THE DIFFERENTIAL EXPRESSED GENES IN THE *PDH47* TRANSGENE-CARRYING RICE UNDER DROUGHT STRESS

Tran Ngoc He[1,†,*], Pham Thi Kim Vang[1,†]

[1]*CuuLong Delta Rice Research Institute, Tan Thanh Commune, Thoi Lai District, Can Tho City, Vietnam.*
[†]*Tran Ngoc He and Pham Thi Kim Vang contributed equally to this work.*
**Correspondence: tranngoche@yahoo.com or tranngoche9@gmail.com (T. N. He).*

ABSTRACT

The development of drought-tolerant DEGs-carrying rice cultivars through a genetic engineering-based transgenic approach is one of the most popular solutions for drought stress. Whole transcriptome analysis of four leaves tissue samples (Transgenic line and Control plant: before and after drought stress) that performed by platform Illumina NextSeq 500 system using reagent Kit 300 cycles PE (paired-end) was used for identification of differentially expressed genes (DEGs) in rice plants. Several different bioinformatics tools were used for the classification and functional annotation of all DEGs. The RNA-Seq analysis revealed significant 942 and 475 DEGs in drought-stress and non-stress conditions for both transgenic rice and untransformed rice, respectively. There were 170 and 386 up-regulated and 772 and 89 down-regulated genes in transgenic rice and untransformed rice under drought-stress and non-stress conditions, respectively. The up-regulated DEGs in transgenic rice are categorized into eleven groups of genes related to drought stress responses, out of these groups five important groups are: (i) Late Embryogenesis Abundant (LEAs), (ii) Dehydrins (DHNs), (iii) Transcription Factors (TFs), (iv) Helicases, (v) The Kinase Activity. In conclusion, our results could be helpful in understanding a global view of gene expression/transcriptome profiling in transgenic rice expressing PDH47 transgene under drought stress. The up-regulated DEGs under drought stress could be considered as a valuable resource for exploring novel drought-responsive genes to support investigating rice plant response during drought stress. This would also open opportunities for effectively improving drought stress response and tolerance in rice and contribute to transgenic breeding efforts in the development of new drought-tolerant rice varieties.

Keywords: Abiotic Stresses, DEGs, drought Stress, pea DNA Helicases 47 (PDH47), rice (*Oryza sativa* L.), RNA-sequencing, transgenic rice.

INTRODUCTION

Ensure that crop production is sufficient to satisfy the needs of a growth of human population in the world that is expected to grow to more than 9 billion and respect to a projected increase in agricultural demand of 70% by 2050 is a tremendous challenge for plant science (http://www.unpopulation.org). Technical advancements brought by genomics may result in an increase in rice productivity, even under suboptimal crop conditions, such as the occurrence of drought periods. This goal is challenging primarily because the average rate of the crop production increase is only 1.3% per year, and it cannot keep pace with the growth of the world's population (Godfray et al. 2010). Furthermore, by connecting the genotype to the phenotype, high-yielding, stress-tolerant plants can be selected far more rapidly and efficiently than is currently possible. In recent years and until now, genetic engineering approaches are the key methods in plants that have opened

opportunities for developing new cultivars including rice with better water-use efficiency or improved drought tolerance is a primary goal in rice breeding programs. Many transgenic rice plants have been produced for various agronomic characteristics, including tolerance for abiotic stresses. However, it is important to select suitable candidate genes for conferring tolerance to drought. Tolerance to drought stress from such stress-responsive genes involves the expression of many other genes since drought is a complex phenomenon and it cross-talks with other abiotic stresses (Shinozaki and Yamaguchi-Shinozaki 2000; Munns 2002; Siddiqui et al. 2014b). Bartels and Sunkar (2005) reported that many stress-inducible genes have been identified to function in abiotic stress tolerance using transgenic plants. Under drought conditions, plant responses related to some biological actions including alterations in gene expression, the accumulation of metabolites such as osmotically active compounds, and the synthesis of specific proteins (e.g. largely hydrophilic proteins, proteins that function to scavenge oxygen radicals, chaperone proteins, etc.) (Reddy et al. 2004). The basic strategy of genetic engineering for drought tolerance is the introduction of stress-responsive genes that are directly involved in these events. Currently, an increasing number of studies focuses on the identification of drought-responsive genes that are differentially regulated in rice genotypes characterized by a contrasting phenotype in response to stress (Degenkolbe et al. 2009; Lenka et al. 2011; Cal et al. 2013; Degenkolbe et al. 2013; Moumeni et al. 2015).

