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ORIGINAL RESEARCH PAPER

COMPUTATIONAL ANALYSIS AND EXPRESSION STUDY OF TREHALOSE 6-PHOSPHATE SYNTHASE (TPS) IN RICE (*Oryza Sativa*)

Abbas Saidi*, Zohreh Hajibarat

Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

*Corresponding author: abbas.saidi@gmail.com

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Abstract: Trehalose-6-phosphate synthase is a key enzyme in trehalose biosynthesis. Trehalose has many applications in plant growth and development and in response to abiotic stresses. In this study, analysis of 11 sequences of *OsTPS* genes downloaded from NCBI was performed in *Oryza sativa*. The analysis of bioinformatics performed included co-expression network, gene expression, chromosomal map, analysis of cis element and phylogenetic relationships for the *TPS* gene in rice. Phylogenetic analysis showed that *TPS* genes were grouped into two classes. Class I and II included *OsTPS1* and *OsTPS2-11* genes, respectively. The gene network analysis showed that OsTPS formed the gene networks and are mostly active in nucleus and chloroplast. According to phylogeny tree, gene structure, and analysis of cis-elements, *OsTPS1* gene was different from other *TPS* genes. Also, according to co-expressed network, OsTPS1 showed to be the central protein encoding protein trehalose-6-phosphate synthase. OsTPS1 has been suggested to play a key role in flowering initiation in rice. It has also been shown to have an important role in response to osmotic and abiotic stresses (low temperature, salinity and anoxia). Our findings can provide a better understanding of molecular mechanisms of *OsTPS* genes and their expression in response to various stresses.

Keywords: *Cis-element, Co-expression gene network, expression study, TPS*

INTRODUCTION

Rice is one of the most important crops in the world. Abiotic stresses have direct or indirect effects on the physiological state of living organisms by altering metabolism, growth and development of plants. Plants respond to drought, salinity and low temperature stresses by accumulation of Trehalose and other compatible solvents [1, 2]. Trehalose plays an important role in metabolic regulation and abiotic stress tolerance in a variety of organisms. Trehalose is considered as a storage carbohydrate and is formed in two stages in various organisms. Trehalose protects against a variety of stresses in most organisms such as bacteria, fungi, plants and insects [3]. Initially, trehalose-6-phosphate synthase (TPS) is catalyzed from the synthesis of trehalose-6- phosphate (T6P) from UDP-glucose and glucose-6-phosphate. Finally, T6P is dephosphorylated to trehalose by trehalose-6-phosphate phosphatase (TPP) [4]. TPS plays a key role in plant response to environmental stresses. It has been shown that T6P, synthesized by TPS1, predicted to be mainly localized in the cytoplasm, promotes AGPase activation in chloroplasts via a thioredoxin-mediated redox reaction [5]. Based on studies carried out on rice it has been shown that *TPS* genes are expressed in response to abiotic stress and low temperatures [6]. The results of reports showed that expression of TPS genes in transgenic plants causes increased tolerance to abiotic stress [7]. Considering the importance of rice as a major crop, creating new cultivars with abiotic stress tolerance would undoubtedly have an enormous impact on global food production. According to some reports, it was shown that biosynthesis pathway of *TPS* genes has been identified in plants such as angiosperms, arabidopsis [8], rice [9], potatoes [10] and cotton [11].
The genes encoding this enzyme have been reported in *Arabidopsis* and rice genome of 11 *TPS* genes and 12 *TPS* Poplar gene. In most studies, these genes are classified into classes I and II [12, 13]. *TPS* gene family is a large gene family with multiple copies and functional diversifications [14]. Class I and II TPS genes revealed distinct characteristics in copy number, gene expression patterns, and physiological and enzymatic functions.
It has been reported that *OsTPS1* gene could affect rice-seed tolerance to low temperature, salt, and drought [4]. Based on research results, *OsTPS1* gene has a TPS enzyme activity due to containing some conserved residues involved in the binding site [15]. Class II contains *OsTPS2-11* genes which are involved in regulatory proteins without a clear enzymatic activity [16]. There is little information about upstream regulators or regulatory network of these genes and their roles in stress tolerance. Therefore, TPS gene expression is an important step in understanding the molecular mechanisms of plant growth and development, and environmental stresses. Transcription regulation involves associations among transcription factors and cis-elements of genes involved in plant biotic and abiotic response [17].
Promoters have an important function in controlling gene expression. Cis- elements are gene regulatory units as they control various stress responses. Though they are expensive and technically challenging, modern techniques such as RNA interference, microarray techniques and other tools have allowed identification and investigation of promoter regions of target genes. For example, *in silico* analysis was used to search promoter regions of various cis-elements responsible for the regulation of genes [18].
In the present paper, an *in silico* study was performed for the phylogenetic analysis, chromosome organization and analysis of cis-elements involved in the *TPS* genes. In

addition, co-expressed genes involved in stress response were surveyed. Finally, expression of *TPS* genes under respective gene promoters is interpreted.

MATERIAL AND METHODS

Characteristics, phylogenetic tree and gene structure of *TPS* gene family in rice

The 11 gene sequences of rice were downloaded from GenBank [19]. Alignment of the sequences of the *TPS* genes was performed using CLUSTALW program in MEGA software version 7, produced by Kumar, Stecher, and Tamura (2002) at Research Center for Genomics and Bioinformatics in Japan [20]. The confidence level of grouping accuracy was estimated with a Bootstrap 1000 method [20]. Gene structures of *TPS* genes were analyzed on the Gene Structure Display Server 2.0 GSDS [21]. Protein properties of *TPS* genes, e.g., the molecular weight (MW), isoelectric point (*p*I), and grand average of hydropathicity (GRAVY) were calculated using ProtParam [22]. The CELLO database was used to identify the cellular status of proteins [23].

