to Cluster: Putative GDA2 protein; O. sativa	1,11	
TC116536	Sb01g030400 : similar to Seven transmembrane protein Mlo2 - cell death	1,01	
TC128498	Sb07g024090 : weakly similar to chr7 scaffold_31, whole genome shotgun sequence - Chlorophyllase family	2,92	
TC132684	Sb06g027770 ATP biosynthetic process	1,80	
TC112268	Sb04g034160 : similar to Ferredoxin--nitrite reductase, chloroplast precursor - Electron transport	1,62	
TC111914	Sb04g034160 : similar to Ferredoxin--nitrite reductase, chloroplast precursor - Electron transport	1,48	
TC112143	Sb09g021810 : similar to Ferredoxin-6	1,44	
TC112166	Sb09g029730 : similar to Putative polyprenyl diphosphate synthase	1,26	
TC115588	Sb08g005430 photosynthesis	1,24	
TC124811	Sb03g041100 : similar to Photosystem II subunit PsbS	1,21	
TC122337	Sb01g032930 : similar to Putative uncharacterized protein OSJNBb0074M06.9 - ubiquinone biosynthetic process	1,19	
TC123489	Sb01g017430 : similar to Actin-1	1,60	

TC115146	Sb09g026210 endoplasmic reticulum	1,39	
TC129786	weakly similar to Cluster: RIKEN cDNA 1700021P22 gene; Mus musculus	5,05	Unknown
TC119387	Sb07g021840 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	3,91	
TC122634	Sb07g021850 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	3,81	
TC127109	Sb07g021840 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	3,79	
TC121587	Sb04g009640 - similar to Cluster: Putative uncharacterized protein B1178F07.4; Oryza sativa Japonica Group	3,58	
TC134131	Sb09g019350 - Sb02g004380: similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	3,31	
TC132562	Sb02g024480 - similar to Cluster: Putative uncharacterized protein P0431A03.17; Oryza sativa Japonica	3,19	
TC125693	Sb06g004280 : similar to OSIGBa0139I12.3 protein - transketolase activity	3,16	
TC126250	Sb01g009730 - similar to Cluster: Putative uncharacterized protein P0492F05.31; Oryza sativa Japonica	3,01	
TC124582	Sb06g025450 - similar to Cluster: OSIGBa0158F05.3 protein; Oryza sativa	2,93	
TC125717	Sb04g031810.1 - similar to Cluster: Os05g0569500 protein; Oryza sativa	2,92	
TC132787	Sb05g022680 - similar to Cluster: PREDICTED: similar to Protein C14orf155; Macaca mulatta	2,88	
TC122544	Sb03g043410 - similar to Cluster: Putative uncharacterized protein P0470A12.6; Oryza sativa Japonica	2,81	
TC111603	Sb10g021910 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	2,79	
TC123657	Sb06g029880.1 - similar to Cluster: H0315F07.12 protein; Oryza sativa	2,77	
TC124794	Sb01g017440 - similar to Cluster: Os10g0509600 protein; Oryza sativa Japonica	2,67	
TC126602	Sb01g017440 - similar to Cluster: Os10g0509600 protein; Oryza sativa Japonica	2,61	
TC117176	Sb03g031950 methyltransferase activity	2,60	
TC125651	Sb02g024480 - similar to Cluster: Putative uncharacterized protein P0431A03.17; Oryza sativa Japonica	2,56	
TC123034	Sb10g027660 - similar to Cluster: Os06g0683700 protein; Oryza sativa	2,55	
TC127367	Sb10g008730 : similar to Os06g0242000 protein - GO:0008168 methyltransferase activity	2,54	
TC134179	Sb06g025450 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2,51	
TC127239	Sb02g013190 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	2,50	
TC126280	Sb08g018660.1 - similar to Cluster: Expressed protein; Oryza sativa	2,47	
TC125719	Sb03g005490 - similar to Cluster: Os05g0119300 protein; Oryza sativa	2,41	
TC115491	Sb03g038330 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	2,38	
TC120369	Sb02g004380 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2,38	
TC118310	Sb09g030780 - similar to Cluster: LigA precursor; Halorubrum lacusprofundi ATCC 49239	2,34	
TC125393	weakly similar to Cluster: Os06g0174300 protein; Oryza sativa Japonica Group	2,32	
TC118178	Sb03g005490 - similar to Cluster: Putative uncharacterized protein P0011G08.23; Oryza sativa Japonica	2,32	
TC132169	similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2,31	
TC122692	Sb03g005490 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group|	2,30	

TC126225	Sb01g009730 - similar to Cluster: Putative uncharacterized protein OJ1499_D04.17; Oryza sativa	2,24
TC126799	Sb06g014330 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	2,21
TC120484	Sb06g015140 similiar to Os04g0403600 protein	2,13
TC119711	Sb03g031940 methyltransferase activity	2,10
TC119248	Sb01g038670 integral to membrane	2,09
TC130610	similar to Cluster: UPI0000DC0177 related cluster; Rattus norvegicus	2,05
TC120984	Sb01g046120 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2,04
TC115195	Sb10g029560 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	2,03
TC126504	Sb06g015290 - similar to Cluster: OSIGBa0142C11.2 protein; Oryza sativa	2,02
TC120556	Sb03g006480 biosynthetic process - homologue to uncharacterized protein P0024G09.1; Oryza sativa Japonica	2,02
TC131794	Sb03g011090 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,96
TC132906	Sb09g001610 - similar to Cluster: Putative uncharacterized protein; Frankia alni ACN14a	1,94
TC112246	Sb01g036580 - homologue to Cluster: Osr40c1 protein; Oryza sativa	1,93
TC120534	Sb01g028080 binding - similar to Cluster: Expressed protein; Oryza sativa	1,93
TC126351	homologue to Cluster: PREDICTED: hypothetical protein; Strongylocentrotus purpuratus	1,93
TC115705	Sb02g038740 : similar to Brittle stalk-2-like protein 7	1,89
TC118537	Sb03g028860 : similar to X1 - catalytic activity	1,87
TC118439	Sb09g019990 - similar to Cluster: Os05g0406100 protein; Oryza sativa Japonica	1,87
TC123799	Sb09g024870 binding - similar to Cluster: Putative protein OJ1123_F01.11; Oryza sativa Japonica	1,87
TC121323	Sb02g004630 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,86
TC127309	Sb03g039450 - similar to Cluster: Os01g0841200 protein; Oryza sativa Japonica	1,85
TC114857	Sb01g020430 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,85
TC125694	Sb10g030900 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,84
TC114073	Sb06g000653.2 - weakly similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,84
TC126406	Sb10g005700 : similar to Cinnamoyl-CoA reductase	1,83
TC116634	Sb02g001940 - weakly similar to Cluster: Os07g0123900 protein; Oryza sativa	1,81
TC127628	Sb06g030295 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,81
TC131816	Sb02g004630 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,79
TC118790	Sb09g022700 : similar to Putative uncharacterized protein	1,76
TC131680	Sb05g016820 - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,76
TC114851	Sb10g000890 : similar to Os09g0407700 protein - catalytic activity	1,75
TC132926	Cluster: Putative uncharacterized protein; Trypanosoma cruzi	1,74
TC112112	Sb08g022970 : similar to Hydrolase, alpha/beta fold family protein	1,73

TC122705	Sb09g030780 : weakly similar to Putative uncharacterized protein	1,73
TC130530	Sb07g021760 transferase activity, transferring acyl groups other than amino-acyl groups	1,73
TC113283	Sb01g044020.1 - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,72
TC129094	Sb02g041980: similar to Putative uncharacterized protein P0496C02.128	1,69
TC117319	Sb06g026660 - similar to Cluster: H0307D04.7 protein; Oryza sativa	1,69
TC133457	Sb03g045710 - similar to Cluster: Putative uncharacterized protein P0466H10.40-1; Oryza sativa Japonica	1,68
TC118281	Sb03g033760 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,67
TC126913	Sb06g021640 : similar to OSJNBa0029H02.19 protein - transferase activity	1,67
TC116067	Sb09g022420 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,66
TC120909	Sb08g009120 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,66
TC128101	homologue to Cluster: KIAA1276 protein; Homo sapiens	1,66
TC124285	Sb08g007130.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,65
TC119346	Sb01g033820 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,63
TC121308	Sb02g007080 transferase activity, transferring hexosyl groups	1,63
TC113108	Sb07g022420 - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,63
TC124380	Sb01g018190 catalytic activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,63
TC127608	Sb06g018700 - similar to Cluster: H0523F07.8 protein; Oryza sativa	1,61
TC130979	Sb06g029880.1 - similar to Cluster: H0315F07.12 protein; Oryza sativa	1,60
TC124977	Sb03g036300 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,59
TC131644	Sb02g039830 - weakly similar to Cluster: Uncharacterized protein At1g78070.1; Arabidopsis thaliana	1,58
TC123467	Sb04g021400 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,58
TC117503	Sb01g019970 - similar to Cluster: Uncharacterized conserved protein; Oryza sativa Japonica Group	1,57
TC114444	Sb08g001400.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,56
TC126371	Sb09g028390 - similar to Cluster: Os05g0561600 protein; Oryza sativa Japonica Group	1,55
TC115576	Sb02g043220.1 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,55
TC120396	Sb06g017200 - similar to Cluster: H0525C06.3 protein; Oryza sativa	1,55
TC133952		1,54
TC125769	Sb02g037340 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,53
TC121467	Sb01g044210 : similar to Os03g0194300 protein	1,52
TC123658	homologue to Cluster: Small MutS related (Smr) family protein; Dictyostelium discoideum	1,51
TC127815	Sb03g046650.1 - similar to Cluster: Putative uncharacterized protein P0483G10.22; Oryza sativa Japonica Group	1,51
TC124070	Sb01g005240.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,49
TC127138	Sb01g001250.1: similar to Putative regulatory protein	1,48

