INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–1431/2019/21–2–375–384
DOI: 10.17957/IJAB/15.0000
http://www.fspublishers.org

Full Length Article

Transcriptomic Profiling of Malaysia Rice Cultivars (*Oryza sativa*) under the Effect of Osmotic Stress Induced by PEG 6000

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Abstract

Drought is one of the abiotic stresses on plants, causing significant detrimental impacts, especially to lowland rice (*Oryza sativa* L.) ecosystems. In order to obtain new insights on osmotic stress in rice, a comparative study using a Next-Generation Sequencing platform was conducted to elucidate osmotic-responsive genes from two local Malaysian rice cultivars, namely the commercially available drought-tolerant MR220 and the drought-sensitive MR211. In the study, 21-day-old seedlings of MR220 and MR211 were exposed to 6% PEG 6000 for 24 h, which produced osmotic stress that mimicked the drought condition. The samples were collected and total RNA extracted. Two transcriptomic libraries were constructed from both rice cultivars using the Illumina HiSeq 2000 platform. A total of 77,964,138 and 92,699,454 raw sequence reads were generated from these libraries. Based on the gene annotation of *O. sativa*, a total of 106 genes were identified as differentially and significantly expressed in drought-tolerant and drought-susceptible cultivars, and a total of 29 genes were categorized as unknown genes. From the 106 differentially expressed genes (DEGs), 14 genes were up-regulated, while another 92 were down-regulated in RM220. Gene Ontology (GO) and KEGG analysis were conducted to obtain the functional and biological role of the differentially expressed genes. Overall, this study gave further insight on rice defense mechanisms system in low water potentials at early stages of stress.

Keywords: Transcriptomic Profiling; Malaysian Rice Cultivars; PEG 6000

Introduction

Rice (*Oryza sativa L.*) is one of the most important crops in the world; over half of world's population consumes rice as a staple food (Jackson, 2016). Over 165 million hectares were planted worldwide in 2013 with about 90 percent of the rice production coming mainly from Asia, of which nearly 51 million hectares were in the Southeast Asia region (FAOSTAT, 2015). In Malaysia, rice is grown on about 673,745 hectares of land, which annually produces 2.6 million tons of paddy grain valued at RM 2 billion (Siwar *et al.*, 2014). However, in meeting world demand, the most limiting factor rice production faces is drought stress (Mostajeran and Rahimi-Eichi, 2009; Ambavaram *et al*., 2014; Todaka *et al*., 2015).

Drought significantly influences many rice-growing regions in the world especially in lowland rice ecosystems (Passioura, 2007; Shukla *et al.*, 2012). Observations have shown that the percentage of land affected by drought has increased by more than a factor of two from 1970s to early 2000s around the world (Isendahl and Schmidt, 2006). The problem has further escalated with the global climate changes in agricultural areas and increased world population (Hongbo *et al.*, 2005). Drought stress at different stages of rice growth gives a different effect on rice physiological traits. For instance, drought stress at the vegetative stage causes the rice plant height to be reduced, which eventually affects the rice yields at mature stage (Sarvestani *et al.*, 2008).

Polyethylene glycol (PEG) is a non-ionic, inert molecule that can have a range of molecular weights and is widely used to induce water stress and consistent water potential in experiments related to drought (Lu and Neumann, 1998; Turkan *et al.*, 2005). PEG stimulates

To cite this paper: Othman, T.A., N.A. Bakar, R.A. Zainal Abidin, M. Mahmood, N.B.B. Saidi and N.A. Shaharuddin, 2019. Transcriptomic profiling of Malaysia rice cultivars (*Oryza sativa*) under effect of osmotic stress induced by PEG 6000. *Intl. J. Agric. Biol*., 21: 375–384

drought (osmotic) stress without the confounding environmental factors that are usually associated with field experiments (Almansouri *et al.,* 2001; Landjeva *et al.,* 2008). Studies have been conducted using PEG as a drought inducer in maize, barley, wheat, potato, sunflower, soybean and rice (Lu and Neumann, 1998; Ahmad *et al.,* 2009; Hassanpanah, 2010; Wani *et al.,* 2010; Jain *et al.,* 2013; Mirbahar *et al.,* 2013; Singh *et al.,* 2013; Ye *et al.,* 2013; Opitz *et al.,* 2014; Mujtaba *et al.,* 2016; Zhang *et al.,* 2016).

A recent surge in the use of the latest genome sequencing technology has increased the availability of sequencing data of important crops including rice. A complete rice genome has been sequenced. Due to its relatively small genome size of about 430 million base pairs, which represents about 30,000 expressed genes, rice is considered as a model plant representing the monocots (Jackson, 2016). These raw information needs to be characterized and functionally annotated. With the completion of the whole genome sequencing of indica and japonica rice, rice research has gained a new pace that has led to several significant scientific achievements in understanding rice development, yield and stress responses for example.

RNA-Seq is a high-throughput sequencing based method, which represents transcriptome and expression studies (Sasaki, 2005). Information which includes the identification of transcription sites and new splicing variants, monitoring of allele expression, cataloguing all transcript species, quantifying expression level of each transcript in different conditions and quantification of exon expression and splicing site can be generated from the transcriptome data (Wang *et al.,* 2009; Nagalakshmi *et al.,* 2010; Yamashita *et al.,* 2011). Thus, RNA-Seq was chosen to identify novel drought related-tolerant transcripts and genes from our local cultivar.

Hence, a study was designed as to characterize the expression profile of drought-induced transcripts via high-throughput sequencing technology. Japonica rice cultivars were chosen to investigate drought response: Malaysian cultivar MR220, a commercial drought-tolerant cultivar, and MR211, which is susceptible to drought. MR220 is also one of most high-yielding cultivars in Malaysia.

Materials and Methods

Seeds of MR211 and MR220 (Abdul *et al.,* 2012) were obtained from the Malaysian Agricultural Research and Development Institute (MARDI). The seeds were sterilized with 5% sodium hypochlorite and then placed in boxes submerged in distilled water for three days in darkness at room temperature to germinate. Three-day-old seedlings were transferred to a hydroponic box as shown in (Fig. 1). After seven days, the seedlings were fertilized with half-strength Yoshida's solution (Yoshida *et al.,* 1976) and grown at 30°C under control light of 12 h light/12 h dark cycles for a week. At fourteen days, seedlings were then treated with full strength Yoshida's solution for another week. The 21-day-old seedlings at the three-leaf stage were treated with 6% of PEG 6000 in Yoshida's solution (Chutia and Borah, 2012), which mimicked the specific drought condition (unirrigated water stress) (Fig. 1). After 24 h of the PEG 6000 treatment, the samples were collected and snap-frozen immediately in liquid nitrogen until further processing.

Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions. The quality and integrity of total RNA extracted were analysed using agarose gel electrophoresis. The quantity of RNA was measured using a NanoDrop ND 1000 Spectrophotometer (Termo Scientific, USA). The RNA integrity was assessed using the Agilent 2100, BioAnalyzer (Agilent Technologies, USA). The total RNA extracted was stored at -80°C for further processing (Chan *et al.,* 2017).

The TruSeq™ RNA Sample Preparation Kit was used for mRNA library preparation. The first step involved the purification of poly-A–containing mRNA molecules using poly-T–oligo-attached magnetic beads. Following the purification steps, the mRNA was fragmented into small pieces using a divalent cation under elevated temperature. The cleaved RNA fragments underwent cDNA synthesis twice: the first strand of cDNA was synthesized using random hexamers primers and reverse transcriptase, and the second strand cDNA was synthesized to replace the RNA template. Ampure XP beads were used to separate the double-stranded cDNA from the second-strand reaction mixture. The cDNA was subjected to an end-repair process, i.e. the addition of a single 'A' base, and lastly, the adapters were ligated into the cDNA. The sequencing process was carried out using HiSeq^{TM} 2000 (Illumina, USA).

The quality of raw sequence reads were examined using FastQC (v0.11.2) (http://www.bioinformatics. babraham.ac.uk/ projects/fastqc). To obtain a high quality of sequence reads for further analysis, the raw sequence reads were trimmed using Trimmomatic (v 0.32) (http://www.usadellab.org /cms/?page= trimmomatic) to remove the adapters.

The high-quality pair end sequence reads for each sample were mapped to the International Rice Genome Sequencing Project (IRGSP-1.0) genome assembly (http://rapdb.dna.affrc.go.jp/) using Bowtie 2 (v2.2.4) (http:// http://bowtie-bio.sourceforge.net/bowtie2) and TopHat (v2.0.14) (http:// ccb.jhu.edu/software/tophat) (Trapnell *et al.,* 2012). The gene annotations of *O. sativa* Japonica were retrieved from Ensemble database (http://plants.ensembl.org/Oryza_sativa/Info/Index) to guide the mapping process. The resulting alignment in BAM format from each sample was assembled using Cufflinks (v2.2.1) (http://cole-trapnell-lab.github.io/cufflinks/) to construct the transcript sequences. Cuffmerge (v2) was used to assemble the transcripts from both samples and construct the consensus transcripts.

Gene expression levels were measured as Fragments per Kilobase per Million Reads (FPKM). The result of FPKM value that is less than 0 was filtered to minimize false positive and negative error. The unique genes from the transcriptomic libraries were analysed using MapMan (3.6.0CR1) (http://mapman.gabipd.org/web/guest/mapman-version-3.6.0). To estimate the differentially expressed genes (DEG) between both samples, Cuffdif (v6) was used and the parameter for transcript DEGs identification was as shown; fold change and less than or equal to 0.05 for false discovery rate (FDR). Statistical analysis of the differential expressed genes was performed using the R package, CummeRbund (v2.12) (http://www.bioconductor.org/packages/release/bioc/html/cummeRbund.html). Finally, the gene annotations of significantly differentially expressed genes were performed based on Rice Annotation Project Database (RAP-DB) gene annotation database using custom-made R script.

To elucidate the molecular function and biological process of significantly expressed genes, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were carried out using Blast2Go program (v3.0.10) (https://www.blast2go.com/blast2go-pro/download -b2g). The KEGG analysis (http://www.genome.ad.jp /kegg/) was used to identify the biological pathways that are active in rice plant by mapping the detected genes to reference canonical pathways. The KEGG pathway database also was used to classify gene function.

Results

Illumina Sequencing and Alignment to the Genome

A total of 92 million sequences reads were generated from MR211, and 77 million sequences read from MR220. After trimming the adapters and filtering out low-quality reads, a total of 73 million and 72 million sequence reads from MR220 and MR211 were obtained, respectively. The GC contents of the samples were 52.51% and 49.53% for MR220 and MR211, respectively. Using Tophat and Bowtie, these reads were mapped onto a rice reference genome, IRGSP-1.0 (http://rapdb.dna.affrc.go.jp/). Approximately 90.50% (MR220) and 90.27% (MR211) of reads were mapped from both of the transcriptomic libraries. The summary of the bioinformatic analysis is shown in (Table 1).

Next, assembly analysis through Cufflink generated 79,209 and 73,746 expressed genes in the MR220 and MR11 libraries, respectively. After being mapped on the International Rice Genome Sequencing Project (IRGSP) /Rice Annotation Project Database (RAP-DB), the genes were clustered into known and unknown genes. Among the known genes, 7,749 and 1,757 unique genes were expressed in MR220 and MR211, as shown in (Fig. 2). The results revealed that during the treatment, MR220 expressed more unique genes than MR211. The unique genes were further analyzed using MapMan (3.6.0CR1), where the reads were mapped on the *O. sativa* Japonica Group/Osa_RAPDB_ Mapping database. The list of the unique genes that were expressed in MR220 and MR211 is shown in (Supplemental Table 1).

Fig. 1: Plants were grown in hydroponic boxes using Yoshida nutrient solution. The 21-old day rice plant were treated with PEG6000 in control condition and harvested after 24 h of treatment

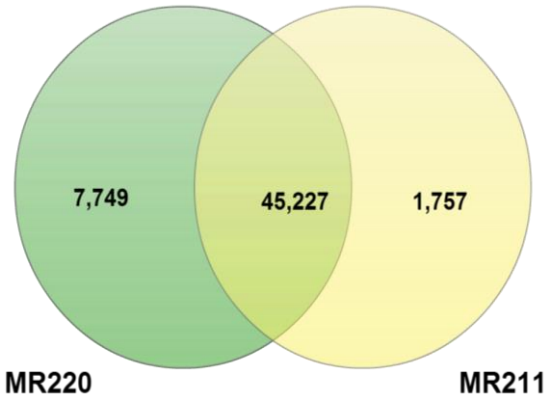

Fig. 2: The diagram of known genes that were expressed in both cultivars. Over 45,227 genes were identified in both MR220 and MR211. There are 7,749 and 1757 genes were only expressed in MR220 and MR211 respectively

Data Analysis and Expression Study

Unique genes from the transcriptome profile of MR220 and MR211 generated using MapMan are shown in (Fig. 3). It shows the distribution of functional genes obtained from the data. Transcription factors, protein modifications and receptor kinase in gene regulation were among the most highly abundant transcripts being identified. The analysis also elucidated that only MR220 expressed abscisic acid (ABA) and benzyladenine (BA) in its hormone distribution pathway whilst in MR211, gene transcripts that are involved in the salicylic (SA) and jasmonate distribution were abundant. However, both cultivars seem to express indole-3-acetic acid (IAA), ethylene and gibberellic acid (GA).

