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Recent Advances and Future Perspectives

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Preface

In nature, plants are constantly challenged by various abiotic and biotic stresses that can restrict their growth, development, and yields. In the course of their evolution, plants have evolved a variety of sophisticated and efficient mechanisms to sense, respond to, and adapt to changes in the surrounding environment. A common defensive mechanism activated by plants in response to abiotic stress is the production and accumulation of compatible solutes (also called osmolytes). These include amino acids (mainly proline), amines (such as glycinebetaine and polyamines), and sugars (such as trehalose and sugar alcohols), all of which are readily soluble in water and nontoxic at high concentrations. The metabolic pathways involved in the biosynthesis and catabolism of compatible solutes and the mechanisms that regulate their cellular concentrations and compartmentalization are well characterized in many important plant species. Numerous studies have provided evidence that enhanced accumulation of compatible solutes in plants correlates with increased resistance to abiotic stresses. New insights into the mechanisms associated with osmolyte accumulation in transgenic plants and the responses of plants to exogenous application of osmolyte will further enhance our understanding of the mechanisms by which compatible solutes help to protect plants from damage due to abiotic stress and the potential roles compatible solutes could play in improving plant growth and development under optimal conditions. Although there has been significant progress made in understanding the multiple roles of compatible solute in abiotic stress tolerance, many aspects associated with compatible solute-mediated abiotic stress responses and stress tolerance still require more research. As well as providing basic up-to-date information on the biosynthesis, compartmentalization, and transport of compatible solute in plants, this book will also give insights into the direct or indirect involvement of these key compatible solutes in many important metabolic processes and physiological functions, including their antioxidant and signaling functions, and roles in modulating plant growth, development, and abiotic stress tolerance.

In this book, *Osmoprotectant-Mediated Abiotic Stress Tolerance in Plants: Recent Advances and Future Perspectives*, we present a collection of 15 chapters written by leading experts engaged with compatible solute-induced abiotic stress

tolerance in plants. The main objective of this volume is to promote the important roles of these compatible solutes in plant biology, by providing an integrated and comprehensive mix of basic and advanced information for students, scholars, and scientists interested in, or already engaged in, research involving osmoprotectant. Finally, this book will be a valuable resource for future environmental stress-related research and can be considered as a textbook for graduate students and as a reference book for frontline researchers working on the relationships between osmoprotectant and abiotic stress responses and tolerance in plants.

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Mohammad Anwar Hossain is a Professor in the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. He received his B.Sc. in Agriculture and M.S. in Genetics and Plant Breeding from Bangladesh Agricultural University, Bangladesh. He also received an M.S. in Agriculture from Kagawa University, Japan, in 2008, and a Ph.D. in Abiotic Stress Physiology and Molecular Biology from Ehime University, Japan, in 2011, through Monbukagakusho Scholarship. As a JSPS Postdoctoral Researcher, he has worked on isolating low-phosphorus stress-tolerant genes from rice at the University of Tokyo, Japan, during the period of 2015–2017. His current research interests include the isolation and characterization of abiotic stress-responsive genes and proteins, physiological and molecular mechanisms of abiotic stress response and tolerance with special reference to oxidative stress, antioxidants and methylglyoxal metabolism and signaling, generation of stress-tolerant and nutrient-efficient plants through breeding and biotechnology, and cross-stress tolerance in plants. He has over 50 peer-reviewed publications and has edited 9 books, including this one, published by CRC press, Springer, and Elsevier.

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Osmoprotectant-Related Genes in Plants Under Abiotic Stress: Expression Dynamics, In Silico Genome Mapping, and Biotechnology

Éderson Akio Kido, José Ribamar Costa Ferreira-Neto, Manassés Daniel da Silva, Vanessa Emanuelle Pereira Santos, Jorge Luís Bandeira da Silva Filho, and Ana Maria Benko-Iseppon

1 Introduction

In nowadays, agriculture faces multiple challenges, including the adoption of sustainable methods to provide food for a growing urban population, in addition to the increase of bioenergy needs. Moreover, plants are subject to a variety of stresses, leading to damages that can negatively influence vegetative and reproductive development and compromise their yields, causing economic losses. In the last decades, the modernization of methods and tools enforced in agriculture has developed simultaneously with civilization. Scientific advances applied in traditional plant breeding have increased genetic gains of cultivated plants improving their yields and their resistance/tolerance to the environmental stresses around the world. Environmental stresses are classified into biotic stresses, those caused by organisms such as bacteria, fungi, viruses, nematodes, insects, or higher eukaryotes (e.g., weed and herbivores), or abiotic stresses, caused by nonliving organisms, including physical or chemicals stressors, such as high or low temperatures, drought or floods, and salinity, among others. These stressors can act alone or often combined, such as droughts and high temperatures.

Plants under stress need to adapt in order to survive. They respond to the environment by modifying the expression of their genes to best suit the stressful situation and minimize the damages. The dynamics of the genes global expression determine the plant response to the stress-derived stimulus.

Briefly, the stress stimulus is recognized by the receptors in plant cell membranes, and a generated signal is transmitted and amplified in a cascade that culminates in the activation of specific genes. Those gene expressions will constitute the plant response to the stress. During the signaling process, enzymes and receptors are activated or deactivated through phosphorylation and dephosphorylation by

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protein kinases and phosphatases (Ardito et al. 2017). Finally, activated transcription factors (TFs) inside the nucleus will recognize specific *cis*-regulatory elements in the promoters of the genes that will be expressed, helping them to modulate their expression. Besides the TFs, gene expressions might be influenced by CoRegs (co-regulator proteins; Burdo et al. 2014). These proteins, unlike TFs, do not interact directly with the DNAs but interfere with gene regulation by protein-protein interactions, even interacting with TFs (Chevalier et al. 2009). They also restrict or release DNA access, behaving as histone modifiers (Fang et al. 2014) or chromatin remodelers (Han et al. 2016). Therefore, gene expression resulting from complex interactions acts on metabolic pathways or other processes such as RNA interference (RNAi, Saurabh et al. 2014), to respond to the triggering plant stress stimulus.

Over time, plant evolving under unfavorable growth conditions presented molecular, biochemical, and physiological adjustments. Some of these alterations were induced by new alleles associated with isoforms neofunctionalizations, increasing plant variability. Such context resulted in a range of combined strategies, which plants access to minimize damages caused by environmental stresses.

In general, plants under abiotic stresses rely on genes from three broad categories (Hossain et al. 2016):

(a) Genes transcribing regulatory proteins, such as kinases and TFs, which are widely reported in plant responses.
(b) Genes related to water channel proteins and ion transporters.
(c) Genes linked to the protection of essential membranes and proteins such as chaperones, heat shock proteins, and osmoprotective osmolytes.

This chapter regards genes related to osmoprotectants reported in plant transcriptomic studies, their regulation under abiotic stress, their genomes mapping, potential pathways, and, finally, their experiences as transgenes in order to improve plant breeding.