Mapping TPS gene on rice chromosomes

To illustrate the location of the *TPS* genes on rice chromosomes, MapChart 2.3 software, produced by Voorrips (2002) in Plant Research International (Wageningen, The Netherlands) was used [24]. A set of 11 TPS genes were mapped onto rice chromosomes [12].

Expression study of *TPS* genes

Genevestigator [25] was utilized to analyze the differential gene expression under environmental stresses and different developmental stages in rice. *TPS* gene expression data was downloaded from *O. sativa* database using Affymatrix Rice Genome Array platform (OS_AFFY_RICE-0) and 'Perturbations' tool. The fold change in gene expression was detected using filter 3 folds as benchmark. The fold changes in the expression of *TPS* genes under environmental stresse conditions were used to draw heatmap using Genevestigator with Red/Green color scheme as markers where "Red" and "Green" colors represent up and down-regulation of respective genes.

Co-expressed genes network and analysis of cis elements

Co-expression of the Pearson correlation coefficients of *TPS* rice genes was obtained from the BAR Database. The interactions among all the 11 genes were determined using the "The rice Interactions Viewer" web based on the publicly available Botany Array Resource (BAR) expression browser tool (http://bar.utoronto.ca/welcome.htm) [26]. PLACE [27] was used to analyze cis-elements and to analyze OsTPS promoter sequences.

RESULTS AND DISCUSSION

Phylogenetic tree, protein properties and gene structure of TPS gene family in rice

In this study we surveyed 11 *TPS* genes using different bioinformatics tools. Protein properties of *TPS* genes, molecular weight (MW), isoelectric point (*p*I), and grand average of hydropathicity (GRAVY) are provided in table 1. Phylogeny analysis revealed that the 11 rice *TPS* genes were divided into two groups. Our results showed that class I and class II included *OsTPS1* and *OsTPS2-11* genes, respectively (Figure 1). The analysis of rice *TPS* genes showed that OsTPS1 belong to class I [4]. Among class I TPS plant proteins, OsTPS1 proved to be involved in plant active TPS enzymes [1 – 9]. OsTPS1 has some conserved residues related to the TPS domain having a key role in substrate binding and catalysis [15]. A previous study has shown that *TPS* genes were grouped into two subfamilies I and II [4].

Table1. *Characteristics of TPS gene family in rice*

No	Locus (MSU)	Accession no.	Number of amino acids	Molecular weight (Kd)	Theoretical point (*p*I)	Subcellular localization
osTPS1	LOC_Os05g44210	HM050424	985	109	6.22	Cytoplasmic
osTPS2	LOC_Os01g54560	HM050425	914	102.4	5.38	Cytoplasmic
osTPS3	LOC_Os01g53000	HM050426	878	99.4	5.54	Cytoplasmic
osTPS4	LOC_Os03g12360	HM050427	860	96.7	5.67	Cytoplasmic
osTPS5	LOC_Os02g54820	HM050428	750	83.4	6.84	Cytoplasmic
osTPS6	LOC_Os05g44100	HM050434	899	100.6	5.53	Cytoplasmic
osTPS7	LOC_Os08g31980	HM050429	862	97.6	6.11	Cytoplasmic
osTPS8	LOC_Os08g34580	HM050430	824	91.4	5.71	Cytoplasmic
osTPS9	LOC_Os09g25890	HM050431	886	96.2	7.37	Cytoplasmic
osTPS10	LOC_Os09g23350	HM050432	885	99.4	5.83	Cytoplasmic
osTPS11	LOC_Os09g20990	HM050433	863	97.8	5.93	Cytoplasmic

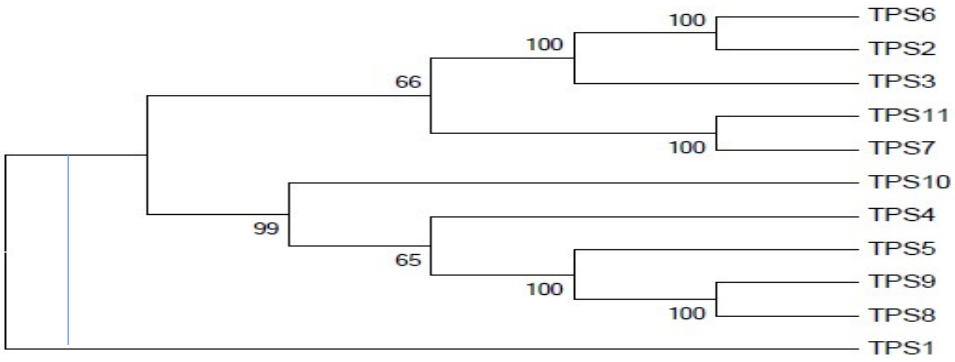

Figure 1. *Phylogeny tree of 11 rice TPS genes. The unrooted tree was drawn using the MEGA v7.0 software with the N-J method*

Class II TPSs from Arabidopsis were categorized as unknown though evidence suggests that they play an important role in controlling cellular morphology and plant growth. Classification of TPS in our results agreed with other research reports [4, 20].