TC131732	Sb01g046470 - similar to Cluster: Expressed protein; Oryza sativa	1,48
TC114011	Sb09g022420 : similar to Putative uncharacterized protein OJ1362_D02.3	1,47
TC132576	Sb01g040410.1 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,47
TC134393	Sb05g005340 binding - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,47
TC134178	Sb05g005980 integral to membrane - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica	1,47
TC122632	homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,46
TC127415	Sb03g033760 - Putative BURP domain-containing protein	1,46
TC125469	Sb02g017220.2 - similar to Cluster: Putative uncharacterized protein P0515A04.8; Oryza sativa Japonica	1,46
TC111437	Sb03g002090 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,45
TC133163	Sb02g029500.1: acyltransferase activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica	1,45
TC130198	Sb06g028110 - similar to Cluster: Uncharacterized protein C10orf95; Homo sapiens	1,45
TC134269	Sb01g009950 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,44
TC120486	Sb10g007160 : similar to Putative Tic22	1,44
TC130020	similar to Cluster: Putative uncharacterized protein; Aspergillus terreus NIH2624	1,44
TC132517	Sb04g004740 - similar to Cluster: Putative uncharacterized protein P0030G02.24-1; Oryza sativa Japonica	1,44
TC117086	Sb03g024850.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,42
TC113902	Sb03g035790 - similar to Cluster: Os01g0770100 protein; Oryza sativa Japonica	1,42
TC123974	Sb07g015410 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,41
TC118404	Sb08g006180 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,40
TC119764	Sb10g005700 catalytic activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,39
TC130944	Sb02g013110 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,38
TC132054	Sb03g004350 - similar to Cluster: Putative uncharacterized protein OSJNBa0089K24.4; Oryza sativa Japonica	1,38
TC121656	Sb02g010080 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,38
TC115838	Sb07g001450 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,37
TC118974	Sb05g026580 acyltransferase activity	1,36
TC127149	Sb03g008870 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,36
TC119848	Sb05g001470 nucleotidyltransferase activity	1,35
TC132221	Cluster: Putative uncharacterized protein; Yersinia pseudotuberculosis IP 31758	1,35
TC122008	Sb09g026630 binding - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,35
TC126634	Sb03g039450.1 - similar to Cluster: Similar to axi 1 protein from Nicotiana tabacum; Arabidopsis thaliana	1,34
TC123186	Sb05g025210 - similar to Cluster: TNP2-like protein; Sorghum bicolor	1,34
TC119114	Sb07g022420 - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,33
TC117956	Sb03g010180 - integral to membrane - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,33

TC122904	Sb04g004100 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,33
TC122066	Sb09g003250 - similar to Cluster: Putative uncharacterized protein OJ1264_A04.8; Oryza sativa	1,32
TC115779	Sb04g024070 - homologue to Cluster: Prohibitin; Zea mays	1,31
TC128339	Sb01g024170.1 - homologue to Cluster: Predicted protein; Physcomitrella patens subsp. patens	1,31
TC113199	Sb06g021640 : similar to OSJNBa0029H02.19 protein - transferring acyl groups other than amino-acyl groups	1,31
TC133821	Sb09g020120 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,31
TC117571	Sb01g015770 - transferase activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,30
TC116404	weakly similar to Cluster: Putative uncharacterized protein; Ustilago maydis	1,30
TC118112	Sb10g007690 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,30
TC131860	Sb03g037990 integral to membrane - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,29
TC129828	Sb09g027060.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,29
TC124177	Sb09g003510 - similar to Cluster: Unknow protein; Oryza sativa Japonica Group	1,29
TC126062	Sb03g035790 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,29
TC122973	Sb04g030560.2 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,28
TC131173	Sb04g037160.2 - weakly similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,28
TC116086	Sb10g007690 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,28
TC115121	Sb03g024820.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,28
TC116273	Sb04g025380 - weakly similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,28
TC121028	Sb02g025470 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,27
TC131105	Sb06g030290 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,27
TC120820	Sb08g004180 - similar to Cluster: Nrap protein expressed; Oryza sativa Japonica Group	1,27
TC119766	Sb10g021910 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,26
TC111429	Sb07g022290 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,26
TC116909	Sb07g025600 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,26
TC121044	Sb04g024330 - homologue to Cluster: OSIGBa0124N08.5 protein; Oryza sativa	1,26
TC129124	Sb03g033760 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,25
TC119039	Sb08g004010 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,25
TC124712	Sb01g043490 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,25
TC123600	Sb10g005340 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,24
TC117515	Sb02g031530 - similar to Cluster: Putative uncharacterized protein P0478E02.19; Oryza sativa	1,23
TC113803	Sb09g022500 : similar to P0482D04.17 protein - Pfam Exostosin family	1,23
TC125251	Sb01g018190: similar to Os10g0497900 protein - catalytic activity	1,23
TC127610	Sb03g039900 - similar to Cluster: Os01g0849500 protein; Oryza sativa Japonica	1,22

TC113236	Sb03g045190 catalytic activity - similar to Cluster: Os01g0935600 protein; Oryza sativa Japonica	1,22
TC126759	similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,22
TC134163	Sb03g004190 - imilar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,22
TC118954	Sb01g014300 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,22
TC115597	Sb05g001340 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,22
TC112753	Sb03g008720.1 - similar to Cluster: Putative uncharacterized protein P0005A05.21; Oryza sativa Japonica	1,21
TC127082	Sb0284s002010.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,21
TC131193	Sb04g036590 - weakly similar to Cluster: Os02g0807100 protein; Oryza sativa Japonica	1,21
TC121445	similar to Cluster: Putative uncharacterized protein; Magnaporthe grisea	1,21
TC130227	Sb07g007500 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,21
TC124195	Sb02g033760 - similar to Cluster: Putative uncharacterized protein; Sporobolus stapfianus	1,21
TC116931	Sb10g007180 - similar to Cluster: CP12 precursor; Pisum sativum	1,20
TC124519	Sb03g045190 catalytic activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,20
TC130372	Sb03g007915 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,20
TC132146	Sb03g007320.1 - similar to Cluster: Putative uncharacterized protein P0408F06.3; Oryza sativa Japonica	1,20
TC130840	Sb09g014016 - homologue to Cluster: Orf107b protein; Beta vulgaris subsp. vulgaris	1,20
TC134416	Sb01g034790.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,20
TC133029	Sb04g000250 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,19
TC120052	Sb03g024850.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,19
TC129530	Sb01g017910.1 - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,19
TC121219	Sb03g027260 integral to membrane - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,19
TC113170	Sb02g025720 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,19
TC125468	Sb04g036740 - similar to Cluster: Putative uncharacterized protein OJ1520_C09.39; Oryza sativa Japonica Group	1,19
TC118114	Sb03g046290 transferring glycosyl groups- similar to Cluster: Putative protein; Oryza sativa Indica Group	1,19
TC127801	Sb08g021820.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,18
TC132513	Sb02g032640.1 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,18
TC115097	Sb01g043520.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,18
TC126154	Sb06g020900 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,18
TC127868	Sb01g011220 - similar to Cluster: Putative uncharacterized protein; Vitis vinifera	1,18
TC129848	Sb02g007350 - similar to Cluster: Putative uncharacterized protein P0022B05.122; Oryza sativa Japonica	1,18
TC115125	Sb05g002060.2 - similar to Cluster: Os12g0133100 protein; Oryza sativa Japonica	1,18
TC131392	Sb02g029500.1 - acyltransferase activity - similar to Cluster: Putative protein; Oryza sativa Indica Group	1,18
TC132749	Sb06g015200 - similar to Cluster: H0502B11.6 protein; Oryza sativa	1,18

TC124630	weakly similar to Cluster: LigA; Methylobacterium sp. 4-46	1,17
TC119700	Sb02g025150 : similar to Fibroin heavy chain (Fib-H) (H-fibroin)-like protein; Oryza sativa Japonica Group	1,17
TC119090	similar to Cluster: Putative uncharacterized protein; Vitis vinifera	1,16
TC127375	Sb10g005770.1 - transferase activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,16
TC132624	Sb01g019150 integral to membrane - weakly similar to Cluster: Putative protein; Oryza sativa Indica Group	1,16
TC115770	similar to Cluster: Sterol 14alpha-demethylase; Pneumocystis carinii	1,16
TC118156	Sb05g024940 - Cluster: Putative uncharacterized protein S126P21.2; Sorghum bicolor	1,16
TC120431	Sb09g028390 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,16
TC125159	Sb04g019560 binding - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,16
TC125095	Sb06g015005 - similar to Cluster: Cobalamin biosynthesis protein CbiD; Burkholderia pseudomallei 1710b	1,16
TC115399	Sb06g031660 - homologue to Cluster: OSIGBa0145G11.6 protein; Oryza sativa	1,16
TC130574	Sb04g035990 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,16
TC117690	Sb01g009660 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,16
TC130377	Sb02g035830 - similar to Cluster: UPI000069F357 related cluster; Xenopus tropicalis	1,15
TC124319	Sb01g042260: similar to Non-symbiotic hemoglobin; Zea mays subsp. parviglumis	1,15
TC113583	Sb05g027610 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,15
TC129830	Sb01g033820 : similar to WD domain, G-beta repeat containing protein	1,15
TC127636	Sb02g003750.1 - similar to Cluster: Os07g0165200 protein; Oryza sativa	1,14
TC125126	Sb01g017330 - similar to Cluster: LigA; Methylobacterium sp. 4-46	1,14
TC113014	Sb01g008280 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,13
TC119425	homologue to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,13
TC121460	Sb01g007470.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,13
TC127779	Sb08g020820.2 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,12
TC128398	Sb04g037160.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,12
TC121035	Sb04g037420 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,12
TC131019	Sb01g001660.3 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,12
TC121773	Sb01g003510.1 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,12
TC124841	Sb05g003260 - similar to Cluster: Os11g0153000 protein; Oryza sativa	1,12
TC132439	Sb02g027580 catalytic activity - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,12
TC119318	weakly similar to Cluster: Putative uncharacterized protein OSJNBa0057L21.16; Oryza sativa	1,12
TC116981	Sb05g008570: hydrolase activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,12
TC115666	Sb06g020710.1 - similar to Cluster: OJ991113_30.3 protein; Oryza sativa Japonica	1,11
TC131964	Sb03g034120 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,11