2 Osmoprotectant Definition, Classification, and Roles

Some inorganic ions in ideal concentrations contribute to the biochemical functions, but in high amounts, they disrupt protein functions. Diversely, osmoprotectants are small, electrically neutral, and highly soluble organic compounds with low toxicity. They can accumulate high amounts in the cells, balancing the intracellular with the external environment when in an unfavorable osmotic condition. Due to their high solubility and little interference in the cellular metabolic pathways, they are also known as compatible solutes. The DEOP database (Dragon Explorer of Osmoprotection Pathways; Bougouffa et al. 2014) is an online resource on osmoprotectants and its associated pathways (http://www.cbrc.kaust.edu.sa/deop/). According to the DEOP web index, osmoprotectants are classified into three distinct classes: (i) those containing quaternary ammonium compounds (QACs) and deriva-

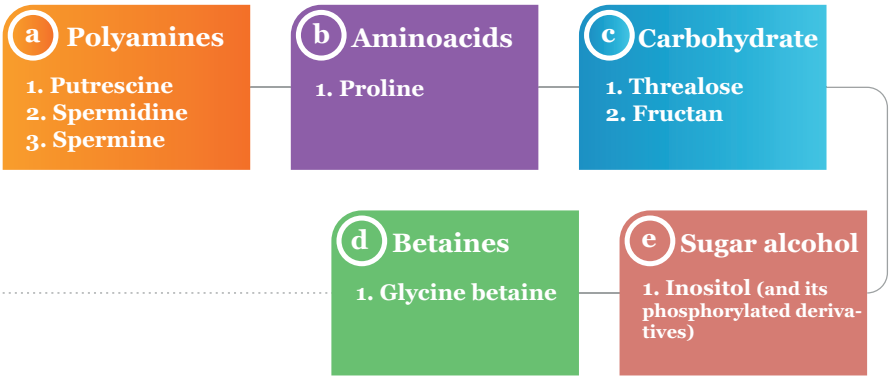

Fig. 1 Main classes of plant osmoprotectants and some representatives compounds

tives, e.g., polyamines and betaines (such as glycine betaine); (ii) those containing amino acids and derivatives, e.g., proline and ectoine; and (iii) those containing sugars and derivatives, e.g., oligosaccharides (sucrose, trehalose, raffinose, stachyose, verbascose), fructan [fructose polymers; oligosaccharides or polysaccharides (>10 units)], and sugar alcohols (polyols: glycerol, inositol, arabitol, maltitol, sorbitol, mannitol, and D-ononitol).

Further, based on the DEOP data, which involved scientific manuscripts published until 2014, covering more than 1160 organisms (including microorganisms, plants, and animals), a total of 135 osmoprotectant compounds were identified (Bougouffa et al. 2014). The major classes of plant osmoprotectants with representatives described in this chapter, whose expressions of their related genes have been reported, are shown in Fig. 1.

Essentially, plants face two situations when under an abiotic stress, such as salt stress (Singh et al. 2015): (a) an osmotic stress due to the higher Na^+ concentration in the rhizosphere, which decreases plant water potential, and (b) a nutritional imbalance caused by ionic stress, in which the higher concentration of Na^+ and Cl^- limits the availability and assimilation of essential nutrients.

Thus, in plants under hypertonic conditions resulting from high NaCl, a flow of water occurs from the inside to the outside of the cell. This situation increases the concentration of the cellular constituents. A high concentration of ions can disrupt proteins, shifting the balance to their unfolded forms. In this case, protective osmolytes accumulated on the surfaces of proteins help to stabilize their structures, tensioning them back to their native structure. Therefore, these osmolytes are recognized as osmoprotectants, due to this protective role against osmotic and saline stresses.

In plants under abiotic stress causing denaturation of macromolecular (proteins and membranes), osmoprotectants such as proline can improve protein stability by binding to hydrogen bonds without affecting the other functions (Slama et al. 2015). Further, trehalose, another osmoprotectant, can stabilize macromolecules, such as the bilayer structure of membranes, by binding to hydrogen bonds in the polar

groups of membranes and proteins, preserving their integrities (Pereira et al. 2004). However, this characteristic varies depending on the osmoprotectant considered. Additionally, some osmoprotectants present chaperone-like activities in order to keep both protein structures and functions. Those compatible osmolytes are also named chemical or molecular chaperones (Slama et al. 2015).

Besides, in plants exposed to salinity and drought, osmoprotectants can accumulate in the cells, helping to maintain cellular turgor and driving the gradient for water uptake to sustain cell volume by osmotic adjustment. In this regulation, the cell tends to compartmentalize ions in the vacuoles; at the same time, it begins to synthesize and accumulate osmoprotectants in the cytoplasm, such as proline, to maintain the osmotic balance between these compartments (Gagneul et al. 2007).

Nevertheless, when a cell undergoes osmotic stress, its redox potential is disturbed, and generated an excessive induction and accumulation of reactive oxygen species (ROS). ROS are by-products of the oxygen metabolism linked to electron transport (Bae et al. 2011). These reactive species [superoxide (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH)] are important in cell signaling once in adequate amounts but in excessive volume cause peroxidation of lipid, oxidation of proteins, and damage of nucleic acids. Further, they can inactivate antioxidative enzymes and even culminate in cells and plant deaths. Therefore, ROS regulation is crucial to avoid cytotoxicity and oxidative damage. Some of the compatible solutes can protect plants from oxidative damages by directly scavenging for ROS or protecting the enzymes from the antioxidative process (Slama et al. 2015).

Other osmoprotectants functions in plant responses to abiotic stresses, especially concerning the drought and salinity tolerance, are found in the review reported by Singh et al. (2015).

3 The Basic of Plant Transcriptomic Studies

A transcriptomic study is an excellent option to look in the potential of osmoprotectants, notably their related genes, in a plant responding to an abiotic stimulus. In general, these studies allow the preview of the gene expression profile of an organism, organ, tissue, or cell, after applying a given stimulus. These global patterns are usually contrasted by comparing the stressed or treated profile with those corresponding to the negative control without the stimulus. Several methodologies (e.g., Northern blot, EST, SAGE and derivatives, microarray, RNA-Seq) can be used to generate libraries expressing these profiles (Kido et al. 2016). Some aspects will be briefly commented below.

Transcriptomic libraries when properly generated and sequenced, using deep-sequencing (NGS, next-generation sequencing) technologies, provide millions of reads. After data quality inspections and the removal of adapters and low-quality bases, these reads allow (a) tag annotation, as those tags (26 pb) generated by the

SuperSAGE technique (Matsumura et al. 2012), or (b) transcriptome assembly (RNA-Seq data), using de novo strategy or based on a reference genome, generating the final assembled transcripts or unigenes (Wang et al. 2009). In both cases, tags or transcripts/unigenes must be adequately annotated, considering similarity levels with previously annotated sequences, using BLAST alignments. In this context, molecular targets involving osmoprotectant-related genes could be identified and selected considering:

(a) The tag or unigene annotation.
(b) The tag or unigene regulation (induced or repressed), considering their frequencies in two circumstantial libraries (stress treatment versus control).
(c) The expression modulated by the tag or unigene; the fold change (FC) value representing the ratio of the normalized frequencies, usually tpm (tags per million), or FPKM (fragments per kilobase of transcript per million mapped reads) considering two comparing libraries.

Furthermore, after appropriated statistical analysis (*p-values*) comparing the normalized frequencies of the tag or unigene based on the contrasted libraries, attention should be given to those identified as differentially expressed gene (DEGs). In this process, the *p-values* are corrected in order to minimize the type I error (Li et al. 2012), using the FDR (false discovery rate) method or similar. That correction diminishes false-positive episodes of differential expressions since the probability of false positives increases due to the high number of tests performed.