Apparently, OsTPS genes can also be grouped into two subfamilies, as are AtTPS1–11 [28]. Only *OsTPS1* gene belongs to class I, whereas Arabidopsis contains 11 genes, four genes (AtTPS1–4) and seven genes (AtTPS5–11), which were placed in class II [11]. The protein sequence length varied from 750 (osTPS5) to 985 (osTPS1) amino acids and the molecular weight of these proteins varied from 83.4 (osTPS5) to 109 (osTPS1) kDa. The isoelectric point of proteins ranged from 5.38 (osTPS2) to 6.22 (osTPS1). Location and function of all genes were observed in cytoplasm using CELLO database (Table 1). It has been reported that most of TPSs were located in cytoplasm [4].

Mapping rice chromosomes and gene structure of OsTPS genes

The distribution of 11 rice genes on rice chromosomes is shown in Figure 2. There are three genes on both chromosomes 8 and 9, whereas only one TPS gene is located on chromosomes 2, 3, and 5. Two TPS genes were found on chromosome 1. All chromosomes contain TPS encoding genes except for chromosomes 4, 6, 7, 10, 11, and 12. No gene cluster was observed in rice chromosomes (Figure 2). Evolutionary relationships of 11 TPS rice genes were drawn using GSDS database. *OsTPS1* gene structure is different and more complicated than the other *OsTPS* genes with longer introns (Figure 3). OsTPS1 probably has a very long N-terminal extension because of enzymatic activity in comparison with other OsTPS proteins [4].

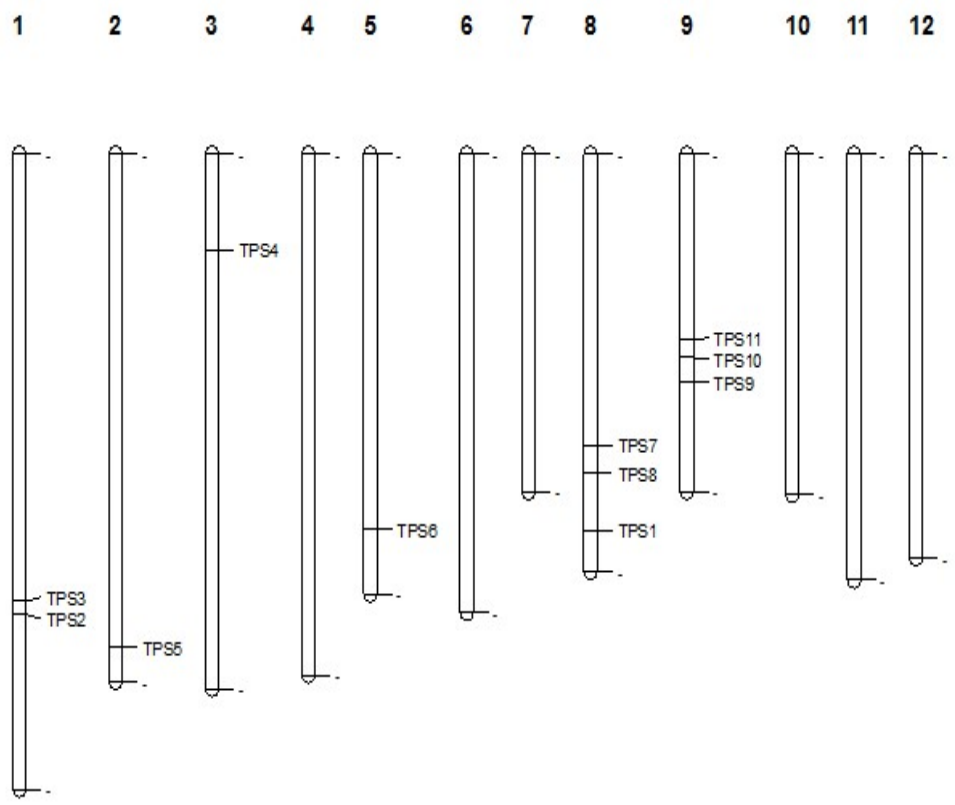

Figure 2. *Chromosomal dispensation of rice TPS genes by Mapchart software*

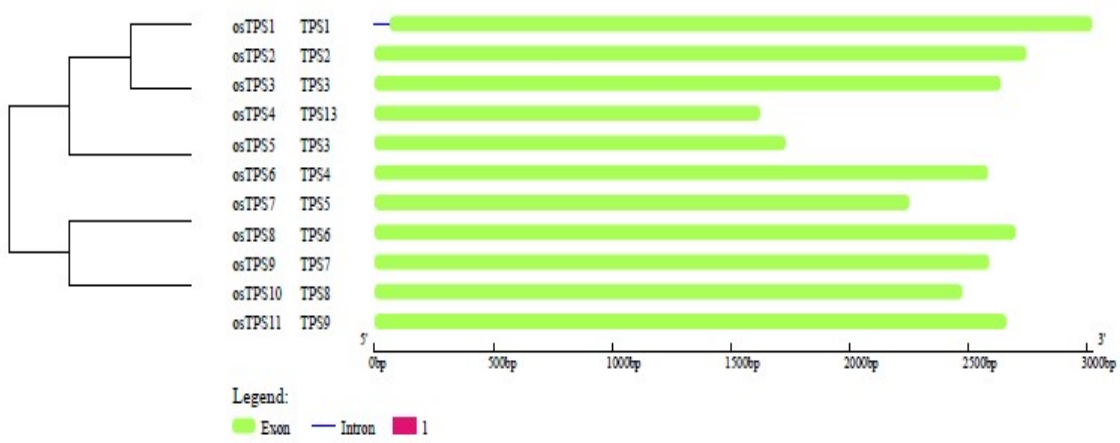

Figure 3. *The exon-intronic structure of rice TPS genes according to their phylogenetic relationship. Green and blue colors represent gene exon and intron, respectively*