Table 1: Summary of sequence reads before and after trimming process, mapping percentages and total number of expressed genes

Sample	Total read before trimming	Total read after trimming	Total Mapped reads	GC (%)	Total expressed genes
Drought tolerant cultivar_MR220	77,964,138	73,546,016	90.50	52.51	79,207
Drought susceptible cultivar_MR211	92,699,454	72,667,254	90.27	49.58	73,746

Table 2: Differential expressed genes (DEGs) in Malaysia rice cultivars, MR220 vs MR211

DEGs	Quantity
Only expressed in MR220	14
Only expressed in MR211	85
Down-regulated in MR220	7

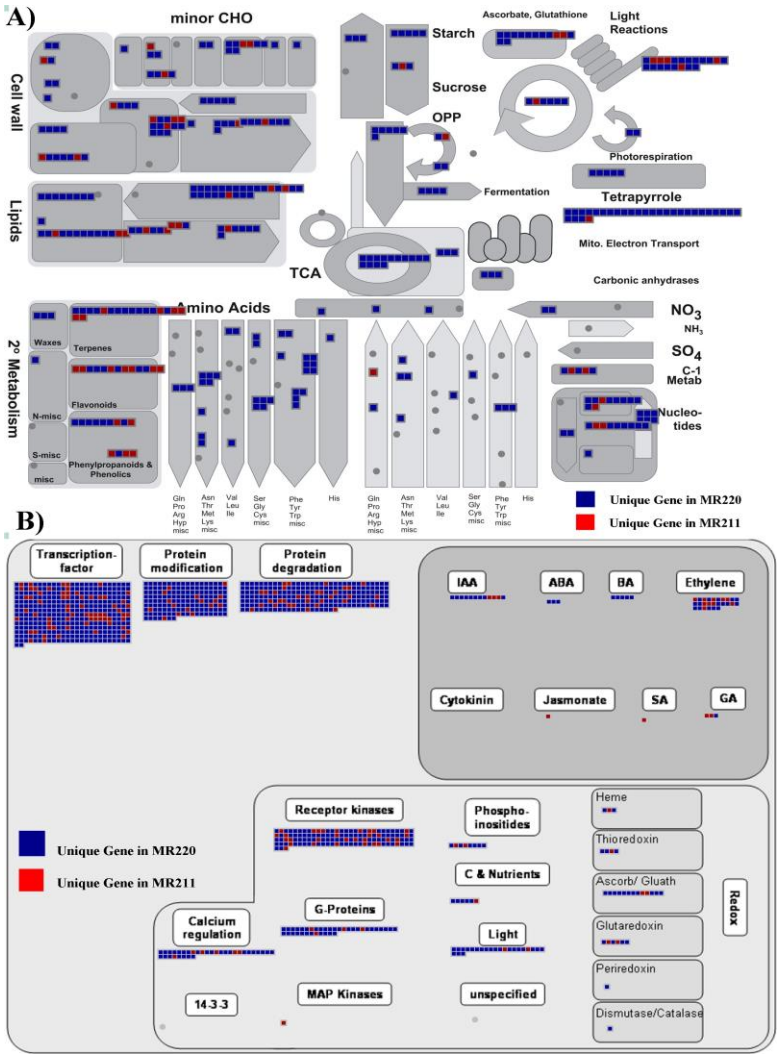

Fig. 3: Classification of uniquely expressed genes of MR220 and MR211 transcriptomic libraries as generated by MapMan software, where (A) is main metabolic pathway group and (B) gene regulations group. Known unique genes were filtered depending on their accession number, locus and FPKM values

To gain a deeper insight into the biology of MR220 and MR211, a number of differentially expressed genes were analysed. A total of 106 differentially expressed genes (DEGs) were identified, of which 14 genes were found to be upregulated and 92 genes downregulated in the treated MR220 (Table 2 and Supplemental Table 2). The DEGs were classified into different groups depending on their IRSGP gene annotation, as shown in (Fig. 4). Most genes were conserved hypothetical genes (40%) followed by well-known genes (30%). Non-coding RNA, cDNA and protein of unknown function contributed 7% each and 2% of the genes were similar to certain proteins.

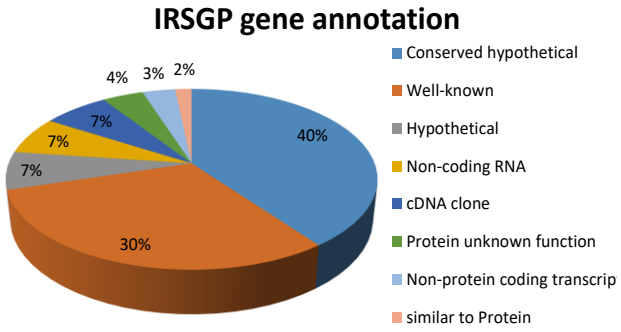

Fig. 4: Distribution of DEGs genes annotated from IRGSP database

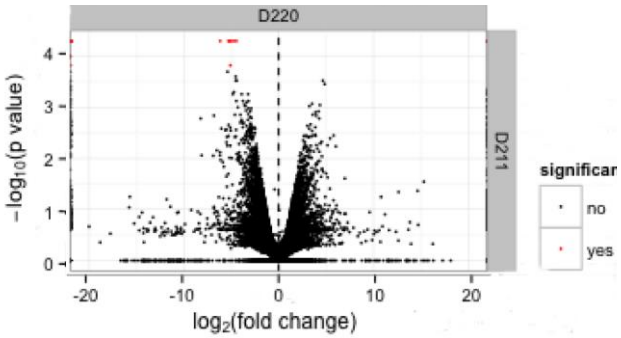

Fig. 5: Comparison of genes in between the two libraries of different drought perception variety, MR220 and MR211 were shown in volcano plot. The expression level were estimated by FPKM value which comparison between $\log_2$ (fold change) X – $\log_{10}$ (p value)

The unknown DEGs annotated from RAP-DB database were blasted again using the *nr* database from NCBI, and the results are shown in (Supplemental Table 3).

Volcano plot were generated regarding to the comparison between $\log_2$ (fold change) X –$\log_{10}$ (p value) which showed as in (Fig. 5). Heat-map diagrams were generated as shown in (Fig. 6). This figure shows the comparison between MR220 and MR211 for the distribution of each gene, based on their FPKM value (LOG_{10} FPKM + 1). Based on this result, we used MR220 for further analysis.