Another advantage of a genomic-scale approach is the fact that many plant genomes and transcriptomes data, including those from crops under experimentally controlled stress, are deposited in public databases. The GenBank at NCBI (the National Center for Biotechnology Information; https://www.ncbi.nlm.nih.gov/), the DNA Data Bank of Japan (DDBJ; https://www.ddbj.nig.ac.jp/index-e.html), or the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) are constantly receiving biomolecules sequences from current projects. Therefore, these substantial databases provide bioinformatic tools, online analysis, and downloads of datasets allowing reliable annotations of DEGs, tags, or unigenes, in addition to other analysis.

Diversely, although transcriptomic studies address the global expression of genes, the identification of those related to osmoprotective osmolytes is not a simple task. In transcriptomic studies, despite the detection of many genes comprising several functional metabolic categories, only a few tags or unigenes have already been more detailed. In general, the most observed are those genes related to DEGs showing relevant modulation based on the in silico analysis. Thus, if osmoprotectants are not the primary focus, then few osmoprotectant-related genes are unveiled. Essentially tags or unigenes that are strongly expressed after an applied stimulus are the mostly noted. Usually, the same set of genes is reported to be associated with plant abiotic stress profile. A compilation of some known osmoprotectants and their potential related pathways are shown in Table 1.

Table 1 Some pathways associated with osmoprotectant biosynthesis in plants

Pathway	Related compound/class
Choline biosynthesis	Amine and polyamine/choline
Ectoine biosynthesis	Amine and polyamine/ectoine
Fructan biosynthesis	Sugar/fructan
Glycine betaine biosynthesis	Amine and polyamine/glycine betaine
L-glutamate biosynthesis	Amino acid/L-glutamate
L-proline biosynthesis	Amino acid/L-proline
Mannitol biosynthesis	Polyol/sugar alcohol
Myo-inositol biosynthesis	*Myo*-inositols
Putrescine biosynthesis	Amine and polyamine/putrescine
Sorbitol biosynthesis	Polyol/sugar alcohol
Spermine biosynthesis	Amines and polyamines/spermine
Sucrose biosynthesis	Sugar/sucrose
Trehalose biosynthesis	Sugar/trehalose

4 Osmoprotectant-Related Genes and Associated Pathways

Despite the importance of osmoprotectants in plants and the scientific advances over the years, a database compiling most of the generated information was not available until 2014, when Bougouffa et al. (2014) performing intensive data mining (more than 900,000 scientific articles) compiled 141 osmoprotectant compounds from 1160 organisms (microorganisms, plants, and animals). The authors connected osmoprotectant with potential pathways (biosynthesis or degradation) affecting these osmolytes (834), including reactions (1883), genes (3529), and proteins (4899). Concerning those compounds, only 34 remained not correlated with the identified pathways or reactions. This unique initiative resulted in the DEOP website (http://www.cbrc.kaust.edu.sa/deop/index.php), which is a database dedicated exclusively to osmoprotectants and their possible associated pathways.

Based on the site's background information, the focus of the authors was to study the potential of microorganisms accumulating osmoprotectants to become cell factories. Another concern was the potential transference of such functional capability into other organisms through synthetic biology. Besides those already mentioned features, the available information provides perspectives covering microorganisms-plant interactions, with both organisms acting together against adverse conditions in the rhizosphere and soil environment. Such information can greatly assist studies of functional, comparative, and evolutionary genomics aspects involving osmoprotective genes.

The searches performed on DEOP relational tables scrutinize pathways derived from the KEGG (Kyoto Encyclopedia of Genes and Genomes; https://www.genome.jp/kegg/) and MetaCyc databases (Metabolic Pathway Database, https://metacyc.org/). The MetaCyc database is a cured bank containing more than 2570 pathways of almost 3000 organisms from the various domains of life (Caspi et al. 2018). A compound can be associated with pathways representing osmoprotectant

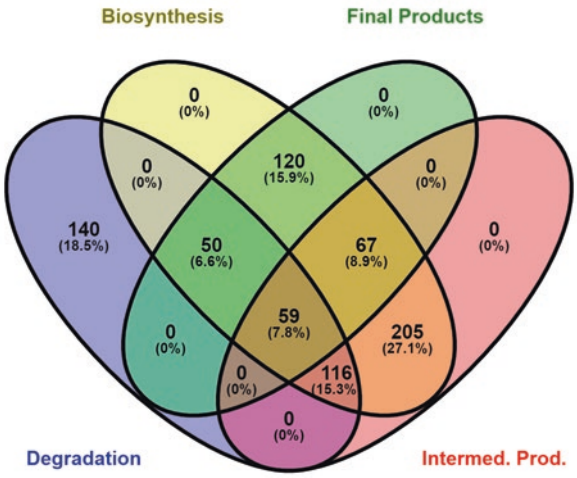

Fig. 2 Venn diagram based on numbers of entries related to osmoprotectant-associated pathways [biosynthesis (final or intermediate product)/ degradation] prospected from DEOP database (http://www.cbrc.kaust.edu.sa/deop/index.php)

biosynthesis (related to the final or intermediate product; also, reversible or not), osmoprotectant degradation, and other osmoregulation. Furthermore, since it is possible to download data from the DEOP site, the entries related to the biosynthesis pathways of osmoprotective compounds as final products (December 2018) totalized 120, while those addressing intermediate products were 205, and those covering degradation were 140 (Fig. 2). Some of the 120 identified entries are listed in Table 1, and the described pathways typically associate sugars and its derivatives (e.g., sugar alcohol), amines and polyamines, and amino acids, highlighting these compounds as osmoprotectants.

According to the DEOP relational tables associated with the applied research, plant species presenting data associated with the pathways presented in Table 1 are listed in Table 2. The identified pathways and plants comprised mostly the biosynthesis of proline (14 plant species), sucrose (9), trehalose (8), and putrescine (8). In turn, plants concentrating studies focusing on those pathways (Table 1) comprised *Nicotiana tabacum* (9), *Arabidopsis thaliana* (7), *Oryza sativa* (7), and *Triticum aestivum* (5) (Table 2).