TC123751	Sb01g013400 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,11
TC115095	Sb01g001660.3 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,11
TC126517	Sb01g019100 - similar to Cluster: Putative uncharacterized protein OSJNBa0093B11.10; Oryza sativa Japonica	1,10
TC116356	Sb01g040410 : similar to Expressed protein - homologue to Cluster: Putative protein; Leishmania major	1,10
TC130029	homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,09
TC118941	Sb07g015030 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,09
TC129500		1,09
TC118459	Sb06g026180 - homologue to Cluster: H0404F02.10 protein;Oryza sativa	1,09
TC112104	Sb01g009950 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,09
TC113788	Sb07g008570.1 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,09
TC124415	Sb06g027320 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,09
TC133853	Sb10g030960.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,08
TC134500	Sb01g012550 binding - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,08
TC121503	Sb01g007470 - homologue to Cluster: Putative sialin; Oryza sativa Japonica Group	1,08
TC129192	Sb10g023190.1 - homologue to Cluster: Putative sporulation protein; Gluconacetobacter diazotrophicus PAl 5	1,08
TC133605	Sb10g025230 integral to membrane - homologue to Cluster: Putative adiponectin receptor 1; Oryza sativa Japonica	1,08
TC129213	Sb03g006520.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,08
TC112834	Sb04g037820 : similar to Putative O-diphenol-O-methyl transferase, Oryza sativa Japonica Group	1,08
TC133252	Sb07g000650.3 -similar to Cluster: Putative ReMembR-H2 protein; Oryza sativa Japonica Group	1,08
TC131254	Sb01g015270 - homologue to Cluster: Chromosome chr12 scaffold_47 whole genome shotgun sequence; V. vinifera	1,07
TC130249	Sb10g029900 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,07
TC134511	Sb02g042180.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,07
TC116920	Sb04g000400.1 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,07
TC123625	similar to Cluster: Os06g0730300 protein; Oryza sativa Japonica Group	1,07
TC130758	Sb07g019840 hydrolase activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,07
TC132564	Sb03g027780 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,07
TC130567	Sb04g003200: intramolecular transferase activity - homologue to Cluster: Putative cycloartenol synthase; O.sativa	1,07
TC121364	Sb07g008810: similar to Putative uncharacterized protein OSJNBa0031J07.41	1,06
TC127216	Sb10g009750.1 - membrane - homologue to Cluster: Os06g0269200 protein; Oryza sativa Japonica Group	1,06
TC123475	Sb06g033820 - similar to Cluster: CAA303719.1 protein; Oryza sativa	1,06
TC127183	Sb01g017970 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,06
TC117347	Sb02g039160 - similar to Cluster: Putative uncharacterized protein P0616D06.115; Oryza sativa Japonica	1,06
TC124074	similar to Cluster: Putative uncharacterized protein P0535G04.1; Oryza sativa	1,06

TC126560	Sb04g025760 transferase activity - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,06
TC113592	Sb06g024600.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,05
TC118584	Sb10g023190 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,05
TC127414	Sb06g000220.1 - Cluster: Mitochondrial uncoupling protein 1; Saccharum officinarum	1,05
TC127913	Sb01g018740.1 - similar to Cluster: GRAM domain containing protein expressed; Oryza sativa	1,05
TC131317	Sb02g042210 - similar to Cluster: Putative tubby related protein; Oryza sativa Japonica Group	1,05
TC118011	Sb09g003510 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,05
TC131427	Sb02g009120.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,05
TC124541	Sb07g021895 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,05
TC113642	Sb02g033760 - homologue to Cluster: Putative uncharacterized protein; Sporobolus stapfianus	1,04
TC123990	Sb07g019840: hydrolase activity - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,04
TC118255	Sb06g033340 - imilar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,04
TC124363	Sb10g030900 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,04
TC125641	Sb02g003750.1 - similar to Cluster: Uncharacterized protein At3g03790.2; Arabidopsis thaliana	1,04
TC118902	Sb01g046720 catalytic activity - weakly similar to Cluster: Putative protein; Oryza sativa Japonica Group	1,04
TC122237	Sb09g021590 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,04
TC118443	Sb06g030760 - similar to Cluster: OSJNBb0059K02.4 protein; Oryza sativa	1,03
TC117094	Sb03g047000 - similar to Cluster: Putative uncharacterized protein P0458E05.22; Oryza sativa Japonica Group	1,03
TC119591	Sb01g045230 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,03
TC121794	Sb08g016060.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,03
TC127782	Sb04g003200 intramolecular transferase activity - similar to Cluster: Cycloartenol synthase; Avena strigosa	1,03
TC131030	Sb09g006340 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,03
TC124624	Sb06g031940.1 - homologue to Cluster: Os04g0665000 protein; Oryza sativa Japonica	1,03
TC116045	Sb03g030820 - similar to Cluster: Os01g0672800 protein; Oryza sativa Japonica	1,02
TC121741	Sb10g005340.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,02
TC130718	Sb03g024250 - homologue to Human ribosomal DNA complete repeating unit	1,02
TC130047	Sb06g014680 - homologue to Cluster: Predicted protein; Monosiga brevicollis MX1	1,02
TC126425	Sb04g037530 binding - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,02
TC117935	similar to Cluster: Os10g0552900 protein; Oryza sativa Japonica Group - catalytic activity	1,01
TC127758	Sb02g041980.1 - similar to Cluster: Putative uncharacterized protein P0496C02.128; Oryza sativa	1,01
TC130928	Sb03g020074 - similar to Cluster: Putative uncharacterized protein; Methylobacterium sp. 4-46	1,01
TC117200	Sb03g003750 membrane - similar to Cluster: Putative protein OJ1174_D05.1; Oryza sativa Japonica Group	1,01
TC132822	Sb01g001660.3 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,01

TC133696	similar to Cluster: Putative uncharacterized protein; Anaeromyxobacter sp. K	1,01
TC128416	Sb06g026620.1 - similar to Cluster: OSJNBa0013K16.11 protein; Oryza sativa Japonica	1,00
TC126364	Sb08g005280.2 - similar to Cluster: Os12g0188900 protein; Oryza sativa Japonica	1,00

DOWN-REGULATED

ID	Annotation	Log$_2$FC	Functional class
BG837255	Sb01g040000.1 Belongs to the small heat shock protein (HSP20) family	3,35	Cell rescue defence and virulence
TC490440	Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	2,22	
TC122625	Sb3219s002010.1 chitinase activity	2,15	
TC123964	Sb04g008590.1 peroxidase activity	2,13	
TC132654	Sb03g006890 : similar to 16.9 kDa class I heat shock protein 1	2,01	
TC481803	Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	1,98	
TC518818	Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	1,80	
EB705607	Sb03g003530 : similar to 17.8 kDa class II heat shock protein	1,69	
TC131839	Sb05g017960 : similar to Oxidoreductase, short chain dehydrogenase/reductase family protein	1,67	
TC113729	Sb03g028620.1 monooxygenase activity Belongs to the cytochrome P450 family	1,59	
CO452191	Sb01g040000.1 Belongs to the small heat shock protein (HSP20) family	1,56	
TC118377	Sb01g006300.1 oxidoreductase activity	1,52	
FL292626	Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	1,50	
TC494026	Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	1,49	
TC466646	Sb03g003530 : similar to 17.8 kDa class II heat shock protein	1,47	
TC128001	Sb01g036770 : similar to DnaJ domain containing protein, expressed	1,42	
TC127759	Sb02g042115 : similar to Os07g0663500 protein - oxidoreductase activity	1,41	
TC115165	Sb03g028620.1 monooxygenase activity Belongs to the cytochrome P450 family	1,40	
TC111550	Sb01g030560 : similar to Oxidoreductase, 2OG-Fe oxygenase family protein	1,34	
TC128545	Sb02g030720 : similar to Putative Cytochrome P450	1,31	
TC120839	Sb06g032110.1 metal ion binding	1,31	
BE051728	Sb03g003530.1 Belongs to the small heat shock protein (HSP20) family	1,30	
TC111986	Sb04g023020.1 similar to Putative uncharacterized protein - MONOOXYGENASE	1,28	
TC129542	Sb06g030940 - peroxidase activity - similar to Cluster: H0212B02.16 protein; Oryza sativa	1,26	
TC111863	Sb02g042860 : similar to Plasma membrane-bound peroxidase 3-1	1,25	
TC122393	Sb10g025110.1 monooxygenase activity Belongs to the cytochrome P450 family	1,24	

TC118217	Sb01g000580 : similar to Gibberellin 20-oxidase	1,22	
TC119642	Sb10g011530 : similar to Os06g0490400 protein - peroxidase activity	1,21	
TC116745	Sb02g003930 : similar to Putative iron deficiency protein Ids3 oxidoreductase activity	1,19	
TC117201	Sb10g001940.1: similar to Pathogenesis-related protein PRMS precursor	1,18	
TC129073	Sb09g022510 : similar to Laccase-12/13 precursor - oxidation-reduction	1,17	
FL338885	Sb01g035350 : similar to Superoxide dismutase [Cu-Zn] 4A	1,16	
TC112594	Sb03g040250.1 monooxygenase activity Belongs to the cytochrome P450 family	1,15	
AI770912	Sb03g003530.1 Belongs to the small heat shock protein (HSP20) family	1,14	
TC130566	Sb02g028910 : similar to Os09g0498500 protein - oxidoreductase activity	1,14	
TC119702	Sb01g012930.1 Belongs to the small heat shock protein (HSP20) family	1,12	
TC114235	Sb01g006420 : similar to DNAJ protein - chaperonine	1,08	
TC125517	Sb04g029040 similar to Mitochondrial aldehyde dehydrogenase RF2B oxidoreductase activity	1,07	
TC132154	Sb01g041180 : similar to Heat shock protein 26	1,07	
TC112612	Sb10g010040.1 peroxidase activity	1,07	
TC124244	Sb01g030990 : similar to Glutathione S-transferase GST 42	1,06	
TC129098	Sb05g008350 : similar to Non-TIR-NBS-LRR type resistance protein	1,03	
TC132959	Sb01g002130 - Pfam:03168 Late embryogenesis abundant protein	1,02	
TC123886	Sb03g015070.1: similar to Glutathione S-transferase GST 8	1,02	
TC114557	Sb06g000660 : similar to Heat shock protein 82	1,00	
TC336339	Sb01g040000 : similar to 17.4 kDa class I heat shock protein 3	1,00	
TC119902	Sb07g028050 : similar to Putative glycerophosphoryl diester phosphodiesterase 2	1,60	Lipid metabolism
TC118015	Sb02g029200 : similar to Acyl-[acyl-carrier protein] thioesteraseSb02g031310 lipase activity	1,53	
TC130924	Sb01g019520 : similar to Beta-ketoacyl-CoA synthase family protein, putative, expressed - lipid biosynthesis	1,44	
TC111842	Sb01g050360.1 lipid transport	1,33	
TC118918	Sb07g028050.1 glycerophosphodiester phosphodiesterase activity lipid metabolic process	1,22	
TC128192	Sb07g024290 : similar to Putative fatty acyl coA reductase	1,16	
TC120110	Sb03g001870.1 lipid metabolic process	1,02	
TC120391	similar to Cluster: Glutamate dehydrogenase; Gramella forsetii KT0803	1,62	Aminoacid metabolism
TC116451	Sb02g003530 - LOC_Os02g20360 : tyrosine aminotransferase, putative	1,19	
TC123728	Sb04g021810 : similar to Cell wall invertase	2,93	Sugar metabolism
TC128911	Sb01g025310 : similar to Glycosyl hydrolases family 17 protein, expressed	2,60	
TC111678	Sb03g045460: similar to Glucan endo-1,3-beta-glucosidase, acidic isoform precursor - carbohydrate metabolic process	2,50	
TC120410	Sb04g021810.3 similar to Cell wall invertase	2,40	