Several hundred such stress-responsive genes have been identified as candidate genes for genetic engineering with the help of DNA microarray technology or RNA-Sequencing (RNA-Seq), which allows the high-throughput analysis of differential messenger RNA expression. With a typical sequencing depth and sufficient sensitivity, the RNA-Seq technique represents the latest and most powerful tool for characterizing the transcriptome (Wang et al. 2009) and is more suitable and affordable for comparative gene expression studies than microarrays and generates ultrahigh-throughput data including many low abundance genes (Bellin et al. 2009; Wang et al. 2009). Several studies have demonstrated that RNA-Seq data represents integrated networks that more closely resemble the cellular biology of many plants (Bleeker et al. 2011; Xu et al. 2012). Taking the advantage of recent high-throughput sequencing methods, RNA-Seq or deep sequencing of RNAs is designed to detect and quantify transcript structure and abundance by sequencing randomly fragmented RNA or cDNA (Cloonan et al. 2008; Mortazavi et al. 2008). Currently, RNA-Seq is becoming a standard and fundamental protocol as well as an efficient method to discover genes (Wang et al. 2011) and the increasing sequence data generated by it has broadened the understanding of genomes for various species in transcriptomic research (Ranney et al. 2014; Tang et al. 2015; Shen et al. 2016). There are distinct bioinformatics algorithms and tools that have been developed for RNA-Seq data analysis, including read mapping and junction discovery (Au et al. 2010; Trapnell et al. 2009). The expression level estimation of genes/isoforms and differential expression analysis (Langmead et al. 2010; Mortazavi et al. 2008), transcriptome assembly from mapped reads (Li et al. 2011a,b; Trapnell et al. 2010) or *de novo* assembly (Peng et al. 2011). Recently, high-throughput sequencing technology has been widely applied to identify and investigate drought stress-inducible transcript in a variety of other plants including rice (Oono et al. 2011), wheat (Li et al. 2012), Lupin (O'Rourke et al. 2013), maize (Xu et al. 2014), peanut (Brasileiro et al. 2015), cassava (Hu et al. 2015), sorghum (Fracasso et al. 2016), coffee (Mofatto et al. 2016), Sudan grass (Zhu et al. 2017), and pearl millet (Jaiswal et al. 2018). In this study, the whole transcriptome sequencing (RNA-Seq) approach was used to study the functional information of the differentially expressed genes (DEGs) during drought stress in the transgenic rice expressing *PDH47* transgene.

Several different bioinformatics tools were used to identify specific, and/or exclusively differentially expressed stress-responsive genes (DEGs) in transgenic rice expressing *PDH47* transgene during drought stress. Summary of the important DEGs involved in drought stress tolerance in rice plants with the various gene groups will be classified according to diverse functions under stress conditions.

MATERIALS AND METHODS

Transcriptomic study (RNA-Seq) in transgenic rice and untransformed rice

The four plant samples (transgenic rice before and after drought stress; control untransformed rice before and after drought stress) were prepared previously in ABT's Lab of AAU and sent to SANDOR LIFE SCIENCES PVT. LTD., BANJARA HILLS, HYDERABAD-500034, INDIA for RNA isolation and sequencing. Illumina sequencing was performed and carried out following the manufacturer's instruments with Illumina NextSeq 500 Sequencing (NGS Platform).

Identification of differentially expressed genes (DEGs)

A workflow for reconstructing of the transcript structures with a series of programs was used as described in **Figure 1** below.

The transcripts were classified as expressed exclusively in before and after drought stresses in transgenic lines and untransformed rice plants. The FPKM (Fragments Per Kilobase of transcript per Million mapped reads) value was calculated as the following by the formula:

$$\mathrm{FPKM} = \frac{10^9 \times \mathrm{C_i}}{\mathrm{N_m} \times \mathrm{L_i}}$$

Where C_i is the number of reads mapping to isoform *i* (the specific gene) on a transcript determined from the high-quality category. N_m is the total number of mappable reads (in millions) which was determined as the sum of the high-quality reads and the highly repetitive reads (in the sample). L_i is the length of transcript/gene to isoform *i* (kb) for the longest splice variant for a particular transcript/gene.

Identification of the significance of DEGs

The log fold change (FC) was calculated by log2 (FPKM of drought stress)/FPKM control) for transgenic rice and untransformed rice. While calculating fold changes, 1 was added to avoid division by 0. The probability that highly expressed transcripts/genes were used and detected as differentially expressed is greater than that for low-count genes (Oshlack and Wakefield 2009). Two tests were used including a threshold p-value of ≤ 0.05 (or false discovery rate (FDR) adjusted p-value) and the absolute value of fold change log2 ratio of FPKM between two samples was tested statistically difference as the threshold to judge and determine whether a particular transcript/gene expression was altered significantly or not. The significance level "yes", was considered as significantly differentially expressed transcripts/genes (DEGs) (Noble 2009). The absolute value of fold change $|\log2\text{ratio}| \geq 2.0$ for up-regulation (high expression level with a positive value) and the value of fold change $|\log2\text{ratio}| \leq 2.0$ for down-regulation (low expression level with a negative value) were determined. The specific and common DEGs with respect to treatments (drought-stressed and control-well-watered) and genotypes (transgenic line and untransformed rice) were also considered. The identified DEGs in four samples were used for downstream analysis.