Moreover, the set of reactions predicted in the pathways listed in Table 1, as well as the others available in the DEOP database, allow the identification of genes and enzymes associated with a specific osmoprotectant compound; that association is supported by the MetaCyc database, a comprehensive source of diagrams showing the enzymes involved in such reactions. Therefore, genes encoding the related enzymes identified above are good candidates to be noted in a transcriptomic study, as well as their expression after a signalized stress. Once some gene candidates are identified as DEGs, its expression still needs to be validated by a second method. Usually, the RT-qPCR (real-time reverse transcription-polymerase chain reaction) technique is performed; after all, it is considered a reference method in such cases (Provenzano and Mocellin 2007). After the validation process is done, the reliable candidates become promising to be applied as functional molecular markers to

Table 2 Plant species presenting osmoprotectants data and associated pathways[a] based on the DEOP database (http://www.cbrc.kaust.edu.sa/deop/index.php)

	Pathways[a]													
Plant species	1	2	3	4	5	6	7	8	9	10	11	12	13	Subtotal
Arabidopsis thaliana					x	x	x		x		x	x	x	7
Avena sativa									x					1
Brassica napus						x								1
Glycine max						x						x	x	3
Helianthus tuberosus						x								1
Hordeum vulgare			x			x			x			x		4
Lycopersicon esculentum												x		1
Malus x domestica									x	x				2
Nicotiana tabacum		x	x	x		x	x		x	x		x	x	9
Oryza sativa			x	x		x		x	x			x	x	7
Phaseolus vulgaris						x							x	2
Pisum sativum						x								1
Populus sp.									x					1
Solanum tuberosum						x							x	2
Spinacia oleracea	x					x						x		3
Triticum aestivum			x			x			x			x	x	5
Vigna aconitifolia						x								1
Zea mays						x						x	x	3
Subtotal	1	1	4	2	1	14	2	1	8	2	1	9	8	54

Biosynthesis pathways[a]: (1) choline; (2) ectoine; (3) fructan; (4) glycine betaine; (5) L-glutamate; (6) L-proline; (7) mannitol; (8) *myo*-inositol; (9) putrescine; (10) sorbitol; (11) spermine; (12) sucrose; (13) trehalose

assist selection steps in plant breeding programs or to be evaluated in transgenic assays, helping breeders to develop new cultivars or varieties.

5 Expression of Osmoprotectant-Related Genes

Plants under abiotic stresses presented osmoprotectant-related genes modulating their expressions after the stress stimulus. Regarding salinity stress, at least 15 scientific articles covering 2015 until the beginning of 2019 presented osmoprotectant-related genes analyzed in 12 plant species. The investigated plant species comprised classic model plants (e.g., *A. thaliana*, *M. truncatula*, *N. tabacum*), important cultivated worldwide crops (e.g., *G. max*, *O. sativa*, *S. bicolor*), and other lesser-known plants (e.g., *Bacopa monnieri*, *Chenopodium album*) (Table 3).

The experimental assays described in those articles were quite diverse, covering plants at different growth stages that were submitted to the NaCl salt, which molarities comprised from 75 to 400 mM, and the time of exposure ranging from less than 1 hour to days or even weeks. Some of the studies also looked at the influence of

Table 3 Published scientific reports presenting regulation of osmoprotectant-related genes in plants under salt stress

Plant species	Stress treatment	Osmoprotectant	Target genes	Reg.	Reference
Arabidopsis	NaCl 300 mM (6 h)	Proline	*P5CS1, P5CS2*	UR	Alavilli et al. (2016)
Arabidopsis/ Gossypium hirsutum	NaCl 300 mM (0, 6, 12 h)	Trehalose	*TPS11*	UR	Wang et al. (2016a)
Chenopodium album/tobacco	NaCl 300 mM (0, 24, 48 h)	Putrescine/polyamine	*ADC1, ODC2*	UR	Wang et al. (2017)
Chenopodium quinoa	NaCl 100 and 250 mM (1 h, 24 h, 15 days)	Glycine betaine	*BADH*	UR	Jiang et al. (2016)
Glycine max	NaCl 150 and 300 mM (30 days)	Glycerol, hydroquinone	*GSTU4*	UR	Kissoudis et al. (2015)
Glycine max	NaCl 50 mM (3 weeks)	Proline	*P5CS*	UR	Ren et al. (2018)
Ipomea batatas	NaCl 200 mM (2, 4, 6, 12, 24, 45 h)	*Myo*-inositol	*MIPS1*	UR	Zhai et al. (2015)
Lilium regale	NaCl 250 mM (2, 4, 6, 9, 12 h)	Proline	*P5CS2*	DR	Wei et al. (2016)
Lilium regale	NaCl 250 mM (2, 4, 6, 9, 12 h)	Proline	*P5CS1, P5CS3*	UR	Wei et al. (2016)
Malus hupehensis	NaCl 200 mM (0.5, 1, 3, 6 h)	Putrescine/polyamine	*ADC2, ODC1*	DR	Gong et al. (2018)
Malus hupehensis	NaCl 200 mM (0.5, 1, 3, 6 h)	Putrescine/polyamine	*ADC1*	UR	Gong et al. (2018)
Malus hupehensis	NaCl 200 mM (0.5, 1, 3, 6 h)	Polyamine	*SAMDC1-5, SPDS2-6*	DR	Gong et al. (2018)
Malus hupehensis	NaCl 200 mM (0.5, 1, 3, 6 h)	Putrescine/polyamine	*SPDS1, SPDS4*	UR	Gong et al. (2018)
Medicago truncatula	NaCl 250 mM (12, 18, 24 h)	Proline	*P5CS2*	UR	Li et al. (2017)
Nicotiana rustica	NaCl 200 mM (3 weeks)	Proline	*P5CS, PDH*	UR	Rajaeian et al. (2017)
Nicotiana rustica	NaCl 200 mM (3 weeks)	Glycine betaine	*BADH*	UR	Rajaeian et al. (2017)
Nicotiana tabacum/Arabidopsis	NaCl 200 and 300 mM (60 days)	Proline	*P5CS1*	UR	Ibragimova et al. (2015)
Oryza sativa	NaCl 400 mM (2, 4, 6 h)	Raffinose	*RS5*	UR	Jung et al. (2017)
Raphanus sativus	NaCl 100 and 200 mM (48 h)	Glycine betaine	*BADH*	DR	Sun et al. (2016)
Raphanus sativus	NaCl 100 and 200 mM (48 h)	Proline	*P5CS*	UR	Sun et al. (2016)
Sorghum bicolor	NaCl 75 mM (0, 14 days)	Putrescine/polyamine	*OAT*	UR	de Freitas et al. (2019)

(continued)

Table 3 (continued)

Plant species	Stress treatment	Osmoprotectant	Target genes	Reg.	Reference
Sorghum bicolor	NaCl 75 mM (0, 14 days)	Proline	*P5CS2*	DR	de Freitas et al. (2019)
Sorghum bicolor	NaCl 75 mM (0, 14 days)	Proline	*ProDH*	UR	de Freitas et al. (2019)
Sorghum bicolor	NaCl 75 mM + proline,30 mM (0.5–14 days)	Putrescine/polyamine	*OAT*	UR	de Freitas et al. (2019)
Sorghum bicolor	NaCl 75 mM + proline,30 mM (0.5–14 days)	Proline	*P5CS2*	DR	de Freitas et al. (2019)
Sorghum bicolor	NaCl 75 mM + proline,30 mM (0.5–14 days)	Proline	*P5CS1, ProDH*	UR	de Freitas et al. (2019)
Arabidopsis	NaCl 300 mM (6 h)	Proline	*P5CS1, P5CS2*	UR	Alavilli et al. (2016)

Reg. (gene regulation), *ADC* (arginine decarboxylase), *BADH* (betaine aldehyde dehydrogenase), *GSTU* (glycerol and hydroquinone), *MIPS* (*myo*-inositol-1-phosphate synthase), *OAT* (ornithine-δ-aminotransferase), *ODC* (ornithine decarboxylase), *P5CS* (Δ1-pyrroline-5-carboxylate synthase), *PDH/ProDH* (proline dehydrogenase), *RS* (raffinose synthase), *SAMDC* (S-adenosylmethionine decarboxylase), *SPDS* (spermidine synthase), *TPS* (trehalose 6-phosphate synthase)

other factors besides NaCl, such as PEG, phytohormones, proline, and ethanolamine, among others, independently or combined with the salt. In that set of reports, the highlighted osmoprotectant compounds comprised the amino acid proline, the glycine betaine, the polyamines (putrescine and spermidine), the sugars (trehalose, oligosaccharides), and sugar derivatives (e.g., *myo*-inositol). Also, a total of 12 different osmoprotectant-related genes were investigated about their regulation after salt-stress exposure.