TC115119	Sb03g012830.1: similar to Putative pectinesterase - cell wall modification	2,35
TC128055	Sb02g022260 : similar to Arbutin synthase-like	2,26
TC128904	Sb04g021810 : similar to Cell wall invertase - carbohydrate metabolic process	2,21
TC117723	Sb09g018750 : similar to Beta-glucanase precursor	2,05
TC123285	Sb01g050070.1 xyloglucan:xyloglucosyl transferase activity	2,02
TC111989	Sb08g019670.1 hydrolase activity, hydrolyzing O-glycosyl compounds Glycosidase	2,01
TC127493	Sb09g018730 : similar to Beta-glucanase precursor - Glycosyl hydrolases family 17	1,97
TC120854	Sb05g006880.1 hydrolase activity, hydrolyzing O-glycosyl compounds Glycosidase	1,87
TC117607	Sb09g018730 : similar to Beta-glucanase precursor - Glycosyl hydrolases family 17	1,78
TC115303	Sb09g023440 : similar to Expansin-A4 precursor - plant-type cell wall organization	1,78
TC121432	Sb01g010825 : similar to Putative beta-glucosidase	1,77
TC127774	Sb03g012830 : similar to Putative pectinesterase	1,74
TC121190	Sb09g018730.4 - : similar to Beta-glucanase precursor - Glycosyl hydrolases family 17	1,74
TC117534	Sb09g018750 : similar to Beta-glucanase precursor - hydrolyzing O-glycosyl compounds Glycosidase	1,74
TC116145	Sb03g038290 : similar to Alpha-expansin 1 precursor	1,72
TC128319	Sb06g030270 : similar to OSIGBa0138H21-OSIGBa0138E01.14 protein - hydrolyzing O-glycosyl compounds	1,61
TC126571	Sb06g030270 : similar to OSIGBa0138H21-OSIGBa0138E01.14 protein - hydrolyzing O-glycosyl compounds	1,61
TC126754	Sb01g024390 - hydrolase activity, hydrolyzing O-glycosyl compounds - glucan 1,3-beta-glucosidase	1,59
TC125745	Sb07g028620 : similar to Alkaline alpha galactosidase 3	1,58
TC124121	Sb0010s007570.1 hydrolase activity, hydrolyzing O-glycosyl compounds Glycosidase	1,56
TC116502	Sb08g004580.1 galactoside 2-alpha-L-fucosyltransferase activity cell wall biogenesis - ATP-binding	1,41
TC125746	Sb04g029420.1 cellulose synthase (UDP-forming) activity Glycosyltransferase	1,38
TC112635	Sb05g002710.1 Glycosyltransferase	1,34
TC128947	Sb01g025310 : similar to Glycosyl hydrolases family 17 protein, expressed	1,26
TC129414	Sb0010s007570 - hydrolase activity, hydrolyzing O-glycosyl compounds	1,25
TC112609	Sb04g032820.1: similar to Expansin-B11 precursor	1,23
CO467669	Sb04g025120.1: similar to Glyceraldehyde-3-phosphate dehydrogenase, cytosolic 3	1,17
TC118101	Sb03g047060.1 hydrolase activity, hydrolyzing O-glycosyl compounds (Fructan exohydrolase - Triticum aestivum)	1,17
TC126380	Sb07g019310 : similar to Putative polygalacturonase isoenzyme 1 beta subunit homolog	1,15
TC509745	Sb04g025120 : similar to Glyceraldehyde-3-phosphate dehydrogenase, cytosolic 3	1,15
TC130086	Sb04g029420 : similar to Cellulose synthase-like protein OsCslE2	1,14
TC114197	Sb10g028570 : similar to Xyloglucan endo-transglycosylase homolog	1,14
TC127506	Sb10g031040 - Pfam sucrose synthase	1,13

TC123368	Sb09g018730 : similar to Beta-glucanase precursor - Glycosyl hydrolases family 17	1,13	
TC125618	Sb05g020400 : similar to Probable xyloglucan endotransglucosylase/hydrolase precursor	1,11	
TC121148	Sb03g045390.1 glycerol-3-phosphate dehydrogenase [NAD+] activity oxidation reduction	1,09	
TC114207	Sb03g045490 : similar to Endo-1,3-beta-glucanase	1,08	
TC121873	Sb02g010730 : similar to Beta-expansin 4 precursor	1,07	
TC121378	Sb01g024390.1 hydrolase activity, hydrolyzing O-glycosyl compounds carbohydrate metabolic process	1,07	
TC123634	Sb01g025310.1 hydrolase activity, hydrolyzing O-glycosyl compounds carbohydrate metabolic process	1,05	
TC112397	Sb02g035520.1 hydrolase activity, hydrolyzing O-glycosyl compounds carbohydrate metabolic process	1,03	
TC297776	Sb10g028570 : similar to Xyloglucan endo-transglycosylase homolog	1,02	
TC114250	weakly similar to Cluster: 2-oxoglutarate dehydrogenase E1 component; Geobacter sulfurreducens - glycolysis	1,01	
TC113845	Sb06g024800.1 lipid transport	2,69	Transport
TC117799	Sb01g011360.1 transporter activity (Nucleobase-ascorbate transporter; Zea mays)	2,69	
TC112437	Sb06g030490.1 lipid transport	2,39	
TC117259	Sb05g018520.1 transmembrane transport	1,96	
TC117249	Sb06g026930.1 metal ion transport	1,66	
TC122101	Sb05g018520.1 transmembrane transport	1,66	
TC120873	weakly similar to Cluster: Putative permease transmembrane protein; Methylobacterium nodulans ORS 2060	1,59	
TC115931	Sb03g043020 - Major Facilitator Superfamily	1,57	
TC119094	Sb01g042690.1 substrate-specific transmembrane transporter activity	1,46	
TC126605	Sb06g023010 : similar to OSIGBa0153E02-OSIGBa0093I20.15 protein - transmembrane transport	1,46	
TC116158	Sb01g047690.1 oligopeptide transport	1,45	
TC112756	Sb01g042690.1 substrate-specific transmembrane transporter activity	1,42	
TC113792	Sb01g011360.1 transporter activity (Nucleobase-ascorbate transporter; Zea mays)	1,41	
TC121736	Sb03g043020 - Major Facilitator Superfamily	1,41	
TC113684	Sb01g047690.1 transporter activity	1,32	
TC125758	Sb03g008060.1 transmembrane transport	1,30	
TC126132	Sb02g036370 : similar to Putative organic cation transporter	1,18	
TC123586	Sb02g028520.1 transmembrane transport	1,12	
TC118930	Sb06g023010.1 transmembrane transport	1,07	
TC112532	Sb07g002260.1 integral to membrane - similar to Cluster: Putative histidine amino acid transporter; Oryza sativa Japonica	1,03	
TC127658	Sb10g011210 - KOG:1339 Aspartyl protease	2,87	Protein fate
TC118568	Sb04g029670.1 aspartic-type endopeptidase activity	2,82	
TC114462	Sb04g029670.1 aspartic-type endopeptidase activity	2,40	

TC120931	Sb02g002260.1 serine-type carboxypeptidase activity	1,62	
TC125109	Sb01g007820 : similar to Cysteine proteinase precursor	1,46	
TC122095	Sb01g007820 : similar to Cysteine proteinase precursor	1,43	
TC129930	Sb03g029930 KOG:0978 E3 ubiquitin ligase involved in syntaxin degradation	1,37	
TC123392	Sb05g008470.1 aspartic-type endopeptidase activity	1,33	
TC117225	Sb04g026900.1 serine-type carboxypeptidase activity	1,32	
TC112387	Sb01g044790.1 proteolysis aspartic-type endopeptidase activity	1,31	
TC115432	Sb02g002260 : similar to Putative serine carboxypeptidase II	1,26	
TC132470	Sb04g026900 : similar to Putative carboxypeptidase D	1,26	
TC129953	Sb05g008470 : weakly similar to Os01g0696800 protein - Aspartyl protease	1,21	
TC113549	Sb06g032320.1 cysteine-type endopeptidase activity	1,10	
TC133717	Sb10g027640 : weakly similar to O-methyltransferase ZRP4	1,02	
TC129855	Sb03g004300 : similar to Os01g0173900 protein - ATPase activity, coupled to transmembrane movement of substances	1,51	Nucleotide binding
TC111416	Sb08g023150 : similar to Cell Division Protein AAA ATPase family	1,50	
TC134360	Sb02g005630 : similar to AAA-type ATPase-like	1,46	
TC132868	Sb06g002070 : similar to OSJNBb0022P19.1 protein - ATPase activity, coupled to transmembrane movement of substances	1,31	
TC119586	Sb01g036020.1 protein serine/threonine kinase activity	1,27	
TC121312	Sb09g025640.1 small GTPase mediated signal transduction	1,25	
TC115442	Sb08g023150 : similar to Cell Division Protein AAA ATPase family	1,21	
TC126763	Sb03g004300 : similar to Os01g0173900 protein - ATPase activity, coupled to transmembrane movement of substances	1,07	
TC134093	Sb01g045940 : similar to Os01g0173900 protein - ATPase activity, coupled to transmembrane movement of substances	1,06	
TC115604	Sb08g023150 : similar to Cell Division Protein AAA ATPase family	1,04	
TC126193	Sb09g029900 - ATP binding	1,02	
TC113389	Sb08g023150 : similar to Cell Division Protein AAA ATPase familyy	1,01	
TC124426	Sb09g030280.1 - similar to Cluster: Putative sigma54 specific transcriptional regulator, Anaeromyxobacter sp. K	2,56	Nucleic acid binding
TC116915	Sb07g005610 regulation of transcription	2,42	
TC131912	Sb07g005610 : similar to Putative NAC domain protein NAC1 - regulation of transcription	2,41	
TC122780	Sb07g005610 : similar to Putative NAC domain protein NAC1 - regulation of transcription	2,36	
TC125406	Sb10g026450.1 sequence-specific DNA binding transcription factor activity - Belongs to the bZIP family	2,08	
TC131066	Sb01g045060 : similar to AP2 domain containing protein	2,01	
TC125997	Sb01g045060 : similar to AP2 domain containing protein - regulation of transcription, DNA-dependent	1,90	
TC116354	Sb01g030570 : similar to MADS-box transcription factor TaAGL7	1,64	
TC115292	Sb03g006590 - Pfam AP2 domain	1,59	

