Identification of resurrection genes from the transcriptome of dehydrated and rehydrated Selaginella tamariscina

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ABSTRACT

Selaginella tamariscina is a lycophyta species that survives under extremely dry conditions via the mechanism of resurrection. This phenomenon involves the regulation of numerous genes that play vital roles in desiccation tolerance and subsequent rehydration. To identify resurrection-related genes, we analyzed the transcriptome between dehydration conditions and rehydration conditions of S. tamariscina. The de novo assembly generated 124,417 transcripts with an average size of 1,000 bp and 87,754 unigenes. Among these genes, 1,267 genes and 634 genes were up and down regulated by rehydration compared to dehydration. To understand gene function, we annotated Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). The unigenes encoding early light-inducible protein (ELIP) were down-regulated, whereas pentatricopeptide repeat-containing protein (PPR), late embryogenesis abundant proteins (LEA), sucrose nonfermenting protein (SNF), trehalose phosphate phosphatase (TPP), trehalose phosphate synthase (TPS), and ABC transporter G family (ABCG) were significantly up-regulated in response to rehydration conditions by differentially expressed genes (DEGs) analysis. Several studies provide evidence that these genes play a role in stress environment. The ELIP and PPR genes are involved in chloroplast protection during dehydration and rehydration. LEA, SNF, and trehalose genes are known to be oxidant scavengers that protect the cell structure from the deleterious effect of drought. TPP and TPS genes were found in the starch and sucrose metabolism pathways, which are essential sugar-signaling metabolites regulating plant metabolism and other biological processes. ABC-G gene interacts with abscisic acid (ABA) phytohormone in the stomata opening during stress conditions. Our findings provide valuable information and candidate resurrection genes for future functional analysis aimed at improving the drought tolerance of crop plants.

Introduction

Selaginella tamariscina is a primitive resurrection plant that has the ability to resist extreme dehydration conditions and can be retained to a normal form upon rehydration.¹ The phenomenon of resurrection has been mostly studied in cyanobacteria and plants.² ³ Among these species, more than 300 species of angiosperms have been known as resurrection plants.⁴ ⁵ Most Selaginella species have the ability to survive under severe water stress, with almost full loss of protoplasmic water.⁶ Resurrection plants undergo several physiological and metabolic mechanisms to sustain desiccation.⁷ Plant modifications such as curling and folding confer drought tolerance by limiting light and forming reactive oxygen species (ROS).⁸ ⁹ During dehydration, the photosynthetic apparatus is damaged;¹⁰ therefore, resurrection plants consist of inducible repair mechanisms that maintain their photosynthetic apparatus.¹¹ ¹² Abscisic acid (ABA) plays an important role during water deficit conditions.¹³ Several ABA response genes have been discovered to date. ABA plays a central role in various stress conditions through network signaling with several gene families.¹⁴ Plants have a mechanism to adapt under light stress through the mechanism attributed to the chlorophyll/b-bind- ing protein (CAB) family to protect chloroplasts.¹⁵ Therefore, early light inducible proteins (ELIPs) protect plant leaves during light stress and play a major role in photoprotection.¹⁶ During dehydration, the rate of chlorophyll synthesis and photosynthesis is reduced;¹⁷ therefore, a large number of pentatricopeptide repeated proteins(PPRs) are required for chloroplast development.¹⁸ When plants undergo drought stress, energy-generating organelle mitochondria are supposed to be damaged.¹⁹ In order to protect, LEA proteins and make biochemical and secondary structures to withstand in desiccation stress.²⁰ Recently, trehalose biosynthesis pathway genes have been studied that respond to drought.²¹ These trehalose-
related genes are responsible for inhibiting sucrose non-fermenting (SNF) proteins to regulate the energy during stress conditions.\textsuperscript{22} SNF proteins form an interaction network with ABA and function during abiotic stress.\textsuperscript{23} During desiccation, ABC transporters regulate hormones and secondary metabolites.\textsuperscript{24} Additionally, ABCG gene families have been identified in the moss \textit{Physcomitrella patens} for adaptation to extreme environmental conditions.\textsuperscript{24}

Resurrection plants are studied to understand and identify the genes related to the mechanism of desiccation tolerance.\textsuperscript{10} To elucidate the mechanism of desiccation, several genomic approaches have been discovered.\textsuperscript{25} In our study, to identify the genes involved in resurrection, we analyzed differentially expressed genes (DEGs) based on comparison of the transcriptome among dehydrated and rehydrated leaves of \textit{S. tamariscina}. Since angiosperm resurrection plants have been extensively studied, we characterized the major genes and their functions involved in desiccation. For this knowledge, the identification of potential candidate genes associated with resurrection based on transcriptome and DEG analyses will improve our understanding of the regulation and function of the gene response to dehydration and rehydration.

