In Silico GENOME MINING FOR SALINITY-RELATED PATHWAYS IN THE *Citrus sinensis* GENOME

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The cultivation of citrus fruits is an important economic agricultural activity in Egypt. It is also the fourth-largest global exporter of citrus fruits almost 10% of total production (FAO Statistics 2017). The newly reclaimed lands account for 68% of citrus cultivated area of the country (MALR Statistics, 2015). Currently, citrus production in these regions is susceptible to challenges including limited water resources and salinity (Al-Yassin, 2004; de Oliveira *et al.*, 2013; WWAP, 2018). The citrus trees require irrigation by non-saline water since they are salt-sensitive plants (Maas, 1990 & 1993).

Salinity stress reduce citrus tree's growth and productions due to two factors: i) osmotic stress, i.e., the high concentration of salts around plant roots which cause reduction in the plant absorption of free water from the soil across root cells membrane; ii) ion toxicity stress and nutritional imbalance (Murkute *et al.*, 2005). These factors can potentially have far-reaching impacts on citrus production in Egypt and worldwide.

Plants can naturally cope with salinity stress by several mechanisms such as synthesis of compatible solutes to help cellular osmotic adjustments such as proline, glycine betaine, and sugars (de Oliveira *et al.*, 2013; Kumar *et al.*, 2017). Additionally, plants activate different antioxidant enzymes such as reactive oxygen species (ROS) which play important role in cells by scavenging ROS chloroplasts, mitochondria, peroxisomes and plasma membrane. Enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) can circumvent the negative effects of salinity (Hossain and Dietz, 2016). Also, abscisic acid (ABA) production is increased under salt stress to mitigate the inhibitory effect of salinity on photosynthesis, growth and translocation of assimilates (Popova *et al.*, 1995; Jeschke *et al.*, 1997). Meanwhile by the salt-overly-sensitive (SOS) pathway as, the *SOS1* gene is controlling the efflux of Na^+ out of the cytoplasm (Shi *et al.*, 2000) and SOS3-SOS2 kinase complex restricting Na^+ entry into the cytosol by inhibiting HKT1 activity. Moreover, the SOS2 kinase positively regulates the activities of NHX1 (a vacuolar Na^+/H^+ exchanger) by transporting Na^+ from the cytoplasm into the vacuole, and CAX1 (a vacuolar Ca^{2+}/H^+ exchanger) by transporting Ca^{2+} from the vacuole into the cytoplasm (Cheng *et al.*, 2004; Qiu *et al.*, 2004).

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The aim of the present work was to *in silico* mining the *Citrus sinensis* genome for salinity-related pathways and its completion ratio compared to referenced pathways in higher plants, toward a better understanding of the salinity tolerance mechanism in *Citrus* sp.

MATERIALS AND METHODS

Genome mining is carried out to derive various information about salinity tolerance genes based on *Citrus sinensis* genome at Geneious software v10.03. Citrus genome and genes files imported directly from the NCBI (GenBank) databases using the NCBI search interface in the Geneious panel. Five widely detected