Comparative analysis of annotated genome of date palm and rice shows proliferation of trehalose biosynthetic genes

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ABSTRACT

Agricultural biotechnology is one of the newly emerging technologies of the 21st century for global food security and to benefit the mankind. In order to improve date palm production efficiency in the arid region, we need to deploy favorable strategies to counteract major environmental stresses, such as salinity and drought. Of the several strategies available, use of transgenic approaches and functional genomic tools, probably hold the most promise toward augmenting its production. Now, we can take the advantage of interdisciplinary research approach to confer high levels of tolerance to different abiotic stress in date palm. Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute and in the stabilization of biological structures under abiotic stress in bacteria, fungi and invertebrates. With the notable exception of the desiccation-tolerant "resurrection plants", trehalose does not accumulate to significant levels in the vast majority of plants. The recent discovery of the genes that encode trehalose metabolism enzymes in higher plants, and its potential role in modulating carbon metabolism and stress protection, offers new opportunities and challenges for researchers in this field. The specific objective of this study is to perform comparative analysis of trehalose biosynthesis related genes in date palm and rice. Our results from the data on phylogenetic analyses of protein sequences derived from the corresponding DNA sequences from the annotated genomes of a date palm and the rice (both indica and japonica type) plant species suggests the presence of gene families for both trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP), indicating the genomic complexity of trehalose biosynthetic genes in plants.

INTRODUCTION

Trehalose [α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside], a dimer of glucose, is present in diverse organisms such as bacteria, fungi, insects, and some invertebrates, and known to have various functions that distinguish it from another non-reducing sugar sucrose [α-D-glucopyranosyl-(1→4)-β-D-fructofuranoside]. There is considerable evidence for a role of trehalose in protection from desiccation, salinity, osmotic stress as well as extreme temperatures by stabilizing dehydrated enzymes, proteins, and lipid membranes efficiently, in addition to protection of biological structures from damage against a variety of environmental stresses (Crowe *et al*. 1992, Crowe 2007). At the same time, details of both the physiological functions and regulation of the trehalose biosynthetic pathway remain largely unknown. In bacteria there are five different trehalose biosynthetic routes, whereas in fungi and plants there is only one (Avonce *et al.* 2006). The single pathway for trehalose biosynthesis that is common to both prokaryotes and eukaryotes consists of two reactions. First, trehalose-6-phosphate is generated from UDP-glucose (UDP-Glu)

and glucose-6-phosphate (G6P) in a reaction catalyzed by trehalose-6-phosphate synthase (TPS, EC 2.4.1.15). T6P is then dephosphorylated to form trehalose via trehalose-6-phosphate phosphatase (TPP, EC 3.1.3.12). TPS and TPP genes were functionally identified in *Arabidopsis thaliana* by complementation of yeast mutants (Blazquez *et al*. 1998). Homologous TPS and TPP genes have now been identified in many other plant species. These results suggest that trehalose synthesis may in fact be ubiquitous among angiosperms, although the levels to which it accumulates are generally low (Goddijn and Van Dun 1999).

MATERIALS AND METHODS

The present study was carried out using the tools of bioinformatics and computational biology. NCBI/TIGR genome data bank (for rice genomic DNA sequencing data) and Weill Cornell Medical College in Qatar date palm sequencing project data was used for our phylogenetic analyses. The rice (*Oryza sativa* L.) genome sequences of the *indica* cultivar (I) 93-11 and *japonica* cultivar (J) Nipponbare and date palm (*Phoenix dactylifera L.*) cultivar Khalas were searched with the BLASTN algorithm for genes with similarity to the TPS = Trehalose-6-phosphate synthase, TPP = Trehalose-6-phosphate phosphatase, and TRE = trehalase genes of *Arabidopsis thaliana* L. ecotype Columbia. Locus names may be accessed at NCBI GenBank database.

RESULTS AND DISCUSSION

Comparative analysis of the genomic sequences of two plant species [*Oryza sativa* (both *indica* and *japonica* cultivars) and *Phoenix dactylifera*] suggests a proliferation of putative genes encoding TPS and TPP enzymes (Table 1). Altogether, there are 11 and 11 putative TPS-like proteins, and 11 and 7 putative TPP-like proteins within the respective genomes. The TPS gene family clusters into two distinct groups, the class I subfamily of TPS genes encodes catalytically active TPS enzymes, whereas the class II TPS genes encode inactive TPS-like proteins with a C-terminal TPP-like domain (Leyman *et al*. 2001, Lunn 2007). In general, the class II genes contain two phosphatase consensus sequence boxes that have been found in all class III TPP genes. In contrast, several of the class I genes from rice and date palm does not contain the phosphatase-specific part in the C-terminal region. Thus, these representative genes probably all may contain only TPS enzyme activity. Amino acid identity between the members of the class I and class II genes is approximately 30-40%. Based on the amino acid sequence similarity, less consistency in tree topology was found in class II TPS, and class III TPP gene families compared to class I TPS genes (Data not shown). The class IV trehalase gene family is much smaller, and often represented by a single gene, and most closely related to those from animals, indicating a eukaryotic origin of this gene. So far, only TPS1 gene from a few plants are known to encode an enzymatically active TPS, which is able to complement the yeast *tps1*Δ mutant and showed restoration of trehalose synthesis and growth on glucose (Blazquez *et al*. 1998, Van Dijck *et al*. 2002). In contrast, class II AtTPS7 or AtTPS8 were unable to complement the yeast *tps1*Δ mutant (Vogel *et al*. 2001), and AtTPS5 shows no TPS activity (Harthill *et al*. 2006). Thus, whether the class II proteins actually have TPS and/or TPP activities remains unresolved, and suggests that there may be fundamental differences in the properties and/or functions of the two distinct subfamilies of TPS genes (Lunn *et al*. 2006).