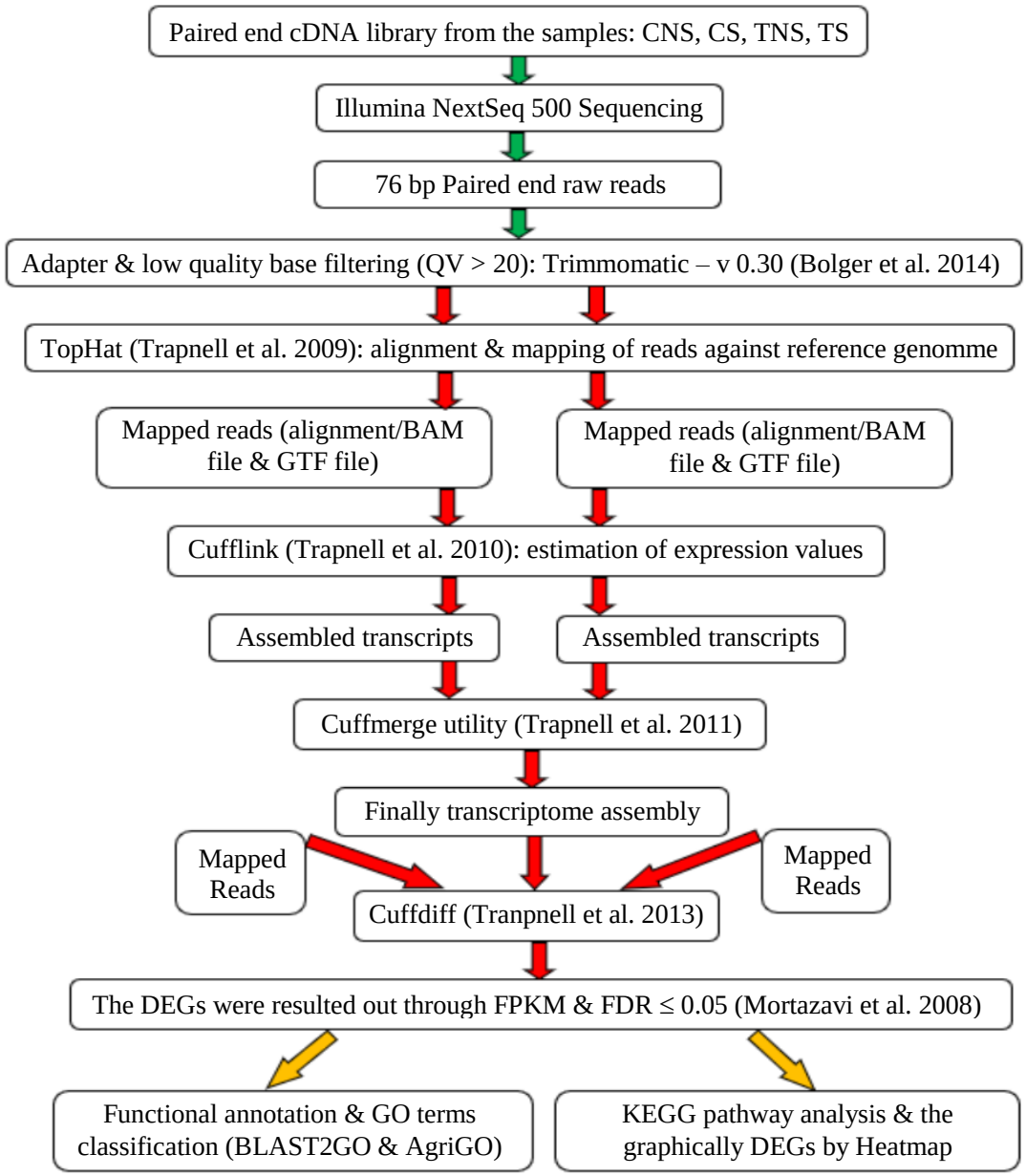

Figure 1. The workflow of NGS and Bioinformatics for DE genes/transcripts (This chart was described more brightly than in the research of Tran Ngoc He et al. 2019).

The graphical representation of DEGs

The two software programs were used for the graphical analysis of DEGs in four samples based on the log-transformed (log2ratio) and the normalized value of genes (FPKM data) using Euclidean distance and Single linkage hierarchical cluster methods. A heat map was generated using the Heatmapper tool (http://www.heatmapper.ca) (Sasha et al. 2016) for the top 100 up-regulated and down-regulated DEGs and for the specifically and commonly up-regulated DEGs between transgenic line and

untransformed rice. Another heat map was also generated using the Clustvis tool (http://www.biit.cs.ut.ee/clustvis/) (Metsalu and Vilo 2015) for a particular group of up-regulated DEGs in a transgenic line.

RESULTS AND DISCUSSION

DEGs encoding *LEA*

There were 7 genes that encode the late embryogenesis abundant protein families such as *LEA3/LEA19* (OS05G0542500) (Jin et al. 2018), *LEA18* (OSJNBA0086O06.12) (Silveira et al. 2015), *LEA14/WSI18* protein induced by water stress (OS01G0705200) (Shim et al. 2018), *LEA*, group 3 (OS03G0168100) (Hanumappa et al. 2013), putative *LEA* protein (OS01G0225600) (Shaar-Morshe et al. 2015), putative *LEA D-34* protein (OS06G0341300) (Moumeni et al. 2015), and *LEA6* (OS06G0110200) (Borah et al. 2017) were identified in T_2 D68/1 transgenic rice under drought stress condition. In previous reports showed that out of which 2 genes, putative *LEA* protein group, and *LEA3* showed low and high expression in transgenic rice as compared to control rice plants during drought stress, respectively. While in this study other 5 genes such as *LEA19*, *LEA18*, *LEA14*, putative *LEA D-34* protein, and *LEA6* were significantly up-regulated in transgenic rice during drought stress **(Figure 2A)**. A heat map of 6 genes between drought-stress and non-stress in transgenic rice and untransformed rice samples involved in *LEA* proteins was depicted in **Figure 2B**. These five *LEA* genes were considered putative genes involved in response to drought stress as compared to the rice/other plant species (Cheng et al. 2002; Figueras et al. 2004; Wang et al. 2006; Kottapalli et al. 2007; Wang et al. 2009; Song et al. 2010; He et al. 2011; Sano et al. 2013; Maruyama et al. 2014; Suzuki et al. 2015; Wang et al. 2016).