Identification of co-expressed genes in the network

The interactive networks analysis of all the significant genes (in total 13) are given in Figure 3. In the network, LOC_Os05g44210 (Os05g44210) seemed to be the central protein encoding "protein trehalose-6-phosphate synthase, putative (OsTPS1)". In network analysis, proteins are located in the chloroplast (green), nucleus (blue), cytoplasm (pink) and vacuole (yellow). Most of the proteins in this network seemed to be localized mainly in nucleus (blue), cytoplasm (pink), and chloroplast (green). They encode mainly for the heat shock proteins such as LOC_Os05g44340 (heat shock protein 101), and LOC_Os08g39140 (heat shock protein). Main proteins localized in the chloroplast included LOC_Os06g30320 (NOC3 - Putative nucleolar complex subunit 3, expressed), LOC_Os07g37650 (GTPase-activating protein, putative, expressed), LOC_Os12g01770 (protein phosphatase 2c, putative, expressed), LOC_Os10g11140 (phosphoglucomutase, putative), and LOC_Os07g37650 (protein GTPase-activating protein, putative).

Proteins expressed in the nucleus include LOC_Os07g10350 (protein S1 RNA binding domain containing protein), LOC_Os07g23740 (protein sterol 3-beta-lucosyltransferase, putative), and LOC_Os12g22900 (protein transposon, putative). The protein located in the cytoplasm included LOC_Os08g39140 (heat shock protein, putative). Protein expressed in the cell membrane included LOC_Os10g35550 (protein pre-mRNA-processing factor 6, putative). In summary, *OsTPS1* gene was involved in the gene network and can interact with themselves in biological processes (Figure 4).

Analysis of gene network is represented by straight lines showing the genes involved in biological processes and relationships among genes. Proteins are essential components of biological processes and co-expression of genes are required for their biological functions. Co-expression network shows that genes are involved in all transcriptional processes such as protein degradation and cell cycle regulation. *OsTPS* genes play a key role in response to environmental conditions (i.e. abiotic stresses) [4]. The identification of the co-expression network is valuable for understanding the cellular and biological processes.

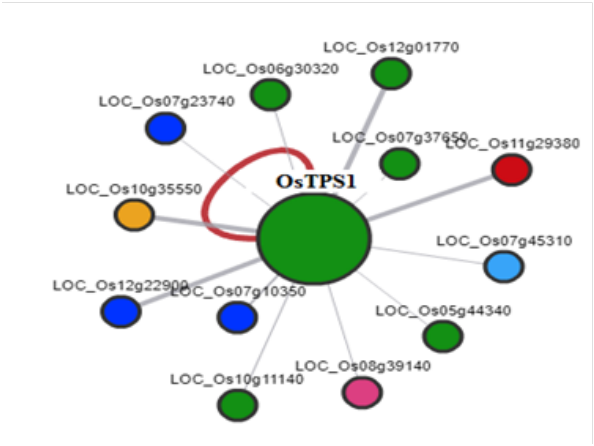

Figure 4. *Co-expressed network of all 11 TPS genes in rice genotypes. The web based tool "Rice Interactions Viewer" [29] was used to predict the interactions*

In silico analysis of TPS genes expression in response to environmental stresses

Expression of *TPS* genes was downloaded by Genevestigator software using rice microarray dataset in response to environmental stresses in rice. Based on our results, a positive correlation between *TPS* promoter regions of genes and presence of TFs was obtained. Similarly, the expression of *OsTPS6* and *OsTPS7* genes depicted high level of similarity upon exposure to several abiotic (cold, dehydration, anoxia and drought) and biotic stresses (*X. oryzea*), and imbibition (Figure 5). Additionally, the gene expression data showed that the gene expression of *OsTPS* gene was up-regulated in response to environmental stresses (i.e., *X. oryzea* and heat).

Overall, *OsTPS11* gene was down-regulated in response to abiotic stresses (drought, heat, and salt) however, it was up-regulated in response to cold. According to gene expression study, *OsTPS1* gene was found different from other *OsTPS* genes. This gene was up-regulated in response to imbibition and anoxia stress. Then, it can be concluded that *OsTPS1* was up-regulated because of high number of EBOX transcription factors in its promoter region. EBOX plays a key role in abiotic stresses like anoxia. It has been reported that loss-of-function mutations in tps1 stimulated anoxia sensibility in some organisms [30].

Overall, *OsTPS7* gene was up-regulated in response to abiotic (cold, heat, drought and dehydration) and biotic (*X. oryzea*) stresses which may be due to the presence of cis-elements in upstream of *OsTPS7* gene (Figure 5). The GCCCORE (GCC box) reported in the promoters of several PR genes, is associated with JA dependent defense responses interacting with Ethylene response factors (ERF). Also, *OsTPS7* gene contains a high copy number of CGCGBOXAT cis-element involved in up-regulation of this gene in response to abiotic and biotic stresses [31]. Our findings agreed with the report by [17] in which GCCCORE element emerged as key regulator of various hormones and stress responses which lead to improved plant survival during stress conditions.