Concerning drought stress, covering the period 2015–2019, at least 14 scientific articles also reported osmoprotectant-related genes modulating their expression after dehydration stress treatment. The experimental assays described in that researches showed different stress application methods, involving natural drought conditions (Yang et al. 2015c), drought simulated by root dehydration (0–72 h; Singh et al. 2015), suppression of irrigation (Rickes et al. 2019; Dastogeer et al. 2018), withholding water assay (Chen et al. 2016), addition of polyethylene glycol (30% PEG 6000 solution; Yadav et al. 2018), and even dehydrated fruits (grape berries) (Conde et al. 2018) (Table 4). These studies embraced 15 plant species, including the reference plants and crops already mentioned, and other lesser-known plants, such as *Stipa purpurea* (Yang et al. 2015c) and *Ziziphus nummularia* (Yadav et al. 2018]. Also, this set of reports encompassed 21 genes related to osmoprotectants, such as the amino acid proline, the glycine betaine, the polyamines (putrescine and spermidine), the sugars (sucrose, raffinose, and trehalose), and polyols (sorbitol and mannitol). Some of the investigated genes are presented in Table 4, taking into account their expression after drought or salt stress.

5.1 Amino Acid Proline

In respect to proline as an osmoprotectant, and considering plants responding to salt stress, eight scientific manuscripts presented expression results of only two genes. One of them encoded *P5CS* (Δ1-pyrroline-5-carboxylate synthase) and the other *PDH* (proline dehydrogenase). Only the first gene takes part in the proline biosynthesis pathway. The P5CS (EC 2.7.2.11/1.2.1.41) reduces glutamic acid to γ-glutamic semialdehyde (GSA), and GSA is converted spontaneously by P5CR (Δ1-pyrroline-5-carboxylate reductase) into Δ1-pyrroline-5-carboxylate (P5C). Finally, P5C is converted to proline by P5CR (Szabados and Savoure 2010). The other gene codifies the enzyme PDH (EC 1.5.5.2) that catalyzes proline degradation after plant dehydration. In general, except for some *P5CS* isoforms and depending on the analyzed tissue, the upregulation of transcripts of both genes are observed in roots after the salt application (Table 3).

Still, considering proline, now taking into account plants responding to drought stress, the same genes were investigated (Table 4). About nine articles presented *P5CS* expression showing upregulation after drought stress, and only one research (Dastogeer et al. 2018) investigated the *PDH* expression, also noting the upregulation of the transcript. Interestingly, Dastogeer et al. (2018) pointed fungal endophytes

Table 4 Published scientific reports presenting osmoprotectant-related gene regulation in plants under water-deficit stress

Plant species	Stress treatment	Osmoprotectant	Target genes	Reg.	Reference
Glycine max	PEG 6000 [osmotic potential: −0,50 Mpa] (10 days)	Proline	*P5CS*	UR	Vaishnav and Choudhary (2018)
Glycine max	PEG 6000 [osmotic potential: −0,50 Mpa] (10 days)	Raffinose	*GolS*	UR	Vaishnav and Choudhary (2018)
Jatropha curcas	PEG 6000 10% (2 days); osmotic potential (−0,50 Mpa)	Proline	*P5CS*	UR	Yang et al. (2015b)
Lilium regale	Withholding water (7 days)	Proline	*P5CS1, P5CS3*	UR	Wei et al. (2016)
Lilium regale	Withholding water (7 days)	Proline	*P5CS2*	ns	Wei et al. (2016)
Malus hupehensis	NaCl 200 mM (0, 1, 3, 6, 12, 24 h)	Putrescine/ polyamine	*ADC1, ADC2, ODC1*	UR	Gong et al. (2018)
Malus hupehensis	NaCl 200 mM (0, 1, 3, 6, 12, 24 h)	Putrescine/ polyamine	*ODC2*	DR	Gong et al. (2018)
Medicago truncatula	Withholding water (9 days)	Proline	*P5CR, P5CS*	UR	Antoniou et al. (2018)
Nicotiana benthamiana	Withholding water (8 days)	Proline	*PDH1*	UR	Dastogeer et al. (2018)
Oryza sativa	Drought (6 days withhold water)	RFO	*GolS2*	UR	Jung et al. (2017)
Prunus persica	Drought (irrigation suppressed; 0, 4, 7, 9 days)	Polyol	*SOT1*	DR	Rickes et al. (2019)
Prunus persica	Irrigation suppressed (0, 4, 7, 9 days)	Proline	*P5CS*	UR	Rickes et al. (2019)
Prunus persica	Irrigation suppressed (0, 4, 7, 9 days)	Raffinose	*SIP1*	UR	Rickes et al. (2019)
Prunus persica	Irrigation suppressed (0, 4, 7, 9 days)	Polyol	*S6PDH*	UR	Rickes et al. (2019)
Spinacia oleracea	Withholding water (0, 5, 8 days) + re-watering (after 1, 4 days). NaHS application in *S. oleracea* seedlings	Glycine betaine	*BADH, CMO, SAMDC*	UR	Chen et al. (2016)
Spinacia oleracea	Withholding water (0, 5, 8 days) + re-watering (after 1, 4 days). NaHS application in *S. oleracea* seedlings	Putrescine/ polyamine	*ADC, CPA, ODC*	UR	Chen et al. (2016)

Spinacia oleracea	Withholding water (0, 5, 8 days) + re-watering (after 1, 4 days). NaHS application in *S. oleracea* seedlings	Sucrose	*FBPase, SPS1, TPS*	UR	Chen et al. (2016)
Stipa purpurea	Naturals drought conditions	Proline	*P5CS*	UR	Yang et al. (2015c)
Stipa purpurea	Naturals drought conditions	Glycine betaine	*BADH*	UR	Yang et al. (2015c)
Triticum aestivum	Withholding water (>11 days); exogenous polyamine application	Putrescine/ polyamine	*ADC, ODC*	UR	Ebeed et al. (2017)
Triticum aestivum	Withholding water (>11 days); exogenous polyamine application	Polyamine	*DHS, SAMDC, SPDS*	UR	Ebeed et al. (2017)
Vitis vinífera	Fruit dehydrated (5, 11 days)	Polyol	*PLT1, SDH*	UR	Conde et al. (2018)
Vitis vinífera	Fruit dehydrated (5, 11 days)	Polyol/ mannitol	*MTD*	n.s.	Conde et al. (2018)
Vitis vinífera	Fruit dehydrated (5, 11 days)	Raffinose	*GolS1*	UR	Conde et al. (2018)
Ziziphus nummularia	PEG 6000 30% (6, 12, 24, 48, 72 h)	Raffinose	*GolS*	UR	Yadav et al. (2018)
Ziziphus nummularia	PEG 6000 30% (6, 12, 24, 48, 72 h)	Proline	*P5CS*	UR	Yadav et al. (2018)
Glycine max	PEG 6000 [with osmotic potential of −0,50 Mpa] (10 days)	Proline	*P5CS*	UR	Vaishnav and Choudhary (2018)