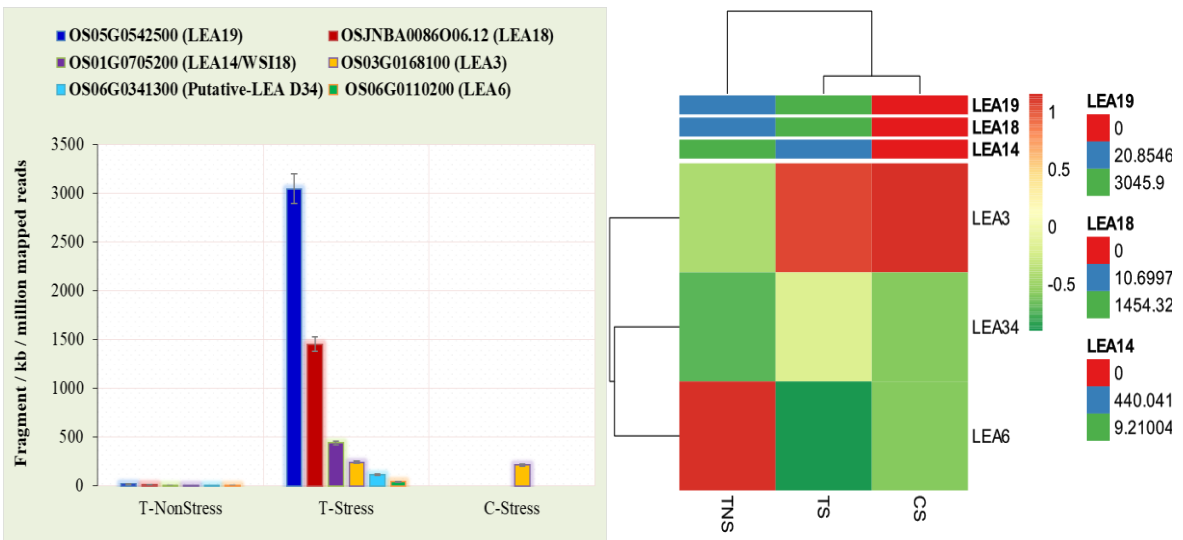

Figure 2. The expression level of drought-related up-regulated genes involved in the LEA proteins in transgenic rice as compared to untransformed rice (Figure 2A-left side). A heat map between drought-stressed and non-stressed samples in transgenic rice and untransformed rice involved in the expression of LEA proteins. Both rows and columns are clustered using Euclidean distance and single linkage (3 rows, 3 columns) (Figure 2B-right side).

DEGs encoding dehydrin

In *S. moorcroftiana*, a selected dehydrins protein was identified under drought stress (Li et al. 2015). Panta et al. (2001) demonstrated that during drought stress the dehydrins were found to accumulate in response to changes in abscisic

acid levels in blueberry (*Vacinium* spp.). The expression level of some dehydrins involved in the ABA accumulation level depends on the duration of stress in other crops such as grapes and barley (Suprunova et al. 2004; Qian et al. 2008; Yang et al. 2012). The transcripts of the dehydrin gene have been identified as up-regulated with high expression levels in peach and tobacco during drought stress conditions (Wisniewski et al. 2006; Dobra et al. 2011). The gene encoding for dehydrins was identified in the deep root of Nagina 22 *Indica* rice (drought tolerant) using ESTs generated at a high level from drought-stress seedlings and during cellular dehydration (Gorantla et al. 2007).

In the current study, 4 genes were identified for encoding the dehydrin protein families in transgenic rice during drought stress **(Figure 3A)**. Out of 4 genes, two genes water stress-inducible protein *Rab21* (OS11G0454300) (Thu et al. 2018), and water stress-inducible protein *Rab21*-like (OS11G0451700) (Kim et al. 2014) were expressed with a higher level in transgenic rice as compared to untransformed rice during drought-stress. While the other two genes dehydrin *Rab16B* (OS11G0454200) (Chen et al. 2015) and dehydrin *Rab16C* (OS11G0454000) (Fu et al. 2017) were significantly up-regulated in transgenic rice during drought stress as compared to non-stress condition. A heat map of 4 genes between drought-stress and non-stress in transgenic line and untransformed rice involved in the dehydrin proteins were depicted in **Figure 3B**.