Gene Ontology (GO) & Enrichment Analysis

Gene Ontology (GO) analysis was performed on the DEGs using NCBI database as shown in (Fig. 7). The GO annotation suggested that the DEGs involved in biological process category 'metabolic process' were the most prevalent sequences (28.0%), followed by 'cellular process' (25.0%). In the molecular function category, both 'cell' and 'organelle' both had the same value of 38.0%, followed by 'membrane' (12.0%). In the cellular component category, 'catalytic activity' was the main function (46.0%), followed by 'binding' (44.0%) and 'molecular transducer activity'

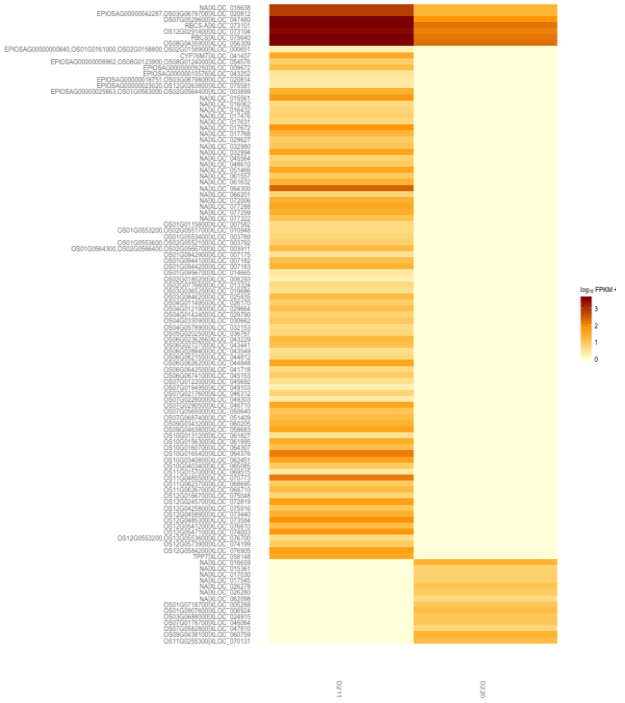

Fig. 6: Cluster of significant differential expression genes in MR211 and MR220 based on log ratio FPKM data. The heat-map diagram referred to FPKM values for differential expressed genes (DEGs) in both cultivars, MR220 and MR211. The different color showed the values of $\log_{10}$ FPKM + 1 for each DEGs

(5.0%). From the enriched DEGs result, eleven pathways involved in DEGs and some of the genes sharing pathways are presented in (Supplemental Table 4). Other interesting pathways such as starch and sucrose metabolism, fructose and mannose metabolism, biosynthesis of antibiotics, T cell receptor signalling, and carbon fixation metabolisms are also highlighted.

Discussion

Plants use a combination of different strategies to cope with drought stress. Drought avoidance and tolerance behaviours such as development of deep root systems, metabolic adjustments and altering gene expression are among of the strategies that plants have adopted when dealing with drought stress. In an attempt to investigate the regulation of genes expression in rice during drought stress and to discover novel transcripts related to plant defense mechanism against this stress, NGS was performed on two selected local rice varieties. A comparative study of the cultivars MR220 (drought tolerant) and MR211 (drought susceptible) were carried out via high-throughput sequencing technology. Data obtained from the differential analysis between these two transcriptome libraries revealed 106 genes that were significantly expressed during the PEG-

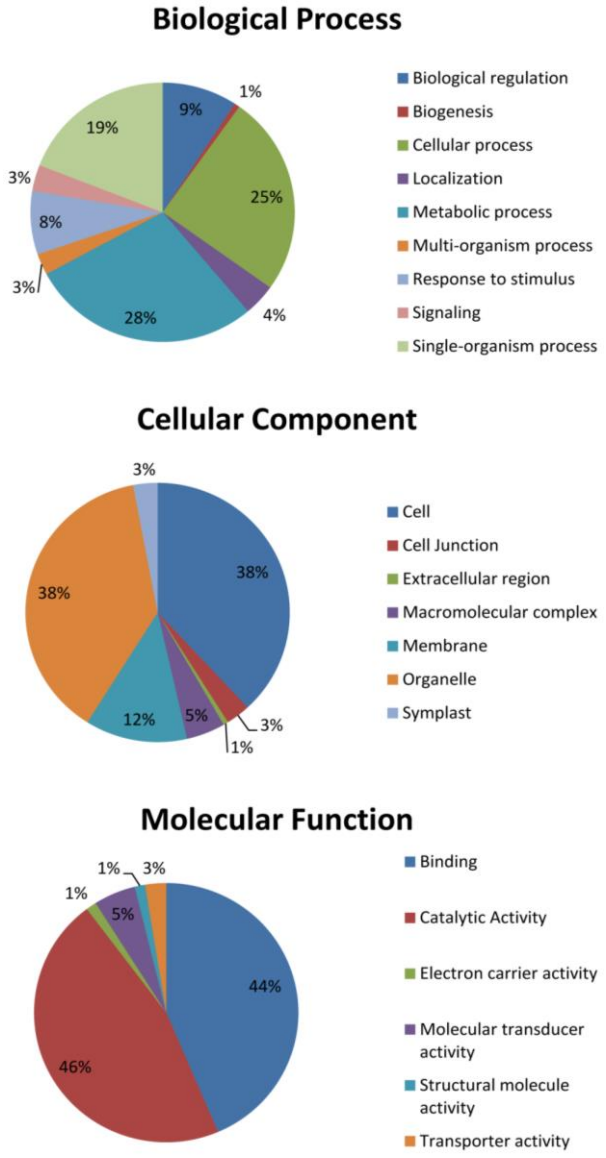

Fig. 7: Functional annotation of differentially expressed genes between MR220 and MR211 within the Gene Ontology (GO) (level2). The differentially expressed genes were divided into three categories: a) Biological process, b) Cellular Component and c) Molecular function. These genes are shown in percentage

induced drought stress treatment. The roles and their potential as well as their functional attributes toward drought stress were further highlighted.

Several genes were shown to be expressed, for instance, secondary signalling molecules such as protein kinases. Protein kinases including mitogen-activated kinase, membrane receptor kinase, and SNF-related protein kinases 2 (SnRK2) have been shown to be activated during drought stress, involved in the regulation of proline accumulation (Kulik *et al.*, 2011; Long *et al.*, 2014; Wu *et al.*, 2015). A study by two groups showed that ABA-responsive SnRKs 2.2, 2.3, and 2.6 in *Arabidopsis thaliana* mutants are important for osmotic stress and ABA-induced proline accumulation (Fujii *et al.*, 2012). Genetic manipulation of mitogen-activated protein kinase (MAPK) signalling adjusts plant tolerance to abiotic stresses and induces proline accumulation. Moreover, receptor-like kinase 1 (RPK1), which is a part of leucine rich repeat family protein was up-regulated by ABA in *A. thaliana* (Teige *et al*., 2004; Kong *et al.*, 2011). It was then suggested that RPK1 is involved in the main ABA signalling pathways and in early ABA perception in *A. thaliana* (Osakabe *et al.*, 2005).