Reg. (gene regulation), *BADH* (betaine aldehyde dehydrogenase), *CMO* (choline monooxygenase), *DHS* (deoxyhypusine synthase), *SPDS* (spermidine synthase), *SAMDC* (S-adenosylmethionine decarboxylase, *PLT* (polyol transporter), *SDH* (sorbitol dehydrogenase), *SOT* (sorbitol transporter), *S6PDH* (sorbitol-6-phosphate dehydrogenase), *MTD* (mannitol dehydrogenase), *P5CR* (Δ1-pyrroline-5-carboxylate reductase), *P5CS* (Δ1-pyrroline-5-carboxylate synthase), *PDH* (proline dehydrogenase), *ADC* (arginine decarboxylase), *CPA* (N-carbamoylputrescine amidohydrolase), *ODC* (ornithine decarboxylase), *GolS* (galactinol synthase/*myo*-inositol 3-alpha-D-galactosyltransferase), *SMO* (sterol C-4 methyl oxidase), *SIP* (raffinose synthase), *SPS* (sucrose-phosphate synthase), *FBPase* (fructose-1,6-bisphosphatase), *TPS* (trehalose 6-phosphate synthase), *RFO* (raffinose)

and inoculated virus conferring drought tolerance to *Nicotiana benthamiana* plants through osmolyte modulation and expression of host drought-responsive genes.

5.2 Glycine Betaine

Regarding glycine betaine (GB) and plants responding to the salt stress, the single gene investigated in the researched period encoded BADH (betaine aldehyde dehydrogenase; EC 1.2.1.8), which presented upregulation in most of the manuscripts (Table 3), and only one *BADH* downregulated (Sun et al. 2016). Besides this DR regulation, the analyzed radish transcriptome (*Raphanus sativus* L.) in response to salt stress (0, 100, and 200 mM NaCl for 48 h) presented 29 induced DEGs associated with osmoprotectants (threshold of $|\log_2 \text{Ratio}| \geq 1$ with FDR ≤ 0.001 and *p-value* ≤ 0.05), including 9 P5CS candidates and 5 (induced) of 7 trehalose-related ones (Sun et al. 2016).

Concerning the drought stress, besides the *BADH* gene, another induced gene evaluated in GB biosynthesis pathway was *CMO* (choline monooxygenase; Chen et al. 2016]. In higher plants, choline is converted by CMO (EC 1.14.15.7) into betaine aldehyde, which is then catalyzed by BADH into GB (Chen et al. 2016; Takabe et al. 2006).

5.3 Polyamines

Concerning the osmoprotectant polyamines (PAs), which have some functions similar to plant growth regulators, five investigated genes covered this issue in plants responding to salt stress: *ADC* (*arginine decarboxylase*), *ODC* (*ornithine decarboxylase*), *OAT* (*ornithine-δ-aminotransferase*), *SAMDC* (*S-adenosylmethionine decarboxylase*), and *SPDS* (*spermidine synthase*) (Table 3). The *ADC*, *ODC*, and *OAT* genes are directly involved with the putrescine biosynthesis pathway, while *SAMDC* and *SPDS* are involved with spermidine pathway.

The osmoprotectant putrescine (Put) can be synthesized directly from ornithine by *ODC* (EC 4.1.1.17) or indirectly, through a series of intermediates following arginine decarboxylation by *ADC* (EC 4.1.1.19). Most of the *ADC* and *ODC* transcripts are induced in responses to salt stresses (Table 3). Upregulation is also observed in *OAT* transcript, target only analyzed by de Freitas et al. (2019) in their study of *S. bicolor* after the stress of 75 mM NaCl, 14 days after the salt application. In respect to drought stress, several manuscripts (Chen et al. 2016; Ebeed et al. 2017; Gong et al. 2018) relate the upregulation of *ADC* and *ODC* transcripts.

The osmoprotectant spermidine (Spd) is synthesized from Put by successive additions of aminopropyl groups catalyzed by SPDS (EC 2.5.1.16). In the other hand, the aminopropyl is provided by decarboxylated *S*-adenosylmethionine, a metabolite synthesized by SAMDC (EC 4.1.1.50). The SAMDC enzyme is also

implicated in cysteine and methionine metabolism, as well as the arginine and proline metabolism (https://www.genome.jp/dbget-bin/www_bget?ec:4.1.1.50). Based on the genome-wide study reported by Gong et al. (2018) in apple (*Malus hupehensis* Rehd.), the *MhSAMDC1* and *MhSPDS1* genes were induced not only by salt but also by other treatments (alkaline, abscisic acid, cold, and dehydration), suggesting that these genes have relevant roles in plant stress responses. About drought stress, also *SAMDC* and *SPDS* presented upregulation after stress application (Chen et al. 2016; Ebeed et al. 2017). However, concerning Chen et al. (2016), the enhancement of plant drought tolerance with effects on polyamines and soluble sugar contents also derived from NaHS application before the stress exposure.

The *CPA* (*N-carbamoylputrescine amidohydrolase*) and *DHS* (*deoxyhypusine synthase*) genes were also investigated concerning responses to drought stress. Both genes are induced in plants under such stress (Chen et al. 2016; Ebeed et al. 2017). The CPA (EC 3.5.1.53) is implicated in Put generation from *N*-carbamoylputrescine (https://www.genome.jp/dbget-bin/www_bget?ec:3.5.1.53), while DHS (EC 2.5.1.46) participates in Spe degradation using it as a substrate. Wang et al. (2003) report that suppression of *DHS* delays premature leaf senescence induced by drought stress in *A. thaliana* among other pleiotropic effects.

5.4 Carbohydrates

Furthermore, in plants responding to salt stress, osmoprotectant carbohydrates (sugars) are the oligosaccharide raffinose and the complex sugar trehalose. Both were represented by the *RS* (*raffinose synthase*) and *TPS* (*trehalose-6-phosphate synthase*) genes, respectively. These genes participate directly in the raffinose and trehalose biosynthesis pathways, and both are induced after the applied salt stress (Jung et al. 2017; Wang et al. 2016a). In relation to plants responding to drought stress, the genes investigated, *TPS* and *SIP* (also a *raffinose synthase*; Rickes et al. 2019), presented upregulation after the applied stress (Chen et al. 2016).

The RS enzyme (EC 2.4.1.82), as predicted in the galactose metabolism (https://www.genome.jp/kegg-bin/show_pathway?ath00052+AT1G55740), converts galactinol into raffinose. In turn, the TPS enzyme (EC 2.4.1.15) participates in the trehalose biosynthesis in plants, generating T6P (trehalose-6-phosphate) from glucose-6-phosphate and UDP-glucose, with the subsequent dephosphorylation of T6P to trehalose by TPP (trehalose-6-phosphate phosphatase) (Cabid and Leloir 1958).