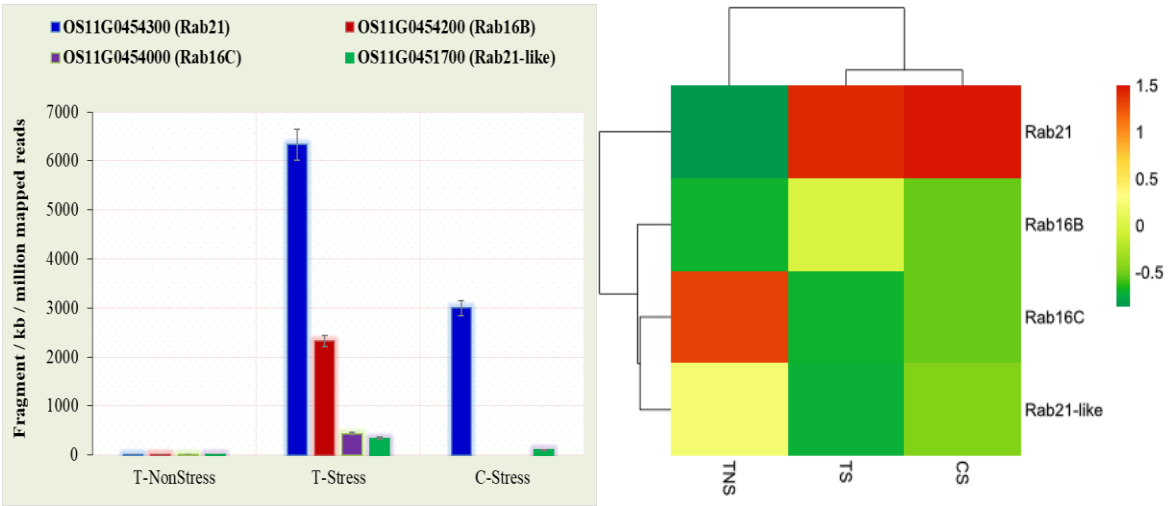

Figure 3. The expression level of drought-related up-regulated genes involved in the dehydrin proteins in transgenic rice as compared to untransformed rice (Figure 3A-left side). A heat map between drought-stress and non-stress samples in transgenic rice and untransformed rice involved in the expression of dehydrin proteins. Both rows and columns are clustered using Euclidean distance and single linkage (4 rows, 3 columns) (Figure 3B-right side).

DEGs encoding transcription factors (TFs)

In the current study, a total of 7 genes that encode transcription factors were identified in the transgenic line under drought stress. Among of which, 6 genes were significantly up-regulated in the transgenic line during drought stress such as putative *DRE* binding factor 2 (OS02G0676800) (Hifzur et al. 2015), *AP2* domain-containing protein *AP29* (P0665C04.30) (Xu et al. 2017), transcription factor *MYB102* (OS07G0558100) (Sircar and Parekh 2015), *NAC* transcription factor 29 (OS12G0123700) (Kitazumi et al. 2018), heat stress transcription factor A-6a

(OS01G0571300) (Kim et al. 2018) and heat stress transcription factor C-2b (OS06G0553001) (Schmidt et al. 2012) **(Figure 4A)**. Only one gene encodes ethylene-responsive transcription factor *ERF109* (OS02G0764700) (Shankar et al. 2016) showed a decrease in expression in transgenic rice as compared to untransformed rice under drought stress. In the present investigation, a heat map of 6 genes during drought-stress and non-stress conditions in transgenic rice involved TFs proteins was described in **Figure 4B**. These 6 genes of TFs families are considered putative genes involved in response to drought stress and other abiotic stresses in transgenic rice and other plant species (Kotak et al. 2004; Cashikar et al. 2005; Seo et al. 2009; Sharoni et al. 2011; Mittal et al. 2012; Pan et al. 2013; Roja 2014; Duan et al. 2015; Michael et al. 2016; Yoo et al. 2017).