TC119236	Cluster: Sigma-54 dependent DNA-binding transcriptional regulator; Burkholderia mallei SAVP1	1,58	
TC133146	Sb06g020810 Contains 1 basic helix-loop-helix (bHLH) domain transcription regulator activity	1,46	
TC126373	Sb03g037940.1 regulation of transcription	1,45	
TC129765	Sb01g030570 : similar to MADS-box transcription factor TaAGL7	1,41	
TC133995	Sb08g001140 : similar to Response regulator 6 - regulation of transcription, DNA-dependent	1,37	
TC131653	Sb10g030770 regulation of transcription	1,37	
TC133385	Sb07g027050 : similar to Putative oligouridylate binding prot. - RNA recognition motif. (a.k.a. RRM, RBD, RNP domain)	1,33	
TC125397	Sb08g020600 : similar to BZIP transcription factor family protein	1,33	
TC112527	Sb01g045060.2 AP2 domain	1,32	
TC116288	Sb09g026830.1 sequence-specific DNA binding transcription factor activity	1,29	
TC112413	Sb03g003370.1 sequence-specific DNA binding transcription factor activity	1,28	
TC129125	Sb03g034140 : similar to Putative endoribonuclease E	1,27	
CO451296	Sb01g012300.2: Glycine-rich RNA-binding protein 2	1,24	
CO440167	Sb08g022740.1: Glycine-rich protein	1,22	
TC131776	Sb01g013340 : weakly similar to OSJNBa0005N02.3, protein argonaute 1B - RNA-mediated gene silencing	1,21	
TC132197	Sb04g027540 : similar to Putative MYB family transcription factor	1,18	
TC125384	Sb07g006195.1 sequence-specific DNA binding transcription factor activity - AP2 domain	1,14	
TC113425	Sb03g003370.1 sequence-specific DNA binding transcription factor activity	1,12	
TC130052	Sb01g028930 : similar to AP2 domain containing protein, expressed	1,08	
TC123407	Sb04g003240.1 regulation of transcription, DNA-dependent auxin mediated signaling pathway	1,07	
FL255856	Sb03g027210 : similar to ETCHED1 protein precursor - DNL zinc finger	1,06	
FL136369	Sb04g022800 : similar to Putative Ligatin - translation initiation factor activity	1,05	
TC127555	Sb03g038200 : weakly similar to WRKY transcription factor 64-like protein	1,03	
TC128178	Sb07g006195 : similar to Ethylene responsive transcription factor - AP2 domain	1,03	
CO454355	Sb08g022740.1: Glycine-rich RNA binding protein	1,02	
TC121390	Sb05g027230.1 protein kinase activity	2,24	Communication and signalling
TC127432	Sb02g035440 : similar to Putative serine/threonine-specific protein kinase	2,22	
TC126479	Sb02g035450.1 protein serine/threonine kinase activity	2,20	
TC116025	Sb02g037310.1: similar to Putative fasciclin-like arabinogalactan-protein	1,92	
TC122156	Sb02g035450.1 protein serine/threonine kinase activity	1,92	
TC124273	Sb09g026180 : similar to Putative receptor like protein kinase	1,86	
TC123158	Sb02g024550.1 calcium ion binding	1,84	
TC119449	Sb06g028760.1 protein serine/threonine kinase activity	1,75	

TC132333	Sb07g005820 : similar to SHR5-receptor-like kinase	1,68	
TC122248	Sb06g003120.1 protein serine/threonine kinase activity	1,68	
TC111925	Sb02g035550.1 - Serine/threonine-protein kinase	1,64	
TC130387	Sb10g002600 : similar to Protein kinase domain containing protein	1,59	
TC133215	Sb05g021320 : similar to Ser/Thr protein phosphatase family protein	1,57	
TC119035	Sb06g003120.1 protein serine/threonine kinase activity	1,47	
TC131290	Sb05g021320 : similar to Ser/Thr protein phosphatase family protein	1,44	
TC116353	Sb02g035560.1 protein serine/threonine kinase activity	1,38	
TC117374	Sb01g034570.1 protein serine/threonine kinase activity	1,36	
TC126917	Sb05g008370 : similar to Serine/threonine protein kinase	1,33	
TC117764	Sb01g034570.1 Serine/threonine-protein kinase	1,29	
TC130295	Sb01g033650 : similar to IQ calmodulin-binding motif family protein, expressed	1,27	
TC129167	Sb06g031600 : similar to H0112G12.4 protein - protein serine/threonine kinase activity	1,21	
TC114238	Sb05g024090.1 Serine/threonine-protein kinase	1,21	
TC122989	Sb04g026010.1 protein kinase activity	1,21	
TC119738	Sb06g031450.1 - (Protein kinase - Sorangium)	1,21	
TC129090	Sb05g008350 : similar to Non-TIR-NBS-LRR type resistance prot. (Leucine Rich Repeat putative; O. sativa Japonica)	1,18	
TC130405	Sb06g021870 - Leucine Rich Repeat	1,16	
TC131538	Sb02g019350.1 - Stress-induced receptor-like kinase 1 (Oryza sativa Indica)	1,15	
TC125832	Sb02g040240.1 calcium ion binding	1,15	
TC134274	Sb09g007250 : similar to OSIGBa0148I18.4 protein - protein serine/threonine kinase activity	1,14	
TC132630	Sb05g008370 : similar to Serine/threonine protein kinase	1,14	
TC130655	Sb08g006710 : similar to Leucine Rich Repeat family protein, expressed - protein phosphorylation	1,13	
TC120721	similar to Cluster: WNK protein kinase; Chlamydomonas reinhardtii	1,11	
TC123792	Sb06g029810: similar to LOC_Os04g54190 : cysteine-rich receptor-like protein kinase 8 precursor	1,09	
TC113576	Sb08g020035.1 protein serine/threonine kinase activity	1,01	
TC466608	Sb01g028750 : similar to Calcineurin B-like protein 1	1,00	
FL136446	Sb03g004890 : similar to Histone H4	1,18	Cell cycle-DNA processing
TC484837	Sb02g032240: similar to histone H4, DNA binding	1,00	
TC133171	Sb01g025970 : similar to Proline-rich protein precursor	3,84	Interaction with the nvironment
TC121800	Sb01g025970 : similar to Proline-rich protein precursor	3,09	
TC500659	Sb09g003050 : similar to Hydrophobic protein LTI6B	1,51	
TC112720	Sb09g030530 : similar to Hypersensitive-induced response protein	1,26	

TC119943	Sb01g038150.1: similar to Streptomyces cyclase/dehydrase family - abscisic acid receptor PYR/PYL family	2,01	Interaction with the environment
TC120887	Sb03g028020.1 oxidoreductase activity - similar to Cluster: Putative auxin-induced protein; Oryza sativa Japonica	1,04	
TC120394	Sb02g024610 : similar to 60S Ribosomal protein l34	1,18	Others
TC125341	Sb02g012040 : similar to Ubiquitin-60S ribosomal L40 fusion protein - translation	1,06	
TC123451	Sb05g004130 : similar to 60S ribosomal protein L3	1,05	
TC134465	Sb07g004290 - KOG:3181 40S ribosomal protein S3	1,05	
TC130137	Sb05g008030 : similar to NB-ARC domain, putative - defense response	1,91	
TC132003	Sb05g008030 : similar to NB-ARC domain, putative - apoptosis	1,84	
TC114256	Sb01g048500 : similar to Seven transmembrane protein Mlo1 - cell death	1,61	
TC114352	Sb01g048500 : similar to Seven transmembrane protein Mlo1 - cell death	1,37	
TC122949	Sb03g027040.1 photosynthesis, light harvesting	1,12	
TC123116	Sb09g004970.1 nutrient reservoir activity	2,70	
TC111495	Sb03g045980.1 nutrient reservoir activity	2,52	
TC122020	Sb04g026020.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	3,18	Unknown
TC120599	Sb09g030280.1 - similar to Cluster: Minus agglutinin; Chlamydomonas incerta	2,74	
TC134574	Sb06g019560 - similar to Cluster: OSJNBa0016O02.17 protein; Oryza sativa	2,67	
TC125771	Sb10g010780.1 - weakly similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2,64	
TC119042	Sb02g035460.2 - weakly similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	2,35	
TC127126	Sb01g008980 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	2,28	
TC114978	Sb05g026890.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	2,27	
TC117420	weakly similar to Cluster: Avena; Gallus gallus	2,21	
TC133428	Sb06g033160 : similar to Os04g0680400 protein - hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	2,14	
TC120921	Sb03g034860.1 - similar to Cluster: Putative uncharacterized protein P0503C12.12; Oryza sativa Japonica	2,07	
TC115192	Sb10g010810.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,89	
TC120814	Sb10g010810.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,84	
TC122655	Sb05g026870.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,81	
TC112125	Sb02g002720.1 - similar to Cluster: Early nodulin 75-like protein; Oryza sativa Japonica Group	1,80	
TC118549	Sb01g010840.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,76	
TC131129	homologue to Cluster: 23S rRNA (Uracil-5-)-methyltransferase RumA; Rhodospirillum rubrum ATCC 11170	1,76	
TC118215	similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,72	
TC124262	similar to Cluster: Drosophila melanogaster GH13383p; Plasmodium yoelii yoelii	1,71	
TC121958	Sb06g019520.1 - weakly similar to Cluster: OSJNBa0016O02.17 protein; Oryza sativa	1,57	
TC126466	Sb09g018730 - similar to Cluster: CDNA FLJ45488 fis clone BRTHA2003759; Homo sapiens	1,54	