Cytochrome P450 is one of the largest protein gene families in the plant genome. Unfortunately, majority of these genes are still uncharacterized (Tamiru *et al.*, 2015). Based on our results, several P450 genes were shown to be significantly expressed. Cytochrome P450 monooxygenase was established as an abscisic acid (ABA) 8'-hydroxylase (Krochko *et al.*, 1998). The transcription factors belonging to the *CYP707A*s family that encoded ABA-8'-hydrolyse were shown to be induced in response to salt, osmotic, and dehydration stresses. This study also revealed that the gene is one of the key genes involved in ABA catabolism (Saito *et al.*, 2004). A study conducted by Degenkolbe *et al.* (2009) found via microarray analysis five genes that encode members of the cytochrome P450 family that were highly induced during moderate drought stress period. Interestingly, a dwarf small seed (dss1) rice mutant line, which had a non-synonymous point mutation at one of the P450 genes, *CYP96B4/SD37*, exhibited tolerance to drought stress, suggesting its involvement in drought stress response. This mutant plant also showed accumulation of ABA and ABA metabolites (Tamiru *et al.*, 2015).

Alpha-amylase inhibitors are a part of large group of protease inhibitor subfamilies. Numerous studies have been conducted on the involvement of alpha-amylase inhibitors in response to abiotic stress. Alpha-amylase inhibitors from *Amaranthus hypochondriacus* have been studied under water stress and their activity was shown to be increased when exposed to water stress (Sánchez-Hernández *et al.*, 2004). Proteomic analysis of drought-tolerant wheat during grain development showed differential expression pattern of alpha-amylase inhibitor which was down-regulated in drought-sensitive wheat (Jiang *et al.*, 2012a). However, our results showed that the inhibitor was significantly expressed in the drought-sensitive cultivar, MR211. The inhibitor might be activated to help plant response to stress at this early stage.

From our comparative study between MR220 and MR211, several up-regulated genes that were suggested as responding to low water potential conditions are cysteine endopeptidase (Os11t0255300-01, Os01t0907600-00), potassium channel kat3 (Os01t0718700-00) and ribosome inactive protein RIP (Os03t0688000-00). The up-regulation of these genes in MR220 suggested their involvement in enhancing the drought tolerance in osmotic stress.

A previous study provided evidence that cysteine protease was expressed during drought stress condition, similar to our comparative study. The role of cysteine protease (CP) from T. *aestivum* has been well studied in abiotic stresses including drought stress. Overexpression of wheat CP in transgenic *A. thaliana* showed stronger drought tolerance and higher CP activity compared to wild-type *A. Thaliana* (Harrak *et al.,* 2001; Zang *et al.,* 2010). A unique ribosome-inactivating protein (proRIP1) from maize was also up-regulated by drought stress conditions (Bass *et al.,* 2004). Furthermore, transgenic rice plants over-expressing *O. sativa* ribosome-inactivating protein gene 18 (OSRIP18) improved tolerance to drought and high salinity stress. However, microarray analysis of global gene expression in the transgenic rice plant identified most of the up-regulated genes was not entirely involved in abiotic stress (Jiang *et al.,* 2012b).

In general, ABA will stimulate a signalling pathway in response to drought stress. This process activates reactive oxygen species (ROS) production that increases cytosolic Ca^{2+}. Subsequently, one of the ion channel proteins, potassium channel KAT3, is activated to reduce turgidity of guard cells and cause stomatal closure (Negi *et al.,* 2008; Vahisalu *et al.,* 2008). In drought conditions, proton pumps coupled with plasma membrane responses mediate stomatal closure by regulating the efflux K^+ and various anions from guard cells. These processes cause the Ca^{2+} to regulate the activity of plasma membrane H^+ -ATPase (Kinoshita *et al.,* 1995).

In our study, the abundance of conserved hypothetical genes and proteins was also assessed. This suggested the possibility of the genes in responding to the stress. These set of genes were further analysed using InterPro (http://www.ebi.ac.uk/interpro/) and are listed in (Supplement Table 5). These genes were identified as hypothetical and did not exhibit clear motif or functional domains due to the low overlapping or lower percentage of sequence identity than the threshold level when analyses were carried out. The unknown genes annotated from RAP-DB database were again blasted against non-redundant (nr) database from NCBI. Several genes showed similarity with gibberellins and abscisic acid stimulus genes. Abscisic acid and gibberellins are found to be regulated in many key processes in plant, including in abiotic stress (Luo *et al.,* 2014; Shu *et al.,* 2016). Further studies need to be carried out, as these genes may represent candidate markers for early drought stress in rice.

The comparative analyses conducted showed more genes were significantly expressed in the susceptible cultivar, MR211 when compared to MR220. Microarray expression profiling analysis conducted in drought sensitive (Nipponbare and Taipei) versus the drought tolerant rice cultivars (IR57311 and LC-93-4) showed more genes were significantly induced in drought sensitive varieties (Degenkolbe *et al.,* 2009). Furthermore, RNA-Seq analyses conducted on two different levels of drought resistance varieties in *Brassica napus* under PEG-6000 treatment demonstrated that 9,542 transcripts were up-regulated while 14,202 were down-regulated in the tolerant cultivar, which was higher in the more tolerant *B. napus* variety (Wang *et al.,* 2015).

Based on the comparative analysis of functional annotation between the up-regulated and down-regulated genes in (Supplement Fig. 1), a group of transporters which were grouped in the molecular function category showed up-regulation in activity. Many plant transporters were shown to be involved in plant adaptation and survival during drought stress. These include such functions as primary ABA transporters, ABA conjugate transporters, ions channels, potassium channels, water channels, and protein regulators (Vahisalu *et al.,* 2008; Kuromori *et al.,* 2010; Burla *et al.,* 2013; Lawson and Blatt, 2014; Osakabe *et al.,* 2014; Wege *et al.,* 2014).

Drought stress can cause retardation of development and growth, reduce productivity and even cause plant death. Plants, however will try to overcome these conditions. This involves complex cross-talk between different regulatory levels, including metabolic adjustment and genes expression for physiological and morphological adaptation. Functional annotation of DEGs revealed that metabolic activity gave the highest percentage, followed by cellular processes and biological processes (Krasensky and Jonak, 2012). In grapes, Principal Component Analyses (PCA) of the response of grapevines with different levels of water stress tolerance indicated that primary metabolism were primarily changed; based on the PCA analyses, the cell component, cell and organelle subgroup were of the highest percent but with similar percentage (Hochberg *et al.,* 2013). Hossain *et al*. (2012) conducted a study on cell organelle proteomics in response to abiotic stress. However, insufficient data on drought sensing and tolerance mechanism at the organelles proteomic level were obtained, although similarly the catalytic activity gave the highest percent for molecular function followed by binding. In our study, the abundance of transcription factor (TF) families found was mainly of the kinase-like protein, leucine repeats family, Zn-Finger and protein phosphatase type. Our findings suggested that the early treatment of drought stress expressed transcription factors that responded to drought stress signalling.