Besides *RS* (*SIP*) gene, the *GolS* (*galactinol synthase/myo-inositol 3-alpha-D-galactosyltransferase*) is another induced gene observed during dehydration events and associated with the galactose metabolism. In the biosynthesis of raffinose family oligosaccharides (RFOs), the enzyme galactinol synthase (EC 2.4.1.123) catalyzes the first step converting UDP-galactose and *myo*-inositol to galactinol, and this will be further converted to raffinose by the RS (SIP) enzyme. Overexpression of *AtGolS2* in transformed Arabidopsis plants showing reduced leaves transpiration

presented increased endogenous galactinol and raffinose (Taji et al. 2002). The overexpression of *GolS* transcripts was observed in several manuscripts (Table 9.4).

Additionally, another two genes associated to sugar biosynthesis were induced in plants responding to drought, but in this case, plant tolerance was improved by NaHS that was applied to *Spinacia oleracea* seedlings as pretreatment (Chen et al. 2016). The investigated genes were *SPS* (*sucrose-phosphate synthase*) and *FBPase* (*fructose-1,6-bisphosphatase*). According to the KEGG database, in the starch and the sucrose metabolism, the substrate UDP-glucose is converted by SPS (EC 2.4.1.14) into sucrose-6-P which is converted by SPP1 (sucrose-phosphatase 1) to sucrose. In turn, FBPase enzyme (EC 3.1.3.11) converts the substrate D-fructose-1,6-bisphosphate into D-fructose 6-P, which is a compound involved with many pathways, including galactose, and also starch and sucrose metabolisms. Based on the results, the NaHS pretreatment improved plant tolerance, modulating the expression levels of genes associated with sugar biosynthesis, and also polyamines, as mentioned before. Sugars, such as sucrose and trehalose, replace water molecules on the surfaces of proteins allowing them to preserve their conformations and, therefore, to restore their functions after rehydration (Hoekstra et al. 2001).

5.5 Sugar Alcohols

When talking about the osmoprotectant sugar alcohol *myo*-inositol, the represented gene is *MIPS* (*myo-inositol-1-phosphate synthase*), in plants responding to salt stress. The induced gene, after the salt-stress exposition, participates directly in the *myo*-inositol biosynthesis pathway (Zhai et al. 2015). The MIPS enzyme (EC: 5.5.1.4) catalyzes the conversion of D-glucose-6-phosphate to 1 L-*myo*-inositol-1-phosphate. The conversion is rate limiting in the biosynthesis of all inositol-containing compounds. *Myo*-inositol plays an essential role as a structural basis for generating second messengers useful in signal transduction (Gillaspy 2011). Also, inositol serves as a crucial component of the structural lipid phosphatidylinositol (PI) and its various phosphates, the phosphatidylinositol phosphate (PIP) lipids. Considering plants responding to drought stress, *MIPS* gene was not investigated in the set of researched articles.

About polyols (polyhydric alcohol), including sugar alcohols such as sorbitol and mannitol, genes associated with these pathways were not presented in the set of manuscripts covering plants responding to salt stress. Nevertheless, Conde et al. (2018) reported a study covering dehydrated grape berries. In that study, most of the genes were associated with sorbitol, and they encoded SDH (sorbitol dehydrogenase, EC 1.1.99.21), S6PDH (sorbitol-6-phosphate dehydrogenase; EC 1.1.1.140), and two polyol transporters, SOT (sorbitol transporter) and PLT (polyol transporter). Based on gene expression results, all of them presented upregulation after the applied stress (Table 4). Another investigated gene encoded MTD (mannitol dehydrogenase, EC 1.1.1.255) and it was also induced (Conde et al. 2018). The MTD enzyme converts D-mannitol into D-mannose, a compound implicated in several

pathways, including galactose, fructose, and mannose metabolisms (https://www.genome.jp/dbget-bin/www_bget?cpd:C00159).

In the polyol pathway, the unused glucose is reduced by aldose reductase to sorbitol, which is subsequently oxidized to fructose by SDH. After that, fructose can be phosphorylated by fructokinase and subsequently metabolized via dihydroxyacetone phosphate or glyceraldehyde to D-glyceraldehyde 3-phosphate, which is a substrate in the glycolysis process. In turn, S6PDH acting on D-sorbitol 6-phosphate generates D-fructose 6-phosphate (fructose and mannose metabolism), a compound implicated in many KEGG pathways (https://www.genome.jp/dbget-bin/get_linkdb?-t+pathway+cpd:C05345). The interrelation between sorbitol and sucrose supply due to its gene expression is observed in transgenic apple altered with S6PDH cDNA (Kanamaru et al. 2004).

6 In Silico Genome Mapping of Osmoprotectant-Related Genes

The osmoprotectants and their genes related to proline (*P5CS1*; *P5CR1*), trehalose (*TPS1*; *TPPB*, *trehalose-phosphatase*), glycine betaine (*BADH1*; *CMO*), cysteine (*SAT1*, *serine acetyltransferase*; *OASTL1*, *O-acetyl serine (thiol) lyase*), and *myo*-inositol (*MIPS1*) were in silico mapped on genomes of six plant species, including model plants (*P. patens*, *A. thaliana*), monocots *(S. bicolor* and *O. sativa*), and dicots (*G. max*, *P. vulgaris*). The mapping allowed a comparative analysis among them. The investigated genes were chosen based on a previous study with transcripts identified using 26 bp SuperSAGE unitags expressed in soybean roots after air dehydration in time intervals ranging from 0 up to 150 min (Kido et al. 2013).

Apart from *P5CS1* which is absent in moss *P. patens* (bryophyte), the most basal species analyzed, the remaining genes were identified in virtual chromosomes of all six plant species (Table 5). The analysis brought up that 33 *loci* were identified in *P. patens* (Table 5) on 21 of 27 chromosomes total (Fig. 3A), while *A. thaliana*, which is a compact angiosperm genome with five chromosomes, due to the loss of DNA by unequal homologous recombination (Devos et al. 2002), mapped 43 *loci* (Table 5 and Fig. 3B). This information points to the relevance of osmoprotectants in cellular homeostasis maintenance through plant evolution. Mosses and flowering plants evolution diverge in more than 400 million years (MYA, Nishiyama et al. 2003).

In turn, considering the two legumes (Fabaceae family), soybean (*Glyxine max*) presented 112 *loci* (Table 5, Fig. 3D), while the common bean (*Phaseolus vulgaris*) presented 58 *loci* (Table 5. Figure 3C). It is worth mention that *G. max* (2n = 40) has almost the double of chromosomes of *P. vulgaris* (2n = 22), as a result of two genome duplications events, at approximately 59 and 13 million years ago (Schmutz et al. 2010). Therefore, soybean is a highly duplicated genome with nearly 75% of the genes present in multiple copies (Schmutz et al. 2010).