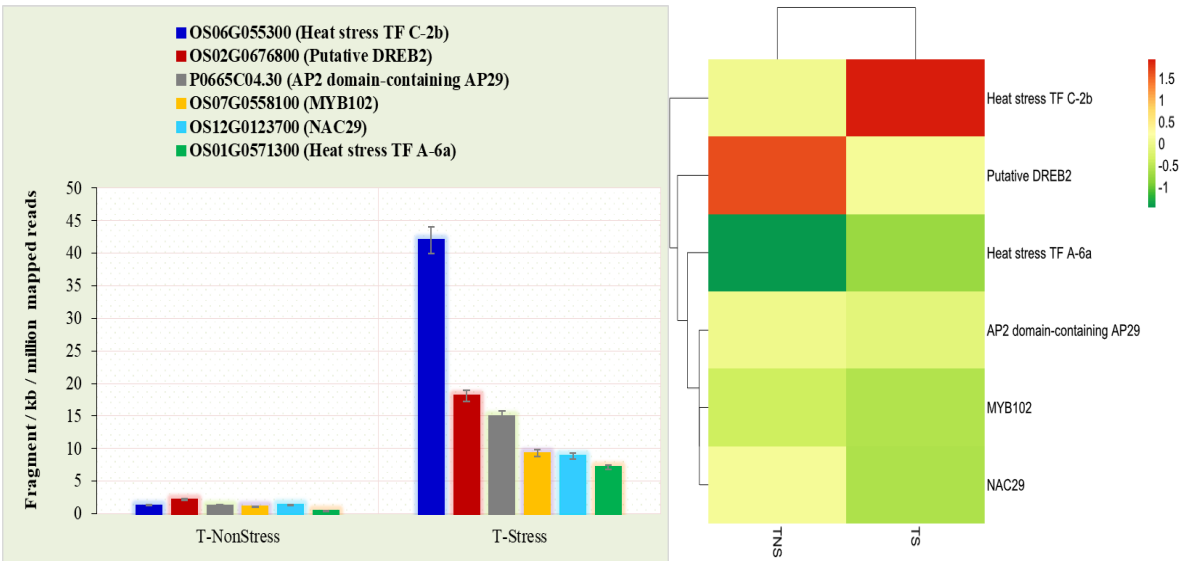

Figure 4. The expression level of drought-related up-regulated genes involved in the transcription factor proteins in transgenic rice (Figure 4A-left side). A heat map between drought-stress and non-stress samples in transgenic rice involved in the expression of TF proteins. Both rows and columns are clustered using Euclidean distance and single linkage (4 rows, 2 columns) (Figure 4B-right side).

DEGs encoding helicases

There were 2 genes that encode the helicases family, such as DEAD-box ATP-dependent RNA helicase 35B (OS06G0697200) and ATP-dependent RNA helicase A (OS03G47270) and 1 gene OS04G0206200 encodes for unknown function protein were identified in transgenic rice during drought stress condition. Out of 3 genes, 2 genes OS03G47270 and OS04G0206200 were significantly up-regulated in transgenic rice under drought stress conditions and another gene DEAD-box ATP-dependent RNA helicase 35B showed low expression in transgenic rice as compared to untransformed rice during drought-stress.

DEAD-box RNA helicase is involved in RNA synthesis, repairing, and regulating genes expression during growth and development as well as environmental stresses response (Owttrim 2013; Mallam et al. 2014; Ma et al. 2016; Vashisht et al. 2005; Vashisht and Tuteja 2006; Gill et al. 2013). The RNA helicase A gene was found at 3.13051-fold up-regulation (p-value 0.00985 $\leq$ 0.05) of the transcript under drought

stress as compared to the non-stress condition under the present study analysis **(Figure 5)**. In the present investigation, under drought stress conditions, the gene OS04G0206200 was significantly up-regulated in the transgenic line at a high 3.25634-fold (p-value 0.0132 ≤ 0.05) as compared to the non-stress condition. This gene is located in chr.4 (7136794-7140421) of rice and is involved in the telomere maintenance; DNA replication; DNA repair; DNA recombination; DNA duplex unwinding and associated with the ATP binding; ATP-dependent 5'-3' DNA helicase activity in the replication fork. OS04G0206200 gene is related to two enzymes adenyl pyrophosphatase/ adenosine triphosphatase (EC:3.6.1.3) and nucleoside-triphosphate phosphatase (EC:3.6.1.15) were annotated in the KEGG database. This gene was also identified as actively participating in two metabolic pathways: purine metabolism (map00230) and thiamine metabolism (map00730) in the KEGG database metabolic pathways. This gene was also identified as a hypothetical protein expressed at a higher level in cv. LaGrue compared to expression in cv. Cypress when treated with high night temperature (Lawson 2016).

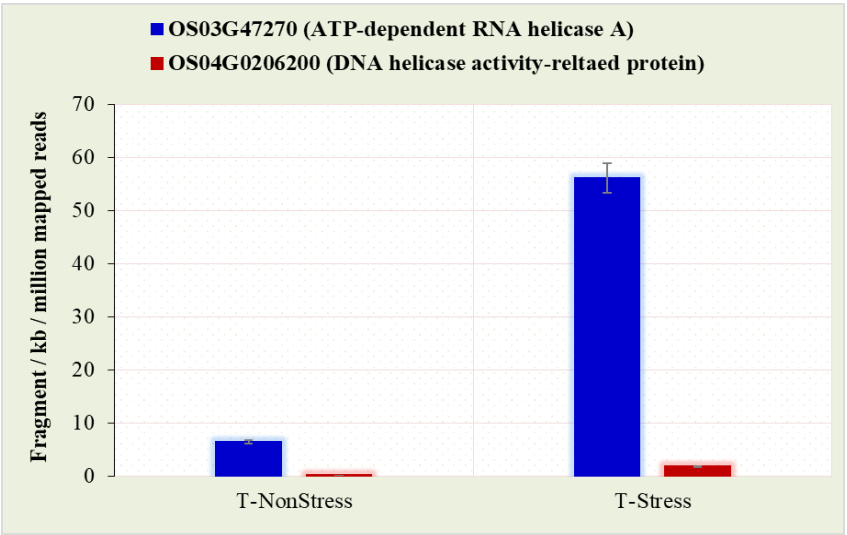

Figure 5. The expression level of drought-related up-regulated gene involved in helicase proteins in transgenic rice.

DEGs encoding kinase activity-involved proteins

In the present investigation, nine genes were related to kinase activity, out of which, 6 genes (OS10G0364900, OS07G0120650, OS08G0192100, P0655A07.28, OS04G0517500, OS09G0115600-*MAP3K*) were identified as significantly up-regulated in transgenic rice during the drought-stress condition, 1 gene (OS10G0505900) and other 2 genes (OS06G0668200, OS03G0423300) were exhibited a high and low level of expression in transgenic rice as compared to untransformed rice during drought-stress, respectively. However, out of 9 genes, 5 genes with high expression levels **(Figure 6A)** as mentioned above considered involved to be drought responsive as reported earlier in rice species described below. A heat map of five genes between drought-stress and non-stress in transgenic rice and untransformed rice samples involved in the transport and transporter activity-related proteins was presented in **Figure 6B**.