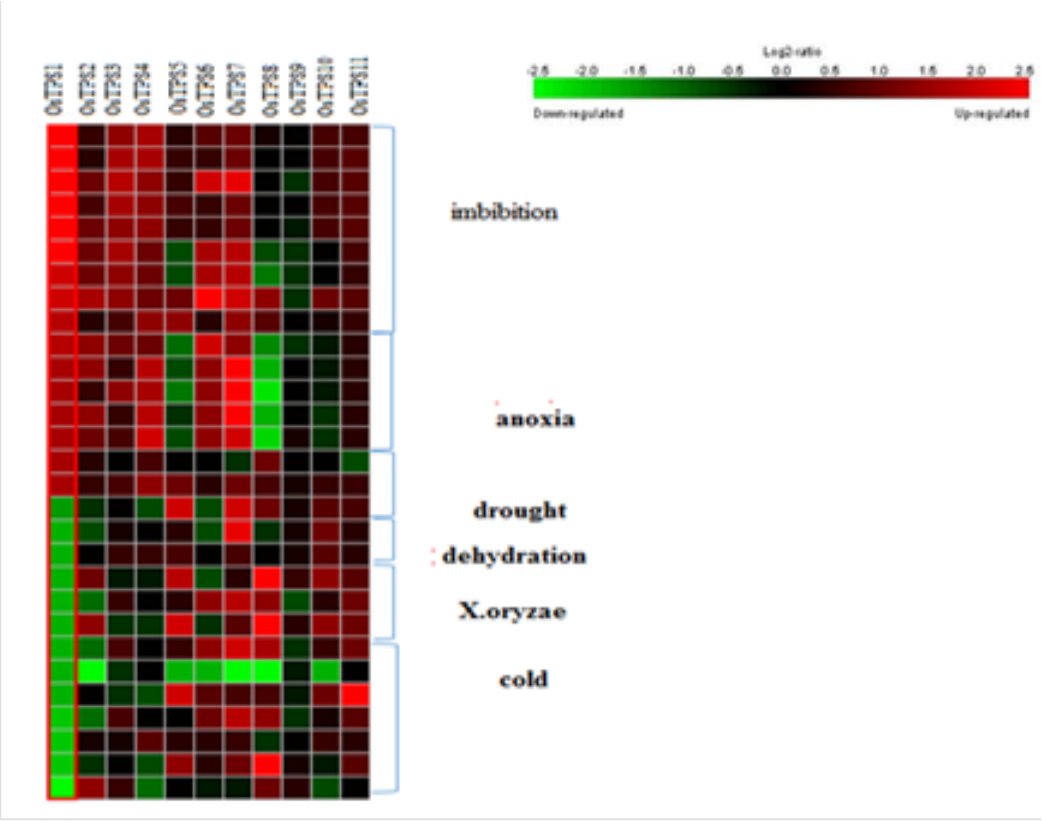

Figure 5. *Heatmap analysis of TPS genes in response to environmental stresses. The microarray data for expression of 11 TPS genes under various abiotic and biotic stress conditions*

Microarray data indicated an up regulation of all expressed *OsTPS* genes during different developmental stages which may be due to the presence of different cis-elements in the promoter regions (Figure 6). As a result, these genes can play key roles in different growth and developmental stages under environmental stresses. *OsTPS1* and *OsTPS7* genes were up-regulated during flowering stage indicating that these genes can play an important role in multiple biological processes, including flower initiation and seed yield [4]. Previous studies have shown that OsTPS1 overexpression in plants will cause delayed flowering. This finding does not agree with our findings [32, 33]. Similarly, OsTPS5 and OsTPS8 were up-regulated in dough stage and *OsTPS3, OsTPS8,* and *OsTPS9* genes were up-regulated during milk stage.