Plant responses under drought stress are a complex process, which involves a wide range of metabolic and synthetic pathways. For KEGG analysis, a very large amount of sequences shared in starch, sucrose, fructose and mannose pathways. Sucrose and glucose are important in plants, which can act as substrates for cellular respiration and as osmolytes to maintain cell homeostasis (Gupta and Kaur, 2005). Metabolites analysis in hybrid Bermuda grass (cv. Tifdwarf) responding to long-term drought stress showed high accumulations of several organic acids, amino acid, sugar compounds including sucrose, fructose and mannose, one nitrogen compound and two sugar alcohols (Gupta and Kaur, 2005). Moreover, the analysis

showed an induced pattern of soluble sugar concentration in drought, salinity, low temperature, and flooding stresses, but a reduced pattern in heavy metals, nutrient shortage, high light irradiance and ozone stresses (Gill *et al.,* 2001). Sucrose non-fermenting 1-related protein kinase 2 and mitogen-activated protein kinase pathways were elucidated as the initial part of stress adaptive to abiotic response (Golldack *et al.,* 2014).

Drought condition normally reduces photosynthesis in plants due to the decline of rubisco activity (Bota *et al.,* 2004). Rubisco activity and concentration in two chickpea cultivars, *Gokce* (tolerant) and *Kusmen* (sensitive) were analysed after being subjected to drought treatment for 10 days. Interestingly, *Kusmen* showed reduced pattern in rubisco activity and rubisco concentration while *Gokce* did not show any significant changes in the activity and concentration of rubisco (Saglam *et al.,* 2014). The rubisco level in leaves is controlled through its synthesis rate and degradation. However, the rubisco activities were shown to be relatively stable even after several days of drought stress (Hoekstra *et al.,* 2001). In our study, the genes related to rubisco enzymes were induced in MR211 cultivars. It was suggested that MR220 have earlier response to drought stress when compared to MR211. However, Flexas *et al.* (2006) found that rubisco activity during water stress was not only caused by drought, but also by low stomatal conductance and choloroplast CO_2 concentration. Furthermore, two positive regulator of ABA signalling in stomata were shown to induce *PLDa1* and *GPA1* expression during stomatal response in *A. thaliana* under moderate drought conditions. Both genes were induced in a 24 h treatment but started to decline after a 72 h of treatment (Harb *et al.,* 2010). Identification of protease inhibitor types in drought stress was also investigated. Interestingly, even though the comparative study conducted showed several different types of protease inhibitors to be differentially expressed and having different FPKM values, the differences were not significant when compared between MR220 and MR211 (The raw data has not been published yet. It is being processed to be published with other supporting data.).

Conclusion

In this study, RNA-Seq was used to investigate the global transcriptomes of shoots from Malaysia-drought tolerant cultivar, MR220 and susceptible cultivars MR211 under osmotic stress treatment. This could generate a useful resource for the Malaysian rice breeders and rice breeders from around the world. The transcripts that were significantly up-regulated were suggested as contributing to osmotic stress, which leads to the drought-tolerance response. This will provide valuable information for future study on molecular mechanisms of the stress. Moreover, the genes and pathways studies related to drought response are useful for plant manipulation in order to improve the adaptation of the plant during stress conditions. In the future, the unique genes and novel genes from both cultivars will be characterized and their roles in drought-related condition and stress response elucidated.

Acknowledgements

The author would like to thank Universiti Putra Malaysia (UPM) and Malaysia Agricultural Research and Development Institute (MARDI) for providing facilities during this study. We also thank Assoc. Prof. Dr. Yahya Awang, UPM for his supportive comments throughout this research. This work was supported by the Ministry of Education under the Fundamental Research Grant Scheme (FRGS-2013-2-5524334).

References

Abdul, R.H., S.K. Zarifth, M.A.R. Bhuiyan, M.K. Narimah, R. Wickneswari, M.Z. Abdullah, L.P.K. Anna, H. Sobri, I. Rusli and A.R. Khairuddin, 2012. International Atomic Energy Agency. Evaluation and Characterization of Advanced Rice Mutant Line of Rice (*Oryza Sativa*), MR219-4 And MR219-9 Under Drought Condition. *In*: *R and D Seminar 2012: Res. Dev. Seminar 2012*, Malaysia

Ahmad, R., M.R. Ahmad, M. Ashraf, E.A. Ashraf and E.A. Waraich, 2009. Sunflower (*Helianthus Annuus* L.) Response to drought stress at germination and seedling growth stages. *Pak. J. Bot.*, 41: 647–654

Almansouri, M., J.M. Kinet and S. Lutts, 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum Desf.*). *Plant Soil.*, 231: 243–254

Ambavaram, M.M., S. Basu, A. Krishnan, V. Ramegowda, U. Batlang, L. Rahman, N. Baisakh and A. Pereira, 2014. Coordinated regulation of photosynthesis in rice increases yield and tolerance references to environmental stress. *Nat. Commun.*, 5: 5302

Bass, H.W., J.E. Krawetz, G.R. OBrian, C. Zinselmeier, J.E. Habben and R. Boston, 2004. Maize ribosome-inactivating proteins (RIPs) with distinct expression patterns have similar requirements for proenzyme activation. *J. Exp. Bot.*, 55: 2219–2233

Bota, J., H. Medrano and J. Flexas, 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress?. *New Phytol.*, 163: 671–681

Burla, B., S. Pfrunder, R. Nagy, R.M. Francisco, Y. Lee and Martinoia, 2013. Vacuolar transport of abscisic acid glucosyl ester is mediated by ATP-binding cassette and proton-antiport mechanisms in Arabidopsis. *Plant Physiol*., 163: 1446–1458

Chan, S., N. Abu Bakar, M. Mahmood, H. Chai-ling, N. Mohamad Dzaki and N.A. Shaharuddin, 2017. Anti-pathogenic properties of plant protease inhibitors (PIs): Cloning and expression of Serpin 1 and Kunitz-type trypsin inhibitor genes from indegenous turmeric, *Curcuma spp*. *Acta Physiol. Plant*., 39: 12

Chutia, J. and S.P. Borah, 2012. Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice *(Oryza sativa Linn*.) Genotypes of Assam, India II. Protein and proline status in seedlings under peg induced water stress. *Amer. J. Plant. Sci.*, 3: 20679

Degenkolbe, T., P.T. Do, E. Zuther, D. Repsilber, D. Walther, D.K. Hincha and K.T. Köhl, 2009. Expression profiling of rice cultivars differing in their tolerance to long-term drought stress. *Plant Mol. Biol.*, 69: 133–153

FAOSTAT, 2015. Available at: http*://www.faostat.fao.org.* (Accessed: 6 Jun 2015)

Flexas, J., M. Ribas-Carbó, J. Bota, J. Galmés, M. Henkle, S. Martínez-Cañellas and H. Medrano, 2006. Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. *New Phytol.*, 172: 73–82

Fujii, H., P.E. Verslues and J.K. Zhu, 2012. Arabidopsis decuple mutant reveals the importance of SnRK2 kinases in osmotic stress responses in vivo. *Proc. Natl. Acad. Sci. USA*, 108: 1717–1722

Gill, P.K., A.D. Sharma, P. Singh and S.S. Bhullar, 2001. Effect of various abiotic stresses on the growth soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulg. J. Plant Physiol.*, 27: 72–84