Materials and methods

Plant materials and sample preparation for transcriptome analysis

\textit{Selaginella tamariscina} plants were grown in pots under a controlled environment plant growth room. The plants were divided into two groups, with a plastic film as a barrier for dehydration and rehydration. The plants were grown with regular watering prior to desiccation treatment. For the desiccation experiment, 2 months old grown plants with similar-sized aerial parts were selected. Water was withheld for 7 days, and the morphology of the plants was observed. After 7 days only one group was watered regularly with bottle sprays until the leaves fully expanded, while the other was left water-deprived. After complete rehydration, leaf tissues were harvested from each group of plants and placed immediately in liquid nitrogen for total RNA isolation.

Water content and phenotype observation

To evaluate the resurrection phenomenon, the leaves were taken from the separately grown rehydrated pots. The leaves (approximately 3–5 g) were plugged from the pot and left on a dry laboratory bench. Different relative water content (RWC) was provided. The fresh leaves were subjected RWC of 70\% and slightly dropped to 30\%. The plant morphology was observed with minimum RWC. Then for rehydration, 30\% RWC leaves were placed on water-soaked facial tissue in a petri dish and covered with lids. The tissues were sprayed with water every 2 hours and 4 hours. The excess water was removed from the surface by blotting with fresh facial tissues. Finally, the morphology of the rehydrated plants was observed.

RNA extraction and sequencing

Total ribonucleic acid (RNA) was extracted from leaf tissues using the RNeasy Plant Mini Kit (Cat No./ID: 79404, Qiagen, USA) according to the manufacturer’s instructions. The quality and concentration of the RNA were assessed using an Agilent Bioanalyzer (Agilent Technology, USA) and a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) with the following parameters: RNA integrity number (RIN) ≥ 7, 28S:18S > 1, and ratio of optical density at 260 and 280 nm (OD260/280) ≥ 2.

RNA-Seqencing was performed using the mRNA isolated from the dehydrated and rehydrated plants leaf, with three biological replicates. cDNA libraries were made using a TruSeq Stranded mRNA kit (Cat. No. RS-122-2101, Illumina, USA). The quality of the sample libraries was assessed using the Agilent Bioanalyzer 2100 system. The libraries were processed for high-throughput DNA sequencing on an IlluminaNextSeq 2000 with the 150 bp paired-end (PE) method.

Transcriptome assembly, annotation, and functional analysis

The next-generation sequencing (NGS) reads were filtered by discarding reads with >20\% N bases, Phred quality ≤ Q20 and length > 50 bp. \textit{De novo} assembly was performed using Trinity (https://github.com/trinityrnaseq)\textsuperscript{26} and CD-HIT (http://weizhonglab.org/CD-hit/).\textsuperscript{27} Transcriptomes were searched for matching sequences in the National Center for Biotechnology Information (NCBI) database using NCBI BLAST, and coding sequence prediction and function prediction were performed with InterProScan (https://www.ebi.ac.uk/interpro)\textsuperscript{28} and TCC (https://github.com/TransDecoder/TransDecoder) programs. Transcriptome quantification and differentially expressed gene (DEG) analyses were carried out using the software applications RSEM (https://github.com/deweylab/RSEM)\textsuperscript{29} and TCC (https://bioconductor.org/packages/release/bioc/html/TCC.html),\textsuperscript{30} respectively. Gene Ontology (GO) enrichment analysis was performed with GO-seq (https://bioconductor.org/packages/release/bioc/html/goseq.html),\textsuperscript{31} with a p-value criterion of < 0.001 for the categories of “molecular function”, “biological process” and “cellular component”. The KEGG pathway was analyzed by the KEGG database (http://www.genome.jp/kegg/pathway.html).