Table 5 *Loci* numbers of genes associated with osmoprotectants∗ biosynthesis in six plant genome species

Genome	*P5CS*1	*P5CR*1	*BADH*1	*CMO*	*TPS*1	*TPPB*	*INPS*1	*OASTL*	*SAT*1	*Loci*
Physcomitrella patens	0	1	11	1	4	7	2	3	4	33
Arabidopsis thaliana	2	1	13	1	10	1	3	8	4	43
Glycine max	7	2	48	1	20	2	4	18	10	112
Phaseolus vulgaris	4	1	13	3	12	9	2	8	6	58
Sorghum bicolor	2	1	11	1	7	13	2	11	1	49
Oryza sativa	2	1	16	1	11	13	2	12	6	64

∗Osmoprotectants [gene(s)]: proline∗ [*P5CS1* (delta(1)-pyrroline-5-carboxylate synthetase), *P5CR1* (delta(1)-pyrroline-5-carboxylate reductase)], Glycine betaine∗ [*BADH1* (betaine aldehyde dehydrogenase), *CMO* (choline monooxygenase)], *Myo*-inositol∗ [*INPS1* (*myo*-inositol 1-phosphate synthase)], Trehalose∗ [*TPS1* (trehalose-6-phosphate synthase), *TPPB* (trehalose-phosphatase)], Cysteine∗ [*SAT* (serine acetyltransferase), *OASTL* (O-acetyl-serine(thiol)lyase)]

The other two analyzed genomes, representing grasses (Poaceae family), presented 49 *loci* (*Sorghum bicolor*; subfamily Panicoidae; Table 5 and Fig. 3E) and 64 *loci* (*O. sativa*; subfamily Oryzoidae; Table 5 and Fig. 3F). Some synteny and collinearity comparing the two genomes showed 1 block involving the chromosomes 1 of sorghum and 10 of rice (same gene order for *P5CS1*, *SAT1*, and *TPS1*) and other 2 blocks (chromosomes 8 of sorghum and 12 of rice and chromosomes 9 of sorghum and 5 of rice). Also, another difference is observed involving the gene *SAT1* presenting only one copy in sorghum, while it shows six copies in rice (Table 5).

Concerning *P5CR1* and *CMO* genes, most of the species had only one *locus*, with *P5CR1* duplicated in soybean, and *CMO* with three *loci* in *P. vulgaris* (Table 5). The consequence of these two extra copies needs further investigation. On the other hand, *BADH1*, *TPS1*, and *OASTL* mapped at multiple *loci* (Table 5), probably reflecting events of duplications, which is one of the sources of new gene generation. Once a duplicate segment is subjected to lower selection pressure in subsequent mutations, it may lead to new functions (Sankoff 2001). Based on this assumption, polyploid species, such as soybean and modern sugarcane (Garcia et al. 2006), which is highly polyploid and aneuploid, as a result of interspecific crosses within the *Saccharum* complex, are valuable sources of genes/alleles with potential to increase plant fitness in response to biotic and abiotic stresses.

7 Osmoprotectant-Related Genes as Transgenes

Regarding the scientific manuscripts addressing osmoprotectant-related genes, they acquired biotechnological relevance for the agriculture area, making them attractive targets to be manipulated also taking into account their participation in plant stress responses (Tables 3 and 4). The impact of this relevance is revealed by data mining

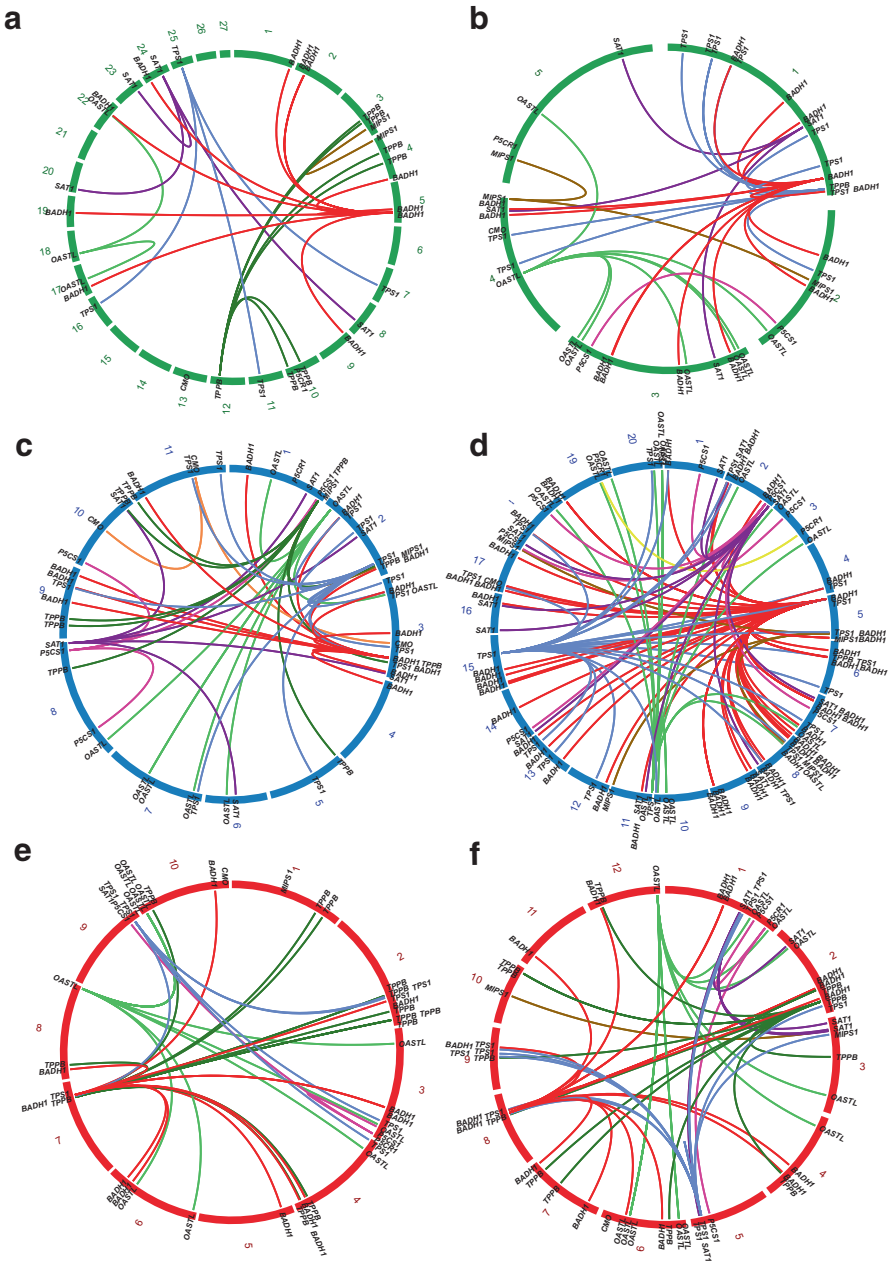

Fig. 3 In silico mapping of loci covering osmoprotectant-related genes in six plant genomes (A, *Physcomitrella patens*; B, *Arabidopsis thaliana*; C, *Phaseolus vulgaris*; D, *Glycine max*; E, *Sorghum bicolor*; F, *Oryza sativa*). Syntenic relationships showed by color lines: red [proline: *P5CS1* (*Δ1-pyrroline-5-carboxylate synthetase*); *P5CR1* (*Δ1-pyrroline-5-carboxylate reductase*)]; purple [glycine betaine: *BADH1* (*betaine aldehyde dehydrogenase*); *CMO* (*choline monooxygenase*)]; orange (*myo*-inositol: *MIPS1* (*myo-inositol 1-phosphate synthase*)); green [trehalose: *TPS1* (*trehalose-6-phosphate synthase*); *TPPB* (*trehalose phosphatase*)]; blue [cysteine: *SAT* (*serine acetyltransferase*); *OASTL* (*O-acetyl-serine(thiol)lyase*)]