Golldack, D., C. Li, H. Mohan and N. Probst, 2014. Tolerance to drought and salt stress in plants: Unraveling the signaling networks. *Front. Plant Sci.*, 5: 1

Gupta, A.K. and N. Kaur, 2005. Sugar signaling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. *J. Biosci.*, 30: 761–76

Harb, A., A. Krishnan, M.R. Madana, Ambavaram and A. Pereira, 2010. Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Plant Physiol.*, 154: 1254–1271

Harrak, H., S. Azelmat, E.N. Baker and Z. Tabaeizadeh, 2001. Isolation and characterization of a gene encoding a drought-induced cysteine protease in tomato (*Lycopersicon esculentum*). *Genome*, 44: 368–374

Hassanpanah, D., 2010. Evaluation of potato advanced cultivars against water deficit stress under *in vitro* and *in vivo* condition. *Biotechnology*, 9: 164–169

Hochberg, U., A. Degu, D. Toubiana, T. Gendler, Z. Nikoloski, S. Rachmilevitch and A. Fait, 2013. Metabolite profiling and network analysis reveal coordinated changes in grapevine water stress response. *BMC. Plant Biol.*, 13: 184

Hoekstra, F.A., E.A. Golovina and J. Buitink, 2001. Mechanisms of plant desiccation tolerance, Trends. *Plant Sci.*, 6: 431–438

Hongbo, S., L. Zongsuo and S. Mingan, 2005. Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (*Triticum aestivum L.*) genotypes at maturation stage, Colloids and Surface B. *Biointerfaces*, 45: 7–13

Hossain, Z., M.Z. Nouri and S. Komatsu, 2012. Plant cell organelle proteomics in response to abiotic stress. *J. Proteome Res.*, 11: 37–48

Isendahl, N. and G. Schmidt, 2006. *Drought in the Mediterranean-WWF Policy Proposals*. A WWF Report, Madrid

Jain, M., M. Mittal and R. Gadre, 2013. Effect of PEG-6000 Imposed Water Deficit on Chlorophyll Metabolism in Maize Leaves. *J. Stress Physiol. Biochem.*, 9: 262–271

Jackson, S.A., 2016. Rice, the first crop genome. *Rice*, 9: 14

Jiang, S.S., X.N. Liang, X. Li, S.L. Wang, D.W. Lv, C.Y. Ma, X.H. Li, W.J. Ma and Y.M. Yan, 2012a. Wheat drought-responsive grain proteome analysis by linear and nonlinear 2-DE and MALDI-TOF Mass Spectrometry. *J. Mol. Sci.*, 13: 16065–16083

Jiang, S.Y., R. Bhalla, R. Ramamoorthy, H.F. Luan, P.N. Venkatesh, M. Cai and S. Ramachandran, 2012b. Over-expression of OSRIP18 increases drought and salt tolerance in transgenic rice plants. *Transgenic Res.*, 21: 785–795

Kong, X., J. Pan, M. Zhang, X. Xing, Y. Zhou, Y. Liu and D. Li, 2011. ZmMKK4, A novel group C mitogen-activated protein kinase kinase in maize (*Zea mays*), confers salt and cold tolerance in transgenic *Arabidopsis*. *Plant, Cell Environ.*, 34: 1291–1303

Kinoshita, T., M. Nishimura and K.I. Shimazaki, 1995. Cytosolic concentration of Ca^{2+} regulates the plasma membrane H^{+}-ATPase in guard cells of fava bean. *Plant Cell.*, 7: 1333–1342

Krochko, J.E., G.D. Abrams, M.K. Loewen, S.R. Abrams and A.J. Cutler, 1998. (+)-Abscisic acid 8'-hydroxylase is a cytochrome P450 monooxygenase. *Plant Physiol.*, 118: 849–860

Krasensky, J. and C. Jonak, 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.*, 1–16

Kulik, A., I. Wawer, E. Krzywińska, M. Bucholc and G. Dobrowolska, 2011. SnRK2 Protein Kinases-Key Regulators of Plant Response to Abiotic Stresses. *OMICS*, 15: 859–872

Kuromori, T., T. Miyaji, H. Yabuuchi, H. Shimizu, E. Sugimoto and A. Kamiya, 2010. ABC transporter AtABCG25 is involved in abscisic acid transport and responses. *Proc. Natl. Acad. Sci. USA.* 107: 2361–2366

Landjeva, S., K. Neumann, U. Lohwasser and M. Borner, 2008. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. *Biol. Plant.*, 52: 259–266

Lawson, T. and M.R. Blatt, 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiol.*, 164: 1556–1570

Long, L., W. Gao, L. Xu, M. Liu, X. Luo, X. He, X. Yang, X. Zhang and L. Zhu, 2014. GbMPK3, a mitogen-activated protein kinase from cotton, enhances drought and oxidative stress tolerance in tobacco. *Plant Cell Tiss. Org. Cult.*, 116: 153–162

Luo, X., Z. Chen, J. Gao and Z. Gong, 2014. Abscisic acid inhibits root growth in Arabidopsis through ethylene biosynthesis. *Plant J.*, 79: 44–55

Lu, Z. and P.M. Neumann, 1998. Water-stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. *J. Exp. Bot.*, 49: 1945–195

Mirbahar, A.A., R. Saeed and G.S. Markhand, 2013. Effect of Polyethylene Glycol-6000 on Wheat (*Triticum aestivum* L.) Seed Germination. *Int. J. Biol. Biotech.*, 10: 401–405

Mostajeran, A. and V. Rahimi-Eichi, 2009. Effects of drought stress on growth and yield of rice (*Oryza sativa L.*) cultivars and accumulation of proline and soluble sugars in sheath and blades of their different ages leaves. *Amer.-Eur. J. Agric. Environ. Sci.*, 5: 264–272

Mujtaba, S.M., S. Faisal, M.A. Khan, S. Mumtaz and S. Khanzada, 2016. Physiological studies on six wheat (*Triticum Aestivum* l.) genotypes for drought stress tolerance at seedling stage. *Agric. Res. Tech.*, 1: 55559

Nagalakshmi, U., K. Waern and M. Snyder, 2010. RNA-Seq: A method for comprehensive transcriptome analysis. *Curr. Prot. Mol. Biol.*, 4: 1–4

Negi, J., O. Matsuda, T. Nagasawa, Y. Oba, H. Takahashi and M. Kawai-Yamada, 2008. CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. *Nature*, 452: 483–486

Opitz, N., A. Paschold, C. Marcon, W.A. Malik, C. Lanz, H.P. Piepho and P. Hochholdinger, 2014. Transcriptomic complexity in young maize primary roots in response to low water potentials. *BMC Genom.*, 15: 741

Osakabe, Y., K. Osakabe, K. Shinozak and L.S.P. Tran, 2014. Mini review article: Response of plants to water stress. *Front. Plant Sci.*, 5: 1