Screening of differentially expressed genes (DEGs)

Expression level analysis was carried out with the filtered high-quality raw reads by counting mapped reads in the unigenic set using RSEM software\textsuperscript{27} and TCC.\textsuperscript{30} The expression value for each gene was calculated with the fragments per kilobase of transcript per million mapped reads (FPKM) method. The significant DEGs were confirmed by Fisher’s exact test (p ≤ 0.05). Additionally, the p-value was adjusted for multiple comparisons by calculating the false discovery rate (FDR) up to 5\%; this Q-value was used to assess differences using multiple test adjustments. Visualization analysis of the volcano plot and heat map clustering of the DEGs were performed by an in-house R script. Finally, the gene response to resurrection was analyzed and selected for further functional analysis.
Results

Resurrection phenomenon of *S. tamariscina*

Two experiments were conducted, one for transcriptome analysis, another for water content phenotype observation. First, the aerial lycophyll leaves of *S. tamariscina* were observed. The water was withheld for 7 days, and the plants were found to be almost dried under dehydration conditions, whereas those irrigated with bottle spray were fully recovered (Figure 1). Second, the resurrection phenomenon was observed in some lycophyll. After water deprivation with different RWC, the plant curled up and changed its appearance. The relative RWC slightly dropped to 70% and severely to 30%, which formed the aerial part into a ball shape. When water was provided again, the aerial part was fully recovered with the highest water content (Supplementary Figure 1). During dehydration and rehydration, we observed morphological changes in the aerial part of the plant, which showed the complete phenomenon of resurrection.

De novo assembly

From two different stages of plants under dehydration and rehydration, lycophyll was used for RNA-Seq analysis. Total RNA from three independent replicates was pooled for mRNA and cDNA synthesis and library preparation. There was no reference genome for *S. tamariscina*, so de novo assembly was selected. The IlluminaNextSeq 2000 150 PE paired sequencing generated 29,233,500 qualified sequence reads with 7,632,894,410 bp length for the rehydration stage and 28,897,467 reads with 7,545,155,886 bp length for the dehydration stage (Table 1). Both sets of filtered reads were pooled to construct a transcriptome reference by de novo assembly using Trinity and CD-HIT software. The de novo assembly generated 124,417 transcripts that varied in size from 224 bp to 19,122 bp, with an average size of 1,000 bp. Of the de novo assembled transcripts, 87,754 were revealed to be unigenes through homology searches using NCBI-BLASTx and InterProScan tools (Table 2, Figure 2). The distribution of the unigenes according to the length obtained is shown in Supplementary Figure 2. Of those obtained unigenes, 63,740 (72.6%) were matched with plant genes with the highest BLAST scores in the BLAST analysis. The average length of the annotated unigenes was 1,136 bp, with a minimum of 224 bp and a maximum of 19,122 bp, and most of the unigenes were less than 1.5 kb in length (Table 2).

Table 1. Summary of raw reads and filtered reads of RNA sequencing.

| Sample       | Raw reads          | Filtered reads       |
|--------------|--------------------|----------------------|
|              | Reads   | Length (bp) | Q30 Bases | Reads    | Length (bp) |
| Rehydration  | 36,801,044 | 11,113,915,288 | 9,152,493,254 | 29,233,500 | 7,632,894,419 |
| Dehydration  | 35,891,739 | 10,839,305,178 | 8,958,726,703 | 28,897,467 | 7,545,155,886 |

Analysis of differentially expressed genes (DEGs)