Moreover, the complete genome sequencing of *Arabidopsis thaliana* and *Oryza sativa* has revealed complex genomic organization of plant trehalose biosynthesis genes (Leyman *et al*. 2001, Lunn 2007, Ramon and Rolland 2007). The Class I (TPS1-4) and Class II (TPS5-11) are most similar to the *E.coli otsA*, except that the catalytic activity of TPS enzymes has not yet unequivocally demonstrated for the latter group (Table1). Class III (TPPA-TPPK) contains a family of smaller proteins similar to the *E.coli otsB* with two conserved phosphatase box. Both rice and date palm contain a single gene encoding for trehalase enzyme (Table1). Based on the comparison of the protein sequences, we found five highly conserved regions in most of the proteins of TPS and TPP in date palm and rice. Also, we found a high degree of conservation of active site residues in the three conserved regions of TPP proteins.

Recently, several research groups have reported on genetic manipulation of trehalose biosynthetic genes in plants and its impact on agronomic traits (Garg *et al.* 2002; Jang *et al.* 2003; Miranda *et al.* 2007; Garg *et al.* 2013). Although these phenotypes indicate that trehalose affects many aspects of metabolism, growth and development, nevertheless, it is difficult to distinguish which of the changes are direct, and which are indirect. Recently, the expression pattern of the 11 AtTPS genes in *Arabidopsis* shows that they are expressed in a developmentally programmed and tissue-specific manner, implying a relevant function in cell metabolism (Avonce *et al*. 2006).

In conclusion, the recent discovery of a plethora of genes that encode trehalose metabolism enzymes in higher plants, and its potential role in modulating photosynthesis, carbon metabolism and stress protection, has led to a series of scientific surprises and offers new challenges for researchers in this field. In view of the latest findings, trehalose research in plants should be seen as an opportunity to use multidisciplinary approaches for the dissection of metabolic networks, including the interface between sugar sensing-signaling and carbohydrate metabolism.

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Table

Table 1: The gene families found for trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) genes from the completely sequenced genomes of *indica* and *japonica* rice, as well as *Phoenix dactylifera*.

Oryza sativa			Phoenix dactylifera	
Indica		Japonica		
Class	Protein name	Locus name	Protein name	Protein name
Class I (TPS)	OsI-TPS1 OsI-TPS2 OsI-TPS3 OsI-TPS4	EAY98715 EAZ09170 EAZ07161 EAY87814	OsJ-TPS1 OsJ-TPS2 OsJ-TPS3 OsJ-TPS4	Pd-TPS1 Pd-TPS2 Pd-TPS3 Pd-TPS4
Class II (TPS/TPP)	OsI-TPS5 OsI-TPS6 OsI-TPS7 OsI-TPS8 OsI-TPS9 OsI-TPS10 OsI-TPS11	EAY89092 EAZ09017 EAY75710 EAY75823 EAY98705 EAZ06991 EAZ08891	OsJ-TPS5 OsJ-TPS6 OsJ-TPS7 OsJ-TPS8 OsJ-TPS9 OsJ-TPS10 OsJ-TPS11	Pd-TPS5 Pd-TPS6 Pd-TPS7 Pd-TPS8 Pd-TPS9 Pd-TPS10 Pd-TPS11
Class III (TPP)	OsI-TPPA OsI-TPPB OsI-TPPC OsI-TPPD OsI-TPPE OsI-TPPF OsI-TPPG OsI-TPPH OsI-TPPI OsI-TPPJ OsI-TPPK	EAY79464 EAZ03880 EAY86968 EAY95105 EAY76459 EAZ00197 EAZ06967 EAZ08844 EAY90273 EAZ04762 EAY82521	OsJ-TPPA OsJ-TPPB OsJ-TPPC OsJ-TPPD OsJ-TPPE OsJ-TPPF OsJ-TPPG OsJ-TPPH OsJ-TPPI OsJ-TPPJ OsJ-TPPK	Pd-TPPA Pd-TPPB Pd-TPPC Pd-TPPD Pd-TPPE Pd-TPPF Pd-TPPG
Trehalase	OsI-TRE1	EAY79237	OsJ-TRE1	Pd-TRE1