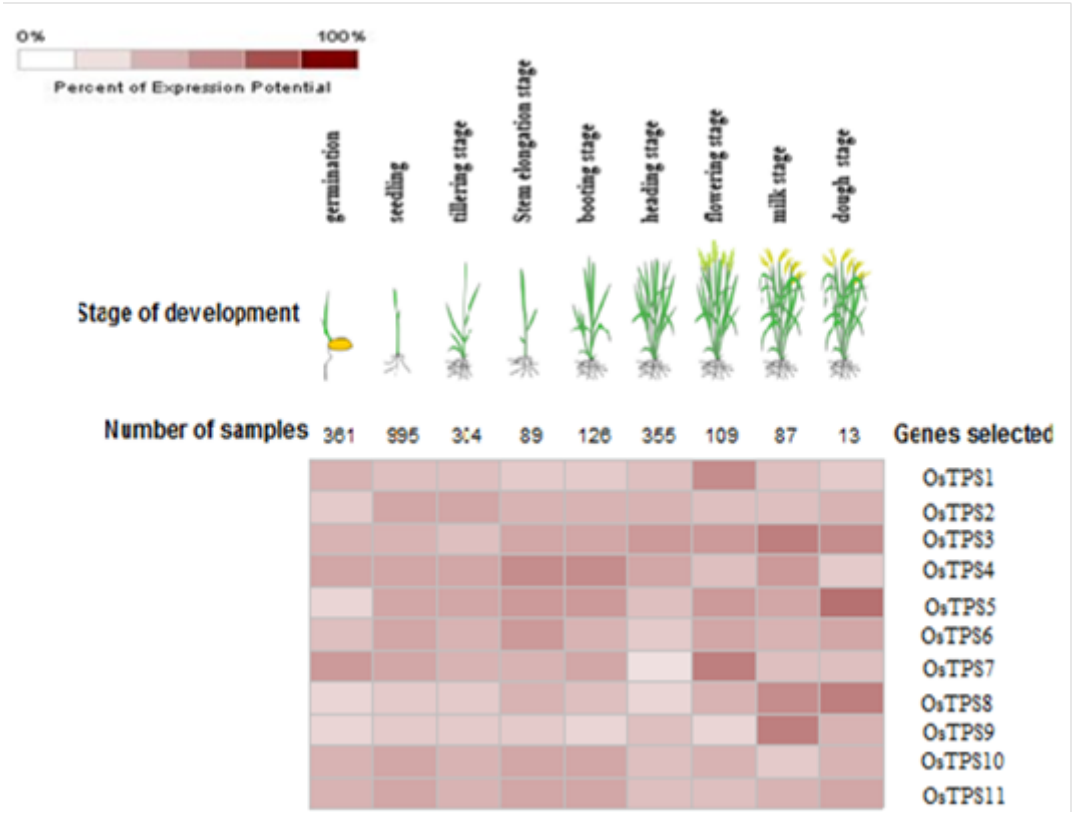

Figure 6. *Heatmap representation of expression analysis of OsTPS genes at different developmental stages of O. sativa*

Analysis of cis elements

To analyze the cis- elements in *OsTPS* genes, 1000 bp upstream region of the eleven genes were extracted from the rice genome and analyzed using PLACE server (Figure 6). Regulatory elements predicted in OsTPS promoters were associated with phyto-hormones, abiotic stress and developmental processes. Cis- elements were distributed in the promoter regions of the *OsTPS* genes (Figure 7). The presence of hormone-responsive elements (ABA, GA, and ET) could be interpreted as an indication that *TPS* genes might be involved in various phytohormones signaling pathway. Among the CREs, CGCGBOXAT was the most abundant element in the promoter region of each gene. This motif was involved in Ca2+/calmodulin regulation. Our results confirmed previous reports that TPS expression levels increased under drought and other abiotic stresses (ex., salt and temperature) in various plants [34 – 36].

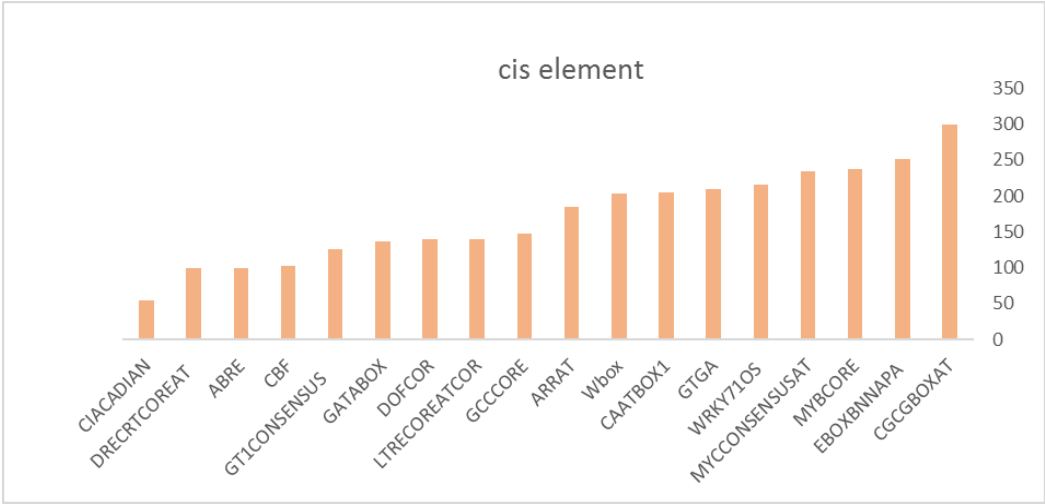

Figure 7*. Cis regulatory elements detected in the promoter region of the genes and their frequencies, the 1000 bp upper region of each gene were searched using the PLACE database*

Our results showed that GCCCORE and GT1CONSENSUS elements were responsive to ethylene signaling and SA hormone, respectively. Genes containing this cis element can be regulated in response to light and salicylic acid [36, 37]. WRKY71OS, a binding element for WRKY71, encodes a transcriptional repressor of gibberillic acid signaling and can be activated by cold, salt and dehydration stress in Musa spp. [31, 38].
Three different dehydration-responsive (DRE) elements including CBFHV, DRE, and DRECRTCOREAT motifs were identified in our study. DREs are important motifs regulated by dehydration, salinity and heat in an abscisic acid (ABA) environment. These elements can improve different plant species (including model and crop plants) in response to multiple stress tolerances [39]. LTRE element (LTRECOREATCOR15) was identified in the promoter sequences of OsTPS. A report has shown that LTRE element is a vital motif regulated in response to cold stress in *OsTPS* genes [40]. MYBCORE and MYCCONSENSUSAT were found in *OsTPS* genes under diverse environmental stresses including biotic and abiotic stresses. MYBCORE and MYCCONSENSUSAT motifs were identified in ABA and drought-responsive genes [41 – 43]. Most of cis regulatory elements predicted in OsTPS promoters are regulated under phytohormone stresses (ABA, gibberellin, ethylene, cytokinin) and abiotic stresses. In particular, OsTPS1 contained the largest number of hormone and abiotic-responsive elements, suggesting an important role in response to phytohormone and abiotic stresses.

CONCLUSIONS

In summary, we surveyed 11 *OsTPS* genes from rice and identified their conserved protein motifs, gene structure, chromosomal distribution and cis-elements in promoter regions. According to phylogeny tree, gene structure and analysis of cis-elements, *OsTPS1* gene was found to be different from other *TPS* genes. OsTPS1 protein has several conserved residues that make it possible to have enzymatic activity. The present study revealed that *OsTPS* promoter contains cis-elements involved in various abiotic

stresses (dehydration, cold) and hormonal (ethylene, ABA, GA, cytokinin and SA) stress regulated in the promoter sequences. Based on heatmap analysis, *OsTPS1* was up-regulated during imbibition and anoxia. It is suggested that this gene can play an important role in response to osmotic and abiotic stresses (low temperature, salinity and anoxia). Also, *OsTPS1* gene plays a key role in flowering initiation in rice.