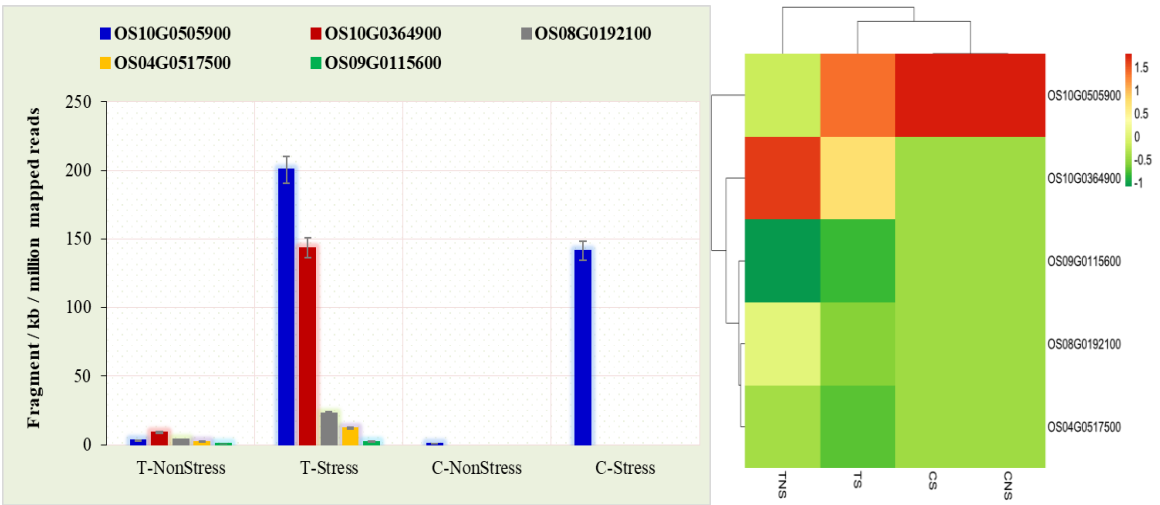

Figure 6. The expression level of drought-related up-regulated genes involved in the kinase activity-related proteins in transgenic rice as compared to untransformed rice (Figure 6A-left side). A heat map of between drought-stress and non-stress samples in transgenic rice and untransformed rice involved in the expression of kinase activity-related proteins. Both rows and columns are clustered using Euclidean distance and single linkage (3 rows, 4 columns) (Figure 6B-right side).

The gene OS08G0192100 significantly up-regulated in T_2 D68/1 transgenic rice during drought-stress with log2 (FC) 2.63857 (p-value 0.0311 $\leq$ 0.05) as compared to non-stress condition. This gene is found in chr.8 (5391859-5392486) of rice and is involved in protein phosphorylation and combined with the function of protein kinase activity. This gene encodes the expressed protein located in module 6 in the shoots at the early stages of water stress especially under the milder PEG treatment and the lower responsivity compared to the roots of *Arundo donax* L. (Fu et al. 2016).

In the current study, during drought stress, the gene OS10G0364900 was significantly expressed in T_2 D68/1 transgenic rice at 4.02551-fold up-regulation (p-value 0.00155 $\leq$ 0.05) with respect to non-stress conditions. This gene is located in chr.10 (11390595-11391123) of rice and is involved in protein phosphorylation and with the function of protein kinase activity, protein binding, and ATP binding. This gene has been identified as an up-regulated gene in response to cell wall removal and regeneration showed merely stress-associated gene (Sharma et al. 2011) and its expression significantly changed in both root and shoot under ABA condition (Kim et al. 2018) and also regulated by low-temperature acclimation in wheat at ambient elevated CO_2 (Kane et al. 2013).

The gene OS10G0505900 (low-temperature-induced 65 kDa protein) showed a higher expression level in T_2 D68/1 transgenic rice as compared to untransformed rice during drought stress with FPKM 200.455 and FPKM 141.47, respectively. This gene also showed an increase in 6.0676-fold up-regulation (p-value 0.00025 $\leq$ 0.05) of the transcript with respect to the non-stress conditions. This gene is located in chr.10 (19346333-19348263) of rice and is involved in response to abscisic acid, protein phosphorylation, protein kinase activity, and ATP binding. Abebe et al. (2009) reported this gene as a drought-induced gene (expressed protein) in barley caryopses and also identified it as a drought stress-related protein gene up-regulated in the *OsMGT1* (*Oryza sativa* MAGNESIUM TRANSPORTER1) knockout rice line (Chen et al. 2012). Maruyama et al. (2014) showed the transcripts level of this gene was significantly higher in dehydration-treated plants than in cold-treated rice plants.