TC113122	Sb02g020830.1 - similar to Cluster: Os09g0307300 protein; Oryza sativa	1,54
TC131851	Sb02g002710 : weakly similar to Early nodulin 75-like protein	1,53
TC123085	Sb10g020870.1 - homologue to Cluster: Putative uncharacterized protein P0438E12.36; Oryza sativa Japonica Group	1,53
TC117021	Sb01g041580.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,51
TC125767	Sb10g010780.1: similar to Embryogenesis transmembrane protein	1,49
TC131402	Sb05g023150 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,39
TC124743	Sb04g030470.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,38
TC130268	Sb06g021860 : weakly similar to OSJNBa0014K14.20 protein	1,38
TC134454	Sb06g029940 : similar to uncharacterized protein OR Sb06g029930: similar to H0410G08.10 protein	1,38
TC125131	Sb05g019070.1 - similar to Cluster: Expressed protein; Oryza sativa Japonica Group	1,36
TC130423	Sb03g008140 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,30
TC129915	Sb04g013580 : similar to Putative uncharacterized protein P0644G05.16	1,29
TC126515	similar to Cluster: Putative uncharacterized protein At1g61000; Arabidopsis thaliana	1,27
TC127208	similar to Cluster: Putative uncharacterized protein; Phaeosphaeria nodorum	1,27
TC118614	Sb03g029930.1 - weakly similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,27
TC128831	Sb03g003310 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,27
TC131877	Sb09g019550 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,26
TC121039	similar to Cluster: Putative uncharacterized protein; Algoriphagus sp. PR1	1,25
TC124931	Sb02g035140.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,23
TC132113	Sb05g006423.1 - similar to Cluster: Chromosome SCAF1938 whole genome shotgun sequence; Tetraodon	1,23
TC121244	Sb09g021420.2 - similar to Cluster: Heavy-chain fibroin; Yponomeuta evonymellus	1,22
TC115421	Sb05g027150 - similar to Cluster: Os08g0413100 protein; Oryza sativa Japonica Group	1,22
TC112712	Sb01g015580.1: similar to Chloroplast post-illumination chlorophyll fluorescence increase protein	1,21
TC122629	homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica	1,21
TC114440	Sb01g039360.1: similar to 33 kDa secretory protein	1,17
TC130887	weakly similar to Cluster: Putative uncharacterized protein; Burkholderia pseudomallei 1106a	1,17
TC131134	similar to Cluster: PxORF20 peptide; Plutella xylostella granulovirus	1,17
TC112464	Sb01g027660.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,17
TC127426	Sb09g021420 : similar to Putative uncharacterized protein OSJNBb0088F07.6	1,15
TC114339	Sb02g025930.1 - similar to Cluster: Putative uncharacterized protein P0046G12.23; Oryza sativa Japonica	1,14
TC116432	Sb08g021090.1 integral to membrane - similar to Cluster: Os12g0610800 protein; Oryza sativa	1,14
TC112878	Sb03g005280.1 - similar to Cluster: Fasciclin-like protein FLA18; Triticum aestivum	1,14
TC121386	Sb01g030600.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,13

TC123048	similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,13
TC131148	Sb04g009200 - homologue to Cluster: Putative uncharacterized protein; Oryza sativa	1,11
TC126426	Sb01g037610.1 catalytic activity - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,11
TC131983	weakly similar to Cluster: Apolipoprotein N-acyltransferase; Chlorobaculum tepidum	1,10
TC119801	weakly similar to Cluster: Predicted protein; Monosiga brevicollis MX1	1,10
TC123421	Sb01g031990 - weakly similar to Cluster: OSJNBb0085F13.3 protein; Oryza sativa Japonica	1,10
TC117656	Sb07g007810.1 catalytic activity - homologue to Cluster: Putative uncharacterized protein; Oryza sativa Indica	1,08
TC123023	Sb10g028380.1 - similar to Cluster: Putative uncharacterized protein P0622F03.3; Oryza sativa	1,08
TC126275	Sb02g036133.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,08
TC116424	Sb06g021870.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Japonica Group	1,07
TC130450	Sb02g003530 - Aminotransferase class I and II	1,07
TC128978	Sb03g034000 : similar to Os01g0737800 protein - catalytic activity	1,06
TC122656	Sb01g039030.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,06
TC112651	Sb04g011100 : similar to Putative DREPP2 protein	1,06
TC131711	homologue to Cluster: Putative uncharacterized protein; Pseudomonas putida W619	1,06
TC122423	Sb04g037330.1 - homologue to Cluster: Putative uncharacterized protein precursor; Roseiflexus sp. RS-1	1,06
TC112177	Sb04g003170.2 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,05
TC119182	Sb08g022530.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,04
DY624944	Sb06g015550.1- alpha/beta hydrolase fold	1,03
TC116421	similar to Cluster: hypothetical protein FG01588.1; Gibberella zeae PH-1	1,03
TC130956	Sb02g004500 - Cupin domain - similar to Cluster: Putative uncharacterized protein; Oryza sativa	1,02
TC116166	Sb06g024250.1 - similar to Cluster: OSIGBa0101C23.5 protein; Oryza sativa	1,02
TC117117	Sb10g023690.1 binding - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,02
TC123636	Sb01g017040.1 - similar to Cluster: Putative uncharacterized protein; Oryza sativa Indica Group	1,01
TC118333	Sb01g048565.1 - similar to Cluster: Os03g0128800 protein; Oryza sativa Japonica Group	1,00

4.2.3 microRNAs expression under drought

Differentially expressed miRNAs were obtained from hybridization of a dedicated 12K Combimatrix microarrays made up of four chambers of about 2K spots. Each chamber contains 61 mature miRNAs sequences of sorghum and 36 of maize. Nine miRNAs of sorghum divided in three miRNA families and two maize miRNAs of two different families resulted differentially expressed under drought stress. The major part of miRNAs resulted up-regulated while only one was down-regulated (ZMA-Mir528a). Water stress appeared to influence miRNAs expression in the earlier phase of stress (FTSW0.37; Tab. 9).

In our experiment, miRNAs SBI-miR164, SBI-miR164b and SBI-miR164c resulted over-expressed only at FTSW 0.37 (Tab. 9). At FTSW 0.15 NAC1 transcriptions factors were found down-regulated in stressed leaves. MiR164 was predicted to target mRNAs encoding members of NAM/ATAF/CUC (NAC) domain transcription family (Guo *et al.*, 2005). *NAC1* is involved in transmitting auxin signals for lateral root development. There is a positive correlation between *NAC1* mRNA levels and lateral root numbers. Transgenic plants and *miR164* mutants had the expected lateral root phenotypes correlating with *NAC1* mRNA abundance (Guo *et al.*, 2005). This could indicate that in severe conditions of water stress plant supress lateral roots production.

Four members of SBI-miR395 family (SBI-miR395a, SBI-miR395f, SBI-miR395k and SBI-miR395l), resulted over-expressed (Log2FC 1.23, 1.13, 1.52, 1.24, respectively) at FTSW 0.37 (Tab. 9). MiR395 was demonstrated to target ATP sulphurylases (*APS1*, *APS4*), that catalyze sulphate assimilation pathway, and a low-affinity sulphate transporter *AST68* mRNAs (Jones-Rhoades *et al.*, 2006). MiR395 is induced by sulphate deprivation while *APS1* transcript levels decrease. Sulphur is an essential macronutrient and is available in the soil as sulphate form. Sulphate is assimilated into cysteine, amino acid involved in primary metabolism, protein synthesis and sulphur-containing defence compounds (SDCs) such as glutathione and thionin (Shukla *et al.*, 2008). Induction of miR395 family miRNAs in the earlier steps of water stress may indicate reduced assimilation and translocation of sulphur into the plant. This may allow to their mRNAs target to be translated in higher levels, and consequently to enhance sulphate uptake. In fact, the Sb01g008450 gene, encoding for an ATP sulphurylase, was effectively up-regulated at FTSW 0.15 (Log2FC 1.2). Plant could use the increased amount of sulphate for the production of glutathione S-transferases, thioredoxins and other compounds involved in detoxification processes of ROS accumulated with the water stress (Rausch *et al*., 2005). Stressed leaves at FTSW 0.15 showed up-regulation of several transcripts encoding detoxifying enzymes (Tab. 8).

SBI-miR399a was the miRNA that up-regulated at all the three water stress levels considered and its expression level increased proportionally with water deprivation (Tab. 9). In the same miR399 family, SBI-miR399b was over-expressed only at FTSW 0.37 (Tab. 9). MiR399 was proposed to target UBC24 mRNA, an ubiquitin conjugating E2 enzyme (Sunkar, and Zhu, 2004). In Arabidopsis, this miRNA was shown to be up-regulated under inorganic phosphate (Pi) deficiency, whereas UBC24 was down-regulated (Lewis *et al*., 2010). Pi starvation strongly induced the expression of all miR399 genes which was rapidly reversed by Pi addiction (Bari *et al*., 2006). Down-regulation of UBC under phosphate stress was important for the attenuation of primary root elongation, induction of high affinity Pi transporters and acquisition of Pi to maintain Pi homeostasis (Fujii *et al*., 2005; Chiou *et al*., 2006). Water stress could interfere with Pi, decreasing its solubility and availability for plants. In this experiment, the gene Sb09g021400, encoding for Pi starvation-induced protein with serine-type endopeptidase activity, resulted up-regulated at FTSW 0.15 (Log_2FC 1.1).

The ZMA-miR827 was up-regulated at the two final levels of water stress (FTSW 0.25 and 0.15) with a Log_2FC 0.80, (Tab. 9). MiR827, as well as miR399 family, was demonstrated to be involved in the response under phosphorus deficiency. Different studies showed that miR827 targets are SPX (SYG1/Pho81/XPR1) proteins (Kuo and Chiou, 2011). This class of protein was implicated in Pi transport or sensing in yeast and in response to changes in external Pi concentration or xylem loading of Pi in plants. Arabidopsis miR827 (Ath-miR827) targets the NITROGEN LIMITATION ADAPTATION (NLA; also named BENZOIC ACID HYPERSENSITIVE1) transcript coding for a protein consisting of an N-terminal SPX domain and a C-terminal RING domain, the latter of which possesses a ubiquitin E3 ligase activity (Peng *et al*., 2007; Yaeno *et al*., 2008; Hsieh *et al*., 2009; Pant *et al*., 2009). The target genes encode proteins involved in the ubiquitin-mediated protein degradation pathway, indicating the importance of post-translational regulation of protein levels in the adaptive responses of P deficiency (Kuo and Chiou, 2011). In this experiment, at FTSW 0.15 a gene encoding for a protein with E3 ubiquitin ligase activity (Sb03g029930) resulted up-regulated in control samples. At FTSW 0.15, an SPX containing protein known to be up-regulated under phosphate starvation (gene Sb01g023270) was over-expressed (Log_2FC 2.3), together with another SPX containing protein involved in vacuolar polyphosphate accumulation (gene Sb03g024480, Log_2FC 1.85). These two genes resulted up-regulated also at FTSW 0.25 with Log_2FC of 1.9, and Sb03g024480 was already over-expressed at FTSW 0.37 (Log_2FC of 1.3). In this experiment, miR827 as well as miR399 were involved in different functions from those of the regulation of genes coding SPX containing protein.

ZMA-miR528a was the down-regulated miRNA at FTSW 0.25 (Log_2FC -0.85). MiR528 resulted under-expressed in *Triticum dicoccoides* exposed to drought stress (Kantar et al., 2010). In maize, miR528 targets copper proteins cupredoxin, multicopper oxidase and laccase genes and thus might play a critical role in regulating physiological processes (photosynthesis and respiratory electron transport) and stress responses (Zhang *et al*., 2009).