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REFERENCES

1. Hare, P., Cress, W.A, Van Staden J.: Dissecting the roles of osmolyte accumulation during stress, *Plant, Cell & Environment*, **1998**, **21**, 535- 553;
2. Wiemken, A.: Trehalose in yeast, stress protectant rather than reserve carbohydrate, *Antonie Leeuwenhoek*, **1990**, **58**, 209-217;
3. Goddijn, O.J., van Dun, K.: Trehalose metabolism in plants, *Trends in Plant Science*, **1999**, **4**, 315-319;
4. Zang, B., Li, H., Li, W., Deng, X.W., Wang, X.: Analysis of trehalose-6-phosphate synthase (*TPS*) gene family suggests the formation of TPS complexes in rice, *Plant molecular biology*, **2011**, **76**, 507-522;
5. Kolbe, A., Tiessen, A., Schluepmann, H., Paul, M., Ulrich, S., Geigenberger, P.: Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase, *Proceedings of the National Academy of Sciences*, **2005**, **102**, 11118-11123;
6. Jin, Q., Hu, X., Li, X., Wang, B., Wang, Y., Jiang, H., Mattson, N., Xu, Y.: Genome-wide identification and evolution analysis of trehalose-6-phosphate synthase gene family in Nelumbo nucifera, *Frontiers in plant science*, **2016**, **7**, 1445-1447;
7. Ge, L.F., Chao, D.Y., Shi, M., Zhu, M.Z., Gao, J.P., Lin, H. X.: Overexpression of the trehalose-6-phosphate phosphatase gene OsTPP1 confers stress tolerance in rice and results in the activation of stress responsive genes, *Planta*, **2008**, **228**, 191-201;
8. Vogel, G., Fiehn, O., Jean-Richard-dit-Bressel, L., Boller, T., Wiemken, A., Aeschbacher, R.A., Wingler, A.: Trehalose metabolism in Arabidopsis: occurrence of trehalose and molecular cloning and characterization of trehalose - 6-phosphate synthase homologues, *Journal of experimental botany*, **2001**, **52**, 1817-1826;
9. Liao, Y., Liu, S., Jiang, Y., Hu, C., Zhang, X., Cao, X., Xu, Z., Gao, X., Li, L., Zhu, J., Chen, R.: Genome-wide analysis and environmental response profiling of dirigent family genes in rice (*Oryza sativa*), *Genes & Genomics*, **2017**, **39**, 47-62;
10. Goddijn, O., Smeekens, S.: Sensing trehalose biosynthesis in plants, *The Plant Journal*, **1998**, **14**, 143-146;
11. Kosmas, S.A., Argyrokastritis, A., Loukas, M.G., Eliopoulos, E., Tsakas, S., Kaltsikes, P.J.: Isolation and characterization of drought-related trehalose 6-phosphate-synthase gene from cultivated cotton (*Gossypium hirsutum* L.), *Planta*, **2006**, **223**, 329-339;
12. Vandesteene, L., Ramon, M., Le Roy, K., Van Dijck, P., Rolland, F.: A single active trehalose-6-P synthase (TPS) and a family of putative regulatory TPS-like proteins in Arabidopsis, *Molecular plant*, **2010**, **3**, 406-419;
13. Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T.: Cytoscape: a software environment for integrated models of biomolecular interaction networks, *Genome research*, **2003**, **13**, 2498-2504;
14. Ramon, M., De Smet, I.V.E., Vandesteene, L., Naudts, M., Leyman, B., Van Dijck, P., Rolland, F., Beeckman, T.O.M., Thevelein, J.M.: Extensive expression regulation and lack of heterologous