Osakabe, Y., K. Maruyama, M. Seki, M. Satou, K. Shinozaki and K. Yamaguchi-Shinozaki, 2005. Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signaling in Arabidopsis. *Plant Cell.*, 17: 1105–1119

Passioura, J.B., 2007. The drought environment: Physical, biological and agricultural perspectives. *J. Exp. Bot.*, 58: 113–117

Saglam, A., R. Terzi and M. Demiralay, 2014. http://www.akademiai.com/doi/abs/10.1556/ABiol.65.2014.2.6 - d23593e68 Effect of polyethylene glycol induced drought stress on photosynthesis in two chickpea genotypes with different drought tolerance. *Acta Biol. Hung.*, 65: 6

Saito, S., H. Hirai, C. Matsumoto, H. Ohigashi, D. Ohta, K. Sakata and M. Mizutani, 2004. Arabidopsis CYP707 as encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiol.*, 134: 1439–1449

Sánchez-Hernández, C., N. Martínez-Gallardo, A. Guerrero-Rangel, S. Valdés-Rodríguez and J. Délano-Frier, 2004. Trypsin and α-amylase inhibitors are differentially induced in leaves of amaranth (*Amaranthus hypochondriacus*) in response to biotic and abiotic stress. *Physiol. Plant.*, 122: 254–264

Sarvestani, Z.T., H. Pirdashti**,** S.A.M.M. Sanavy and H. Balouchi**,** 2008. Study of water stress in effects in the different growth stages on yield and yield component of different rice *Oryza sativa L.* cultivar. *Pak. J. Biol. Sci.*, 11: 1303–1309

Sasaki, T., 2005. The map-based sequence of the rice genome. *Nature*, 436: 783–800

Shu, K., X.D. Liu, Q. Xie and Z.H. He, 2016. Two faces of one seed: hormonal regulation of dormancy and germination. *Mol. Plant.*, 9: 34–45

Shukla, N., R.P. Awasthi, L. Rawat and J. Kumar, 2012. Biochemical and physiological responses of rice (*Oryza sativa L.*) as influenced by *Trichoderma harzianum* under drought stress. *Plant Physiol. Biochem.*, 54: 78–88

Singh, A.K., V. Sharma, A.K. Pal, V. Acharya and P.S. Ahuja, 2013. Genome-wide organization and expression profiling of the NAC transcription factor family in potato (*Solanum tuberosum L.). DNA Res.*, 20: 403–423

Siwar, C., N.D.M. Idris, M. Yasar and G. Morshed, 2014. Issues and challenges facing rice production and food security in the granary areas in the east coast economic region (ECER). *Malays. Res. J. Appl. Sci. Eng. Tech.*, 7: 711–722

Tamiru, M., J.R. Undan, H. Takagi, A. Abe, K. Yoshida, J.Q. Undan, S. Natsume, A. Uemura, H. Saitoh, H. Matsumura, N. Urasaki, T. Yokota and R. Terauchi, 2015. A cytochrome P450, OsDSS1, is involved in growth and drought stress responses in rice (*Oryza sativa* L.). *Plant Mol. Biol.*, 88: 85–99

Teige, M., E. Scheik, T. Eulgem, R. Doczi, K. Ichimura, K. Shinozaki, J.L. Dang and H. Hirt, 2004. The MKK2 pathway mediates cold and salt stress signaling in Arabidopsis. *Mol. Cells.*, 15: 141–152

Todaka, D., K. Shinozaki and K. Yamaguchi-Shinozaki, 2015. Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Front. Plant Sci.*, 6: 84

Trapnell, C., A. Roberts, L. Goff, G. Pertea, D. Kim, D.R. Kelley, H. Pimentel, S.L. Salzberg, J.L. Rinn and L. Pachter, 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Prot.*, 7: 562–578

Turkan, I., M. Bor, F. Ozdemir and H. Koca, 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought tolerant *P. Acutifolius* Gray and drought sensitive *P. vulgaris L.* subjected to polyethylene glycol mediated water stress. *Plant Sci.*, 168: 223–231

Vahisalu, T., H. Kollist, Y.F. Wang, N. Nishimura, W.Y. Chan and G. Valerio, 2008. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. *Nature*, 452: 487–491

Wang, D., C. Yang, L. Dong, J. Zhu, J. Wang and S. Zhang, 2015. Comparative transcriptome analyses of drought-resistant and -susceptible *Brassica Napus L.* and development of EST-SSR markers by RNA-Seq. *J. Plant Biol.*, 58: 259–269

Wang, Z., M. Gerstein and M. Snyder, 2009. RNA-Seq: A revolutionary tool for transcriptomics. *Nat. Rev. Genet.*, 10: 57–63

Wani, S.H., P.A. Sofi, S.S. Gosal and N.B. Singh, 2010. In vitro screening of rice (*Oryza sativa* L) callus for drought tolerance. *Commun. Biometr. Crop Sci.*, 5: 108–115

Wege, S., A. Angeli, M.J. Droillard, L. Kroniewicz, S. Merlot and D. Cornu, 2014. Phosphorylation of the vacuolar anion exchanger AtCLCa is required for the stomatal response to abscisic acid. *Sci. Signal.*, 7: 65

Wu, F., P. Sheng, J. Tan, X. Chen, G. Lu, W. Ma, Y. Heng, Q. Lin, S. Zhu, J. Wang, J. Wang, X. Guo, X. Zhang, C. Lei and J. Wan, 2015. Plasma membrane receptor-like kinase leaf panicle 2 acts downstream of the drought and salt tolerance transcription factor to regulate drought sensitivity in rice. *J. Exp. Bot.*, 66: 271–81

Yamashita, R., N.P. Sathira, A. Kanai, K. Tanimoto, T. Arauchi, Y. Tanaka, S.I. Hashimoto, S. Sugano, K. Nakai and Y. Suzuki, 2011. Genome-wide characterization of transcriptional start sites in humans by integrative transcriptome analysis. *Genom. Res.*, 21: 775–789

Ye, J., S. Wang, F. Zhang, D. Xie and Y. Yao, 2013. Proteomic analysis of leaves of different wheat genotypes subjected to PEG 6000 stress and rewatering. *Plant Omics J.*, 6: 286–294

Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*. 83. *IRRI*, Las Banos, Laguna, The Philippines

Zang, O.W., C.X. Wang, X.Y. Li, Z.A. Guo, R.L. Jing, J. Zhao and X.P. Chang, 2010. Isolation and characterization of a gene encoding a polyethylene glycol-induced cysteine protease in common wheat. *J. Biosci.*, 35: 379–388

Zhang, D., J. Tong, X. He, Z. Xu, L. Xu, P. Wei, Y. Huang, M. Brestic, H. Ma and H. Shao, 2016. A novel soybean intrinsic protein gene, GMTIP2;3, involved in responding to osmotic stress. *Front. Plant Sci.*, 6: 1237

(Received 15 December 2017; Accepted 30 July 2018)