Tab. 9: Log_2FC values at $p<0.05$ of miRNA genes differentially regulated in sorghum IS19453 at three different drought stress levels (FTSW 0.37, 0.25 and 0.15).

miRNA	up at 0.37	up at 0.25	down at 0.25	up at 0.15
SBI-miR164	0.60			
SBI-miR164b	0.76			
SBI-miR164c	0.64			
SBI-miR395a	1.23			
SBI-miR395f	1.13			
SBI-miR395k	1.52			
SBI-miR395l	1.24			
SBI-miR399a	0.61	0.86		1.31
SBI-miR399b	0.80			
ZMA-miR528a			-0.85	
ZMA-miR827		0.80		0.81

4.3 Identification of differentially expressed genes under drought stress in green house experiment

In the genotypes tested in this experiment (BR505, BR501, Mpwekwa, SDS19483, IS33350) the number of differentially expressed genes between stressed and control plants increased with the intensity of the water stress: 674, 296, 69, 341, 618 genes resulted differentially expressed in BR505, BR501, Mpwekwa, SDS19483, IS33350, respectively, with p value <0.05, at 0.10 FTSW (Tab. 10). At 0.3 FTSW few genes resulted differentially expressed (p value <0.05) in IS33350, BR501, Mpwekwa and SDS19483 (data not shown). In BR505, 163 genes were differentially expressed at 0.30 FTSW.

No differences were observed in the number of down-regulated genes respect the up-regulated ones at the beginning of stress and at 0.1 FTSW in IS33350, BR501, Mpwekwa and SDS19483. Otherwise, Mpwekwa showed a slight and slowed gene expression response to drought stress.

On the contrary, in BR505 the number of down-regulated genes was greater respect the up regulated ones (120 against 43 at 0.3 FTSW; 401 against 273 at 0.10 FTSW).

The differentially expressed sequences were classified into 12 functional categories according to the MIPS FunCat database. The class “Others” included genes involved in the protein synthesis, cell fate, energy, subcellular localization and storage protein. About 34% in average of differentially expressed genes were unknown or not annotated (Tab. 10).

In the higher phase of stress, the functional class “Cell rescue, defence and virulence” was the most represented but also genes involved in other pathways, such as lipid and sugar metabolism, communication and signalling, regulation of transcription became important.

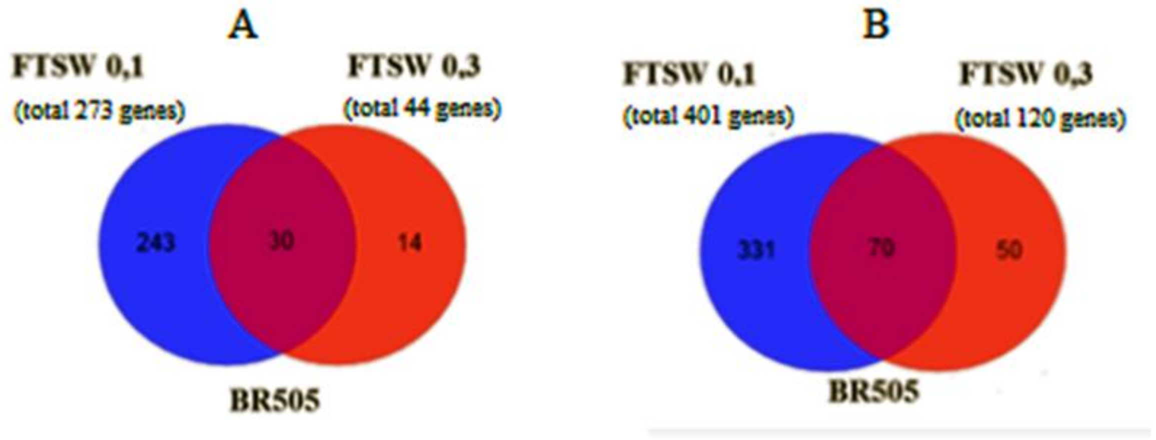
Tab. 10: Functional categories and number of up and down-regulated genes in sweet sorghum IS33350, BR501, BR505, Mpwekwa, SDS19483 leaves sampled at two different FTSW values during dry-down experiment.

	BR505				**BR501**		**Mpwekwa**		**SDS19483**		**IS33350**	
Functional Category	**0.3**		**0.1**		**0.1**		**0.1**		**0.1**		**0.1**	
	up	**down**	**up**	**down**	**up**	**down**	**up**	**down**	**up**	**down**	**up**	**down**
Cell rescue, defence and virulence	7	13	46	43	22	19	8	2	14	11	35	40
Lipid metabolism	1	/	6	12	5	4	3	/	2	6	12	10
Aminoacid metabolism	2	3	7	8	1	2	/	1	4	1	10	9
Sugar metabolism	6	7	22	22	16	19	5	2	13	18	35	16
Transport	7	5	22	20	8	17	/	4	4	13	32	31
Protein fate	2	6	15	20	3	6	/	2	8	13	21	20
Protein binding	/	/	3	16	/	/	/	/	9	3	8	6
Nucleotide binding	3	5	9	12	3	14	1	/	10	13	13	18
Nucleic acid binding	2	18	21	56	7	8	3	2	31	9	30	26
Communication and signalling	2	2	8	24	3	8	/	1	3	4	13	20
Cell cycle-DNA processing	/	1	3	1	4	/	/	/	7	2	4	3
Interaction with the environment	/	1	5	5	2	2	1	1	1	5	3	3
Others	1	3	10	15	3	12	1	/	13	6	5	2
Unknown	11	56	96	147	52	56	15	17	62	56	84	109
Total	43	120	273	401	129	167	37	32	181	160	305	313
Total of differentyally expressed genes	163		674		296		69		341		618	

As shown by the Venn diagrams, in BR505 genotype, 44 and 273 genes were up-regulated at FTSW of 0.30 and 0.10, respectively. 30 common genes were up regulated in stressed condition (Fig. 10 A). Likewise, 120 and 401 genes were down-regulated at FTSW of 0.30 and 0.10, respectively (Fig. 10 B). 70 down-regulated genes were expressed during two stress level.

BR501 genotype showed 3, 166 down regulated genes at FTSW of 0.30, 0.10, respectively. At 0.1 FTSW, the functional classes “Sugar metabolism, Transport, Nucleotide binding” were the most represented but also genes with unknown function were present.

Fig. 10: Venn diagrams showing numbers of overlapping and unique genes induced (A) and repressed (B) at 0.30 and 0.10 FTSW in leaves of sweet sorghum BR505. Results based on three of four biological replicates considering 1-fold or higher fold change (Log_2FC).

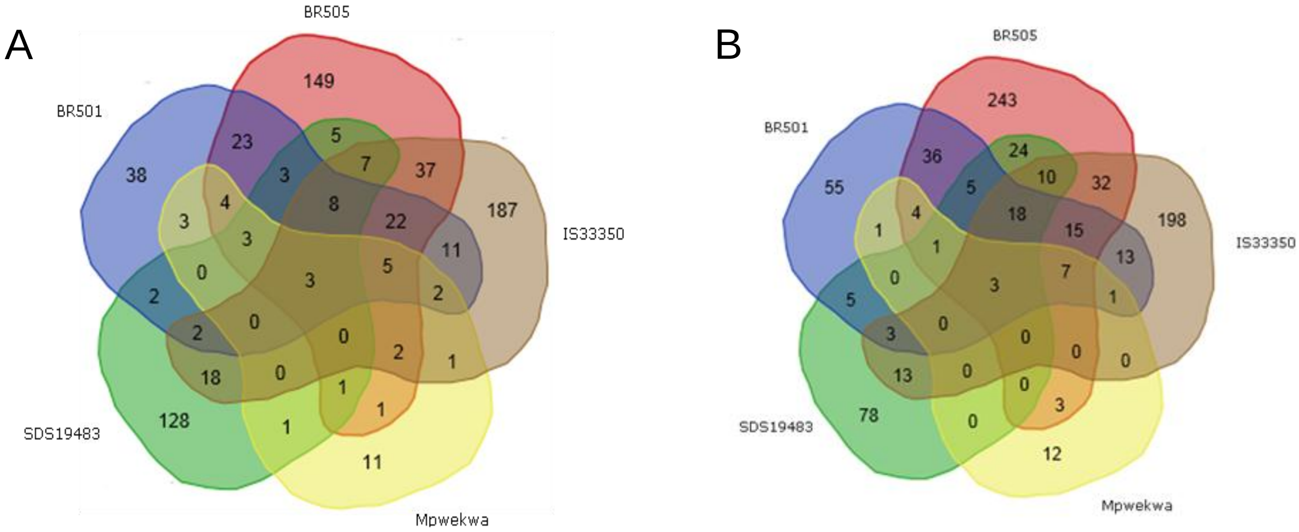


A gene encoding for a transcriptional regulator, mainly MADS-box protein (Sb04g000500) resulted up-regulated in in both BR505 and Mpwekwa genotypes (data not shown).

Three genes were induced in all five genotypes grown in green house and IS19453 in growth chamber at FTSW 0.1. Two genes (Sb10g008820, Sb02g006320) encoding for sugar metabolism-related proteins, and one involved in cellular response to water deprivation were validated and up-expressed in all genotypes (Fig. 11 A). .

At stress level of FTSW 0.1, three common down regulated genes were found (Fig. 11B). Two common genes with unknon function (Sb09g030280, Sb01g039360) were identified, between genotypes grown both in green house and growth chamber. Among these, Sb02g003530), encoding for a tyrosine aminotransferase, was found down-regulated.

Fig. 11: Venn diagrams showing numbers of overlapping genes induced (A) and repressed (B) at FTSW 0.1 in 5 sorghum genotypes. Results based of three biological replicates considering 1-fold or higher fold change (Log$_2$FC).