enzymatic activity of the Class II trehalose metabolism proteins from *Arabidopsis thaliana*, *Plant, cell & environment*, **2009**, **32**, 1015-1032;
15. Gibson, R.P., Turkenburg, J.P., Charnock, S.J., Lloyd, R., Davies, G.J.: Insights into trehalose synthesis provided by the structure of the retaining glucosyltransferase OtsA, *Chemistry & biology*, **2002**, **9**, 1337-1346;
16. Blazquez, M.A., Santos, E., Flores, C.L., Martínez−Zapater, J.M., Salinas, J., Gancedo, C.: Isolation and molecular characterization of the Arabidopsis TPS1 gene, encoding trehalose-6-phosphate synthase, *The Plant Journal*, **1998**, **13**, 685-689;
17. Kaur, G., Pati, P.K.: Analysis of cis-acting regulatory elements of Respiratory burst oxidase homolog (Rboh) gene families in Arabidopsis and rice provides clues for their diverse functions, *Computational biology and chemistry*, **2016**, **62**, 104-118;
18. Wittkopp, P.J., Kalay, G.: Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence, *Nature Reviews Genetics*, **2012**, **13**, 59-60;
19. Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. and Sayers, E.W.: GenBank, *Nucleic acids research*, **2012**, **41**, D36-D42;
20. Kumar, S., Stecher, G., Tamura, K.: MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, *Molecular biology and evolution*, **2016**, **33**, 1870-1874;
21. Hu, B., Jin, J., Guo, A.Y., Zhang, H., Luo, J. Gao, G.: GSDS 2.0: an upgraded gene feature visualization server, *Bioinformatics*, **2014**, **31**, 1296-1297;
22. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M.R., Appel, R.D., Bairoch, A.: Protein identification and analysis tools on the ExPASy server, in *The proteomics protocols handbook* (editor: Walker, J.M.), Humana Press Inc., Totowa, **2005**, 571-607;
23. Chary, S.N., Hicks, G.R., Choi, Y.G., Carter, D., Raikhel, N.V.: Trehalose-6-phosphate synthase/phosphatase regulates cell shape and plant architecture in Arabidopsis, *Plant Physiology*, **2008**, **146**, 97-107;
24. Voorrips, R.E.: MapChart: software for the graphical presentation of linkage maps and QTLs, *Journal of Heredity,* **2002**, **93**, 77-78;
25. Grennan, A.K.: Genevestigator. Facilitating web-based gene-expression analysis, *Plant physiology*, **2006**, **141**, 1164-1166;
26. Taniguchi, M., Sasaki, N., Tsuge, T., Aoyama, T. and Oka, A.: ARR1 directly activates cytokinin response genes that encode proteins with diverse regulatory functions, *Plant and Cell Physiology*, **2007**, **48**, 263-277;
27. Higo, K., Ugawa, Y., Iwamoto, M. Higo, H.: PLACE: a database of plant cis-acting regulatory DNA elements. *Nucleic acids research*, **1998**, **26**,358-359;
28. Leyman, B., Van Dijck, P., Thevelein, J.M.: An unexpected plethora of trehalose biosynthesis genes in *Arabidopsis thaliana*, *Trends in plant sci*ence, **2001**, **6**, 510-513;
29. Ho, C.L., Wu, Y., Shen, H.B., Provart, N.J. and Geisler, M.: A predicted protein interactome for rice, *Rice*, **2012**, **5**, 1-14;
http://bar.utoronto.ca/interactions/cgi-bin/rice_interactions_viewer.cgi;
30. Chen, Q., Ma, E., Behar, K.L., Xu, T., Haddad, G.G.: Role of trehalose phosphate synthase in anoxia tolerance and development in Drosophila melanogaster, *Journal of Biological Chemistry*, **2002**, **277**, 3274-3279;
31. Saidi, A., Hajibarat, Z.: Characterization of cis-elements in hormonal stress-responsive genes in *Oryza sativa*, *Asia-Pacific Journal Molecular Biology and Biotechnology*, **2019**, **27**, 95-102;
32. van Dijken, A.J., Schluepmann, H., Smeekens, S.C.: Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering, *Plant physiology*, **2004**, **135**, 969-977;
33. Gómez, L.D., Gilday, A., Feil, R., Lunn, J.E., Graham, I.A.: AtTPS1-mediated trehalose 6-phosphate synthesis is essential for embryogenic and vegetative growth and responsiveness to ABA in germinating seeds and stomatal guard cells, *The Plant Journal*, **2010**, **64**, 1-13;
34. Layne, R.E.C.: Peach rootstocks. IN/Rootstocks for Fruit Crops, RC Rom and RF Carlson, **1987**; 185-216;
35. Bielsa, B., Jiwan, D., Marti, A.F., Dhingra, A., Rubio-Cabetas, M.J.: Detection of SNP and validation of a SFP InDel (deletion) in inverted repeat region of the Prunus species chloroplast genome, *Scientia Horticulturae*, **2014**, **168**, 108-112;
36. Saidi, A., Hajibarat, Z.: *In silico* Analysis of floral MADS-BOX gene in *Brachypodium distachyon*, *Bionature*, **2018**, **36**, 366-375;

37. Gradziel, T.M.: Almond (*Prunus dulcis*) Breeding, in *Breeding Plantation Tree Crops: Temperate Species*, (Editors: Priyadarshan, P. M., Mohan Jain, S.), Springer-Verlag New York, **2009**, **104**, 1-31;
38. Zhang, Z.L., Xie, Z., Zou, X., Casaretto, J., Ho, T.H.D., Shen, Q.J.: A rice WRKY gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells, *Plant Physiology*, **2004**, **134**, 1500-1513;
39. Shekhawat, U.K.S., Ganapathi, T.R., Srinivas, L.: Cloning and characterization of a novel stress-responsive WRKY transcription factor gene (MusaWRKY71) from Musa spp. cv. Karibale Monthan (ABB group) using transformed banana cells, *Molecular biology reports*, **2011**, **38**, 4023-4035;
40. Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K.: Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought-and low-temperature-responsive gene expression, respectively, in Arabidopsis, *The Plant Cell*, **1998**, **10**, 1391-1406;
41. Dunn, M.A., White, A.J., Vural, S., Hughes, M.A.: Identification of promoter elements in a low-temperature-responsive gene (blt4. 9) from barley (*Hordeum vulgare L.*), *Plant molecular biology*, **1998**, **38**, 551-564;
42. Gotah, A., Whang, K., Garcia, C.V.: Determination of aflatoxins in rice using quechers and fluorescence HPLC*, Scientific Study & Research. Chemistry & Chemical Engineering*, *Biotechnology, Food Industry*, **2018**, **19**, 133-141;
43. Madinzi, A., Aboulhassane, M.A., Assou, M., Fakidi, H., Bahlaoui, M.A., Souabi, S., Hafidi, M.: Biological treatment of stabilized landfill leachate produced by the public discharge of the city Mohammedia (Morocco), *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2014***,* **15**, 155-167.