The gene OS04G0517500 encodes phosphoenolpyruvate carboxylase kinase (EC:2.7.11) was an significantly up-regulated gene in T_2 D68/1 transgenic rice under drought-stress with log2 (FC) 2.47173 (p-value 0.0434 ≤ 0.05) as compared to non-stress condition. This gene is located in chr.4 (25867806-25869187) of rice and is involved in the protein phosphorylation and associated with the protein serine/threonine kinase activity; ATP binding; transferring phosphorus-containing groups. *PEPC* kinase related to the phosphorylation of a conserved serine residue close to the amino-terminal end of the *PEPC* polypeptide is essential to its activity by reducing sensitivity to the feedback inhibitor malate and a catalyst in rice (Wang et al. 2009). This gene also identified in the *OsAP2-39* transgenic rice leaves involved in controlling key interactions between ABA and GA in rice and in turn regulates plant growth and seed production (Yaish et al. 2010). Another member of this gene, *CAMK* (calcium/calmodulin-dependent protein kinases) involved in salinity responsive expression pattern of a selected gene in the contrasting finger millet genotypes and in other crops and also up-regulated in *Porteresia coarctata* species (Rahman et al. 2014).

CONCLUSIONS

In summary, in the current study, the expression profiling of the important up-regulated DEGs groups such as Late embryogenesis abundant (LEA) proteins, Dehydrin (DHD) proteins, Transcription factors proteins (TFs), Helicases proteins, and Kinase activity proteins was studied in detail in T2 D68/1 transgenic rice and untransformed rice before and after drought stress. These results indicate that these genes play an important role during drought stress response and/or drought tolerance in rice and other plant species. Hence, these results could be served as useful information for future studies on drought stress *via* genetic/molecular mechanisms of the identified DEGs in rice. The biological functions of the identified DEGs of rice could be the significant information sources for the development of new drought-tolerant rice varieties.

LIST OF ABBREVIATIONS

DNA Deoxyribose Nucleic Acid

DEGs Differentially Expressed Genes

FPKM Fragments Per Kilobase of transcript per Million mapped reads

NGS Next Generation Sequencing

PDH47 Pea DNA Helicase 47

RNA Ribose Nucleic Acid

COMPETING INTERESTS

The authors declare they have no conflict of interest, financial or otherwise.

AUTHOR'S INFORMATION AND CONTRIBUTIONS

Tran Ngoc He performed the research works. Tran Ngoc He wrote the final paper and manuscript. Pham Thi Kim Vang is the first reviewer of this manuscript as well has read and approved the final paper and manuscript with very good notes, all the authors discussed the results.

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GEN ĐƯỢC BIỂU HIỆN KHÁC NHAU Ở CÂY LÚA MANG GEN *PDH47* TRONG ĐIỀU KIỆN KHÔ HẠN

Phát triển các giống lúa mang gen chịu hạn thông qua phương pháp chuyển nạp gen dựa trên kỹ thuật di truyền là một trong những giải pháp phổ biến và hiệu quả nhất đối với tình trạng hạn hán. Phân tích toàn bộ sự phiên mã gen của bốn mẫu mô lá lúa (dòng lúa chuyển gen và đối chứng: trước và sau khi khô hạn) được thực hiện bằng hệ thống Illumina NextSeq 500 trên nền tảng sử dụng Kit 300 cycles PE (paired-end) được sử dụng để xác định các gen (DEG) được biểu hiện khác nhau ở cây lúa. Một số công cụ tin sinh học khác nhau đã được sử dụng để phân loại và định danh chức năng của tất cả các DEG. Phân tích RNA-Seq cho thấy 942 và 475 DEG biểu hiện một cách có ý nghĩa trong điều kiện stress khô hạn và không stress khô hạn, tương ứng đối với dòng lúa biến đổi gen và đối chứng không biến đổi gen. Trong đó, 170 và 386 gen được điều hòa biểu hiện cao và 772 và 89 gen được điều hòa biểu hiện thấp tương ứng với dòng lúa biến đổi gen và đối chứng, trong điều kiện khô hạn và tự nhiên. Các DEG được điều hòa biểu hiện cao ở dòng lúa biến đổi gen được phân loại thành 11 nhóm gen liên quan đến phản ứng stress khô hạn, trong đó 5 nhóm quan trọng nhất là: (i) Late Embryogenesis Abundant (LEAs), (ii) Dehydrins (DHNs), (iii) Transcription Factors (TFs), (iv) Helicases, (v) The Kinase Activity. Tóm lại, kết quả nghiên cứu này có vai trò quan trọng trong việc tìm hiểu một cách toàn diện về sự biểu hiện gen/thành lập thông tin phân tử về bộ gen phiên mã ở cây lúa chuyển gen PDH47 dưới áp lực stress khô hạn. Các DEG ở lúa được điều hòa biểu hiện cao trong điều kiện khô hạn có thể được xem là một nguồn tài nguyên quý giá để khai thác các gen mới phản ứng với stress khô hạn, nhằm hỗ trợ trong việc khám phá các phản ứng phân tử của cây lúa trong suốt điều kiện stress khô hạn. Điều này cũng sẽ mở ra cơ hội cải thiện khả năng chống chịu và ứng phó với hạn hán một cách có hiệu quả trên lúa, góp phần vào các nỗ lực chọn tạo giống lúa chuyển gen, đặc biệt trong việc phát triển các giống lúa mới chịu hạn.

Từ khóa: *Stress phi sinh học, DEG, stress khô hạn, DNA Helicase đậu (PDH47), lúa (Oryza sativa L.), giải trình tự RNA, lúa chuyển gen.*