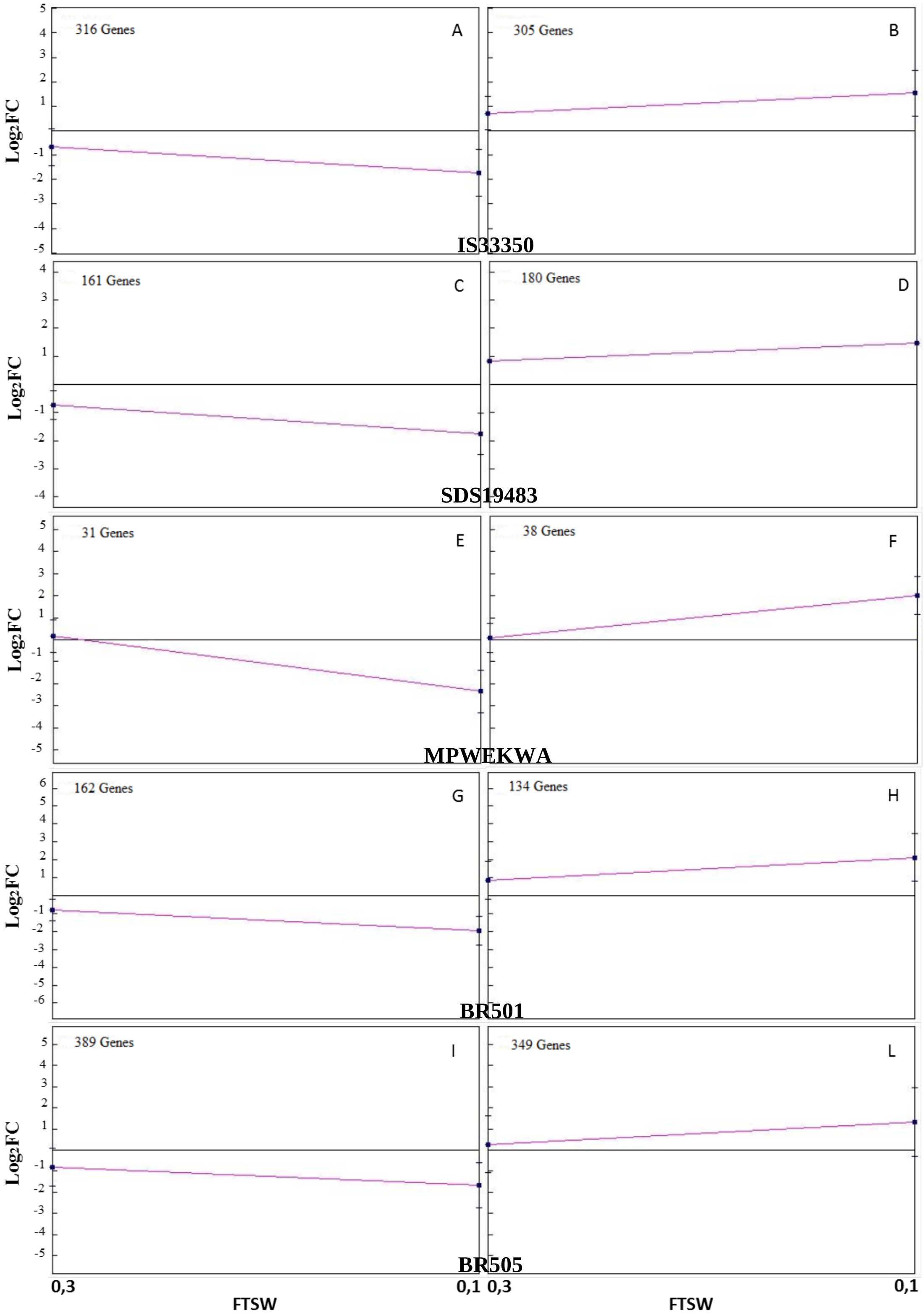


All differentially expressed genes identified in IS33350, SDS19483, Mpwekwa, BR501, BR505 at two different levels of drought stress (FTSW 0.30 and 0.10) were clustered. 621, 341, 69, 296, 738 genes were characterized in IS33350, SDS19483, Mpwekwa, BR501, BR505 genotypes, respectively. The genes resulted differentially expressed at least in one of the stress level considered, were processed. For each genotype, differentially expressed genes were grouped in two different clusters, each showing a specific trend at the increasing of water stress (Fig. 12 A-L). In detail, 316, 161, 31, 162, 389 probes exhibited a progressive decreasing expression level with a marked down regulation at FTSW 0.10. In particular, Mpwekwa (Fig. 12 E) yielded a specific profile that differed from other genotypes: only 31 probes exhibited a progressive decreasing expression level with down regulation at FTSW 0.10.

Likewise, for each genotype, up regulated genes were clustered. In detail, 305, 180, 134 genes showed a progressive increment in expression level at the increasing of stress in IS33350, SDS19483, BR501, respectively (Fig. 12 B, D, H).

Each genotype showed a balanced down and up gene regulation: the abundance of up and down regulated genes was almost the same. Only Mpwekwa genotype showed a slight and slowed gene expression response to drought stress.

Fig. 12: Cluster analysis of gene expression profiles of sweet sorghum IS33350, SDS19483, Mpwekwa, BR501, Br505, based on the mean $\mathrm{Log_2FC}$, at two water stress levels (0.3, 0.1 of FTSW). Four each genotype two different clusters were detected. Fig. A-C-E-G-I: genes decreased progressively their expression level; fig. B-D-F-H-L genes progressively incremented their expression level at the increasing of water stress. Vertical bars represent standard deviation.


316 Genes
A
305 Genes
B
IS33350
161 Genes
C
180 Genes
D
SDS19483
31 Genes
E
38 Genes
F
MPWEKWA
162 Genes
G
134 Genes
H
BR501
389 Genes
I
349 Genes
L
BR505
Log₂FC
0,3
0,1
FTSW
0,3
0,1
FTSW

5. Conclusions

In this study the response of sweet sorghum genotypes to progressive water stress was monitored in "dry-down" experiments. The dry-down approach was an excellent method to assess the physiological response of sorghum to the progressive reduction of water availability in different stages of plant development in growth chamber and in green house condition.

This approach enables to measure gravimetrically soil moisture content, as soil evaporation is suppressed, together with whole-plant transpiration rate (Luquet *et al.*, 2008). IS19453 sorghum genotype was recognized as drought tolerant because of its high pre- and post-flowering drought tolerance in field trials (Braconnier S., personal communication) and it was chosen in a first growth chamber experiment for a preliminary identification of drought related genes in young seedlings.

Subsequently, a greenhouse dry-down experiment was carried out. IS33350, BR501, BR505, Mpwekwa, SDS19483 were tested to combine the evaluation of physiological-molecular parameters in seedlings in a more advanced phenological stage.

In this study microarray analysis was applied to evaluate sorghum plants in presence of different amount of soil available water, giving a detailed and informative description of the quality and quantity of genes involved in the response of young sorghum plants at the increasing of water depletion. The new sorghum array revealed to be a high sensitive and powerful instrument for measure gene expression: the qRT-PCR analysis for IS19453 genotype confirmed the results obtained with the microarray analysis, with similar Log_2FC values, indicating the high sensitivity of the high density microarray technology.

Genes involved in drought tolerance were identified using a high density microarray, containing all available sorghum TC sequences and several rice and maize TC sequences known to be involved in response to water deficit. Gene expression analysis of sorghum under water stress using cDNA libraries (Pratt *et al.*, 2005), microarrays (Buchanan *et al.*, 2005), or RNA-Seq technology (Dugas *et al.*, 2011) have been reported. In particular, Pratt and colleagues (Pratt *et al.*, 2005) studied a post-flowering (stay-green) drought tolerant genotype (B35), while the other works investigated drought response using BTx623 seedlings grown in presence of PEG (Buchanan *et al.*, 2005; Dugas *et al.*, 2011).

In IS19453 genotype, as shown from microarray data, the response to drought started between 0.25 and 0.15 of FTSW, when plant transpiration rate drastically decreased. Until this moment the major part of differentially expressed genes resulted down-regulated, indicating that stress incoming induced a reduction of some metabolic functions, while relatively few genes started to be over-expressed. At 0.15 FTSW about 74% of differentially expressed genes resulted up-regulated indicating the activation of relative high number of mechanisms involved in adaptation and tolerance to very low water amounts in the substrate. The 1736 drought-related genes included transcription factors and regulators involved in signal transduction (ABREs, MYBs, HOXs, calcineurin-like phosphoesterases, kinases and phosphatases), functional genes involved in carbon metabolism (NADP-malic enzyme), detoxification (cytochrome P450 families, GST, AKRs), osmoprotection mechanisms (P5CS) and stability of proteins and membranes (DHN1, LEA proteins, HSPs). Some of them, LEA proteins and DHNs, P5CS, ABA-responsive elements, are known to enhance tolerance to drought if constitutively over-expressed or used to transform plants (Ashraf *et al*., 2010).

The expression analysis of miRNA revealed three different pathways: nine miRNAs were up-regulated in the early phases of water stress; one miRNAs (SBI-miR399) increased in expression progressively with water deficit; one miRNAs (ZMA-miR827) was up-regulated, remaining stableduring the two final levels of water stress.

The IS19453 genotype of sorghum showed tolerance to very low water soil content, at early stages of development, rendering it a potential source of genes for improving drought tolerance in sorghum.

In greenhouse conditions where genotypes were maintained for three days at FTSW 0.3 and 0.1 and reached the phenological stage about of 12 leaves, the response to drought started at 0.1 of FTSW.

At 0.3 FTSW where IS33350, BR505, Mpwekwa, BR501, SDS19483 reached transpiration rates (grams/day) equal to 642, 573, 613, 618, 506, respectively; sorghum plants did not show active genes regulation mechanisms. The slow gene response at 0.3 FTSW could be linked to the fact that in relation to the progressive decrease of the available water, it has not been found a sudden decrease in transpiration rate.

In all genotypes, the response to drought started at 0.1 FTSW, when plant transpiration rate drastically decreased: IS33350, BR505, Mpwekwa, BR501, SDS19483 reached transpiration rates equal to 145, 116, 138, 114, 210 grams/day, respectively.

Mpwekwa differed from other genotypes showing a very slight and slowed gene expression response to drought stress, indicating tolerance to very low water soil content.

Three common genes between IS19453, IS33350, BR501, BR 505 Mpwekwa, SDS19483 were up regulated in the higher stress level: Sb02g006320 encoding for Raffinose synthase, Sb10g008820 encoding for the subunit alpha of pyruvate dehydrogenase E1 and Sb09g028710 encoding for a soluble hydrophilic dehydrin WCOR stress-inducible protein.

Given that the expression of common gene in the same drought stress condition but in different stage of growth, it can be assumed that these five genes are regulated by water stress unconditionally and that their expression is independent of the phenological stage.

This study stands in the context of methodology development for drought phenotyping, particularly for physiological and molecular, process-based traits that are not accessible through morpho-physiological observations.

The present preliminary results will serve to the identification of Single Nucleotide Polymorphisms (SNPs) in candidate genes sequences in order to map the same genes. SNPs identification may be carried out on genotypes with contrasting response to drought in order to develop molecular markers.

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