To gain a comprehensive overview of the *S. tamariscina* transcriptome, a study of differentially expressed genes was conducted. Based on the number of reads mapped onto the reference, the expression level and quantification of each gene were calculated. A total of 124,427 transcripts generated from the sequencing 61,927 were expressed in the dehydrated stage, and 87,755 were expressed in rehydrated stage, among which 43,923 were
commonly expressed in both stages (Figure 2a, Supplementary Table 1). From a total of 87,754 annotated genes, 66,590 were expressed in hydrated tissues, and 30,462 were expressed in dehydrated tissues, accounting for 45.7% of the expressed genes in hydrated tissues. Additionally, 46,940 genes were expressed in dehydrated tissues, and 10,812 were expressed only in the dehydrated tissues, which accounted for 23% of the genes expressed in dehydrated tissues (Figure 2b). The number of genes with reduced expression in dehydrated tissues compared to hydrated tissues was 1.67 times greater than the number of genes with increased expression. The number of DEGs was reduced to 84,247 at a fold change threshold of 2. Then, it was further reduced to 1,901, in which 1,267 were up regulated and 634 were down regulated in the rehydrated state at a p-value ≤ 0.05. Of the 1,901 DEGs, the number with reduced expression in rehydrated tissues was approximately half the number with increased expression (Table 3). The expression of DEGs between dehydration and rehydration was visualized with a volcano plot (Figure 3a) and heat map with fold change ≥ 2 and p-value ≤ 0.05 (Figure 3b), which shows that most of the genes were regulated in rehydration. Among the total DEGs, we found that the maximum number of unigenes was up regulated during rehydration. This indicates that the maximum number of genes was responsible for the regeneration of the plants after rehydration.

**Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway analyses**

To determine the association of DEGs involved in resurrection, we performed functional GO annotation and KEGG pathway analysis. Using the GO-Seq platform, we classified DEGs of dehydration and hydration into “biological process”, “cellular component” and “molecular function” categories. There was no visual difference between transcripts from hydrated and dehydrated tissues. The numbers of DEGs among the transcripts from hydrated tissues were 94,913

| Assembly data | Transcripts | Genes |
|---------------|-------------|-------|
| Total transcript length (bp) | 124,384,611 | - |
| Number of Reads (contigs) | 124,417 | 87,754 |
| Average length | 1,000 | 1,136 |
| Minimum length (bp) | 224 | 224 |
| Maximum length (bp) | 19,122 | 19,122 |

**Figure 2.** Differentially abundant transcripts and annotated genes upon dehydration and rehydration. a) The Venn diagram shows the expression of total transcripts between rehydration and dehydration leaves obtained from the assembly. b) The statistics expression of total annotated genes between rehydration and dehydration leaves. A total of 124,417 transcripts were obtained from the de novo assembly, among which 87,754 unigenes were annotated through different homology searches.

**Figure 3.** Expression profiles of differentially expressed genes (DEGs) in dehydrated and rehydrated samples. a) Volcano plot of DEGs, red dots are the genes that are up regulated and blue dots are the down regulated genes in rehydrated samples. The x-axis plots the distribution of fold change and the y-axis plots the logarithm of p-value of each identified DEGs. b) Schematic representation of heat map clustering of DEGs. Green color indicates the lowest expression, black as intermediate expression, and red indicates the highest expression within the DEGs.

**Table 3. Statistical status of differentially expressed genes between dehydrated and rehydrated samples.**

| Dehydrated vs. rehydrated | Transcripts | Unigenes (LogFold change (LFC) ≥ 2) | DEGs (LFC ≥ 2, p-value ≤ 0.05) |
|----------------------------|-------------|------------------------------------|---------------------------------|
| Up-regulated               | 65923       | 55480                              | 1267                            |
| Down-regulated             | 39594       | 28767                              | 634                             |
| Total                      | 124417      | 84247                              | 1901                            |
under cellular components, 75,690 under molecular functions, and 45,373 under biological processes. Among the 1,901 DEGs, 30 unigenes were classified under biological processes and 15 under molecular functions, and there were only 7 functional categories under cellular components (Figure 4). Both up regulated and down regulated DEGs showed almost the same functional classifications. Under biological processes, approximately 60% of the DEGs were in the top four functional categories of “cellular process”, “metabolic process”, “response to stimulus” and “biological regulation”. Moreover, there were only two predominant functional categories each under molecular functions and in cellular components: “catalytic activity” (43%) and “binding” (43%) for the latter and “cellular anatomical entity” (50–52%) and “intracellular” (40–41%). We found that more unigenes were involved in biological processes and that a minimum number of unigenes were involved in cellular components during resurrection. Among the total DEGs, 29 unigenes were annotated to different pathways. Among them, 2 unigenes were annotated to the starch and sucrose metabolism pathway, 18 unigenes were annotated to the purine metabolism pathway, and 9 unigenes were annotated to the thiamine metabolism pathway (Table 4). These are the pathway genes related to the resurrection phenomenon in S. tamariscina. The family member genes TPP4 and TPS1 encode trehalose-phosphate phosphatase-4 and alpha-alpha trehalose-phosphate synthase-1, respectively, which are involved in the trehalose synthesis pathway (Figure 5a). KEGG pathway analysis showed the products of ABCCG were involved in thiamine metabolism and purine metabolism (Figure 5b, c).

**Identification of resurrection genes**

We analyzed the functional annotations of the DEGs that exhibited the greatest difference in expression in response to dehydration and rehydration. The top unigenes that were significantly expressed are listed in Table 5. We shortlisted reported genes that play a significant role in drought tolerance. Early light inducible proteins (ELIPs), pentatricopeptide repeat-containing protein (PENTA/PPR), late embryogenesis abundant proteins (LEAs), sucrose non-fermenting proteins (SNFs), trehalose phosphate phosphatase (TPP), trehalose phosphate synthase (TPS), and ABC transporter G family member (ABCG) were abundantly expressed during the resurrection process. Our results showed that a large number of DEGs were enriched during the rehydration process. Most of the identified genes are up regulated by resurrection. The unigenes encoding ELIP found to be down regulated by rehydration, whereas the unigenes encoding PENTA/PPR, LEA, SNF, TPP, TPS and ABCG were found up regulated by rehydration. These are the genes reported in previous research under different desiccation conditions with resurrection phenomena. We predict that these genes play a significant role during the rehydration process and keep plants alive under desiccation conditions.

**Discussion**

*S. tamariscina* species have been extensively studied in relation to desiccation tolerance. Resurrection phenomenon studies in *S. tamariscina* have revealed the relationship between the morphology and desiccation tolerance mechanism. Therefore, the
comparative RNA-Seq analysis in our study included the characterization of the unigenes response to the desiccation tolerance based on previously reported research. We analyzed the DEGs between dehydrated and rehydrated tissues among the DEGs we identified and discussed the expression of the genes involved in desiccation tolerance and subsequent rehydration.

Generation of ROS is amplified by drought, inhibiting the photosynthetic activity. This effect is encountered by a desiccation-related ELIP gene, which is regulated by light and ABA. The expression of the ELIP gene family has higher expression during drought stress in resurrection plant *S. lepidophylla* and *B. hygrometrica*. According to, ELIPs showed low expression in rehydrated tissues and helped plants resynthesize chlorophyll. Expression of *ELIP* transcripts was found in one of the moss species *Syntrichis* during environmental stress. When plants return to normal water content, desiccation-tolerant species show decreased expression of *ELIPs*. We obtained similar results in our study, the unigenes encoding *ELIPs* showed lower expression during the fully watered condition of *S. tamariscina* leaves (Table 5), indicating that lycophyll in dehydration is more likely to protect chlorophyll.

Pentatricopeptide repeat-containing proteins (PPRs) are involved in ABA signaling and play an important role in drought tolerance, cold stress and salinity. In response to rehydration, there is high expression of *PPR* genes in

### Table 5. Significantly expressed DEGs involved in resurrection phenomenon of *S. tamariscina*.

| Family | Gene ID | Gene name | Description | p-value | Log2FC |
|--------|---------|-----------|-------------|---------|---------|
| ELIP   | Unigene049319 | ELIP3_2 | LHC-related protein | 0.0013 | −5.04 |
|        | Unigene049320 | ELIP3_2 | LHC-related protein | 0.0003 | −5.62 |
|        | Unigene049321 | ELIP | High molecular mass early light-inducible protein HV58, chloroplastic | 0.0009 | −5.39 |
| PPR    | Unigene021227 | PPR49_ARATH | Pentatricopeptide repeat-containing protein | 0.0045 | 14.1 |
|        | Unigene021561 | PCMP-H79 | Pentatricopeptide repeat-containing protein | 0.0057 | 14 |
|        | Unigene032931 | EMB2745 | Pentatricopeptide repeat-containing protein | 0.0061 | 14 |
|        | Unigene047771 | ATC401 | Pentatricopeptide repeat-containing protein | 0.0038 | 14.2 |
|        | Unigene047777 | ATC401 | Pentatricopeptide repeat-containing protein | 0.0002 | 15.4 |
|        | Unigene05316 | MRL1 | Pentatricopeptide repeat-containing protein MRL1, chloroplastic | 0.0020 | 14.5 |
|        | Unigene05393 | PPR86_ARATH | Pentatricopeptide repeat-containing protein | 0.0002 | 8.43 |
|        | Unigene05394 | PP166_ARATH | Pentatricopeptide repeat-containing protein At2g20710, mitochondrial | 0.0038 | 14.2 |
|        | Unigene056623 | PPR36_ARATH | Pentatricopeptide repeat-containing protein At1g12300, mitochondrial | 0.0022 | 14.5 |
|        | Unigene060706 | PCMP-H24 | Pentatricopeptide repeat-containing protein At4g02750 | 4.19E-05 | 16.2 |
| LEA    | Unigene061603 | LEA | late embryogenesis abundant hydroxyproline-rich glycoprotein | 0.00083 | 14.9 |
|        | Unigene061616 | SNF4 | Sucrose nonfermenting 4-like protein | 0.0029 | 14.3 |
|        | Unigene061620 | SNF4 | Sucrose nonfermenting 4-like protein | 0.005 | 14.1 |
|        | Unigene061624 | SNF4 | Sucrose nonfermenting 4-like protein | 0.0094 | 13.7 |
|        | Unigene061634 | SNF4 | Sucrose nonfermenting 4-like protein | 0.0023 | 14.4 |
| TPP    | Unigene011013 | TPP4 | Probable trehalose-phosphate phosphatase 4 | 0.0012 | 7.1 |
|        | Unigene020138 | TPS1 | Alpha, alpha-trehalose-phosphate synthase | 0.0002 | 15.5 |
|        | Unigene035960 | TPSS | Alpha, alpha-trehalose-phosphate synthase | 0.0054 | 12.4 |
| ABCG   | Unigene050298 | ABCG39 | ABC transporter G family member 39 | 0.0005 | 15.2 |
|        | Unigene050301 | ABCG39 | ABC transporter G family member 39 | 0.0001 | 16.1 |
|        | Unigene050303 | ABCG39 | ABC transporter G family member 39 | 0.0001 | 16 |
|        | Unigene050312 | ABCG39 | ABC transporter G family member 39 | 0.0013 | 14.7 |
|        | Unigene050319 | ABCG39 | ABC transporter G family member 39 | 0.000 | 17.8 |
|        | Unigene050328 | ABCG39 | ABC transporter G family member 39 | 0.000 | 16.4 |
|        | Unigene050331 | ABCG39 | ABC transporter G family member 39 | 0.0048 | 5.35 |
|        | Unigene050339 | ABCG39 | ABC transporter G family member 39 | 0.001 | 14.9 |

Figure 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. a) Trehalose biosynthesis in starch and sucrose metabolism pathway. This pathway consists of the KEGG enzyme number information of trehalose. The Black highlighted boxes represent trehalose phosphate synthetase (TPS1) and the grey box indicates trehalose phosphate phosphatase (TPP4). These two genes (2.4.1.15 and 3.1.3.12) are responsible for the formation of trehalose necessary for desiccation tolerance. b) Purine metabolism pathway and c) thiamine metabolism pathway involving ABC-G genes that interacts with different enzymes information of phosphate. Phosphate promotes rapid recovery of plant physiological functions when re-watering after drought stress. The number in the boxes are KEGG enzyme and black highlighted boxes represent the ABC-G gene.
S. tamariscina, in which plants are involved in chloroplast development. In Arabidopsis, up regulation of PPR gene negatively regulates NADH dehydrogenase activity and enhance defense mechanism under abiotic stress found up regulation of PPR protein SOAR1 enhances ABA sensitivity, and overexpression of this gene strongly increases the drought tolerance ability of Arabidopsis. In this study, we identified a high number of up regulated DEGs under rehydration that belong to the PPR gene family (Table 5). This indicates that PPR genes might play a potential role in rehydrating plants from dehydration through maintenance and development of chloroplast.

In plants, LEA proteins are well-known ion scavengers which function in reducing oxidative damage generated by abiotic stress in soybeans. Significant up regulation of LEA genes protects the plants during drought stress. Overexpression of transgenic rice and wheat LEA genes are regulated by ABA which resulted drought tolerance. Similarly, overexpression of the Oryza sativa LEA gene improved drought resistance with high yield in field conditions. In our study, the LEA gene family are up regulated in rehydration, which shows its important role in the protection and regeneration of plants after dehydration. Sucrose non-fermenting (SNF) proteins have been widely studied in several plants and play an important role in physiological resistance. In Arabidopsis, SNF4 regulates ROS in pollen and helps pollen hydration. Overexpression of SNF-related kinase 2 in transgenic tobacco improved drought stress and increased the survival rate through an improved antioxidant system. The expression of SNF genes is increased during hydration, which might help in the regeneration of carbohydrate metabolism and starch biosynthesis in plants. We found similar result in our study, unigenes encoding SNF proteins are upregulated during rehydration.

Trehalose is a disaccharide sugar consisting of two glucose molecules that functions in sugar transport during dehydration. Trehalose is known to exert a strengthening effect on biological structures by forming a glass-like structure after dehydration. The trehalose synthase complex is involved in the formation of trehalose from the substrate UDP-glucose. TPS encodes the enzyme trehalose-6-phosphate synthase, which catalyzes the conversion of UDP-glucose to trehalose-6-phosphate, and TPP encodes the enzyme trehalose-6-phosphate phosphatase, which catalyzes the conversion of trehalose-6-phosphate to trehalose (Figure 5a). During dehydration, TPS1 and TPP4 play significant roles in stabilizing proteins in plants, which helps during dehydration. The mutant of Arabidopsis lacking the TPP gene resulted in a drought-sensitive phenotype, and overexpression of the same gene increased the drought tolerance. demonstrated that TPS1 mRNA was constitutively expressed in Selaginella lepidophylla, which is known as a resurrection plant. When S. lepidophylla TPS1 and SITPS1 were introduced to yeast, the transformed yeast showed tolerance at high temperatures. In Arabidopsis, reported that overexpression of AtTPS1 displayed dehydration tolerance; on this basis, they posited that trehalose-6-P synthase involving AtTPS1 plays a pivotal role in the regulation of glucose and ABA signaling during vegetative development. In our results, we found that TPP and TPS coding unigenes are upregulated in the fully rehydration condition.

ABC transporter are one of the largest and oldest protein families in prokaryotes and eukaryotes. The G sub-family of ABC transporters is the largest known family in the context of protein structure. In pathway analysis, ABCG genes are found in purine metabolism and thiamine metabolism, which converts thiamine diphasosphate to thiamine phosphate (Figure 5 b, c). Thiamine metabolism was modulated under the condition of abiotic stress in Zea mays seedlings. ABCG genes are essential for vascular development in A. thaliana. Overexpression of ABCG25 gene in A. thaliana reduced the rate of water loss, indicating that AtABCG25 facilitates ABA in guard cell-enhancing stomata closure. The mutant of abcg40 in Arabidopsis reduced the role of ABA, and plants were found to be more susceptible to drought stress. In the present study, the ABCG gene showed up regulation during rehydration, indicating that ABCG might play an important role in the deregulation of stomata opening during resurrection.

**Conclusion**

Using the Illumina platform, we analyzed the gene expression of dehydrated and rehydrated lyochyll of S. tamariscina. Comparative gene expression identified 1901 DEGs involved in resurrection. More number of DEGs were upregulated in rehydration compared to dehydration. The selected genes are mostly involved in ABA hormone signaling and play important roles in drought tolerance – especially in chloroplast protection, reducing oxidative damage, accumulation of sucrose and trehalose, and vascular development in plants under the acquired environmental period (dehydration and rehydration). The up regulation of these genes relates to increased tolerance to desiccation. In this study, we provide the most promising resurrection genes and their functions that could be improved biotechnologically to obtain drought-tolerant plants.

**Disclosure statement**

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**Authors’ contributions**

N.-SK and I.-YC designed the project and wrote the manuscript. EK, J.-HK and KH prepared the samples and analyzed the data. K.-CP, PB and TU analyzed the data and performed the wet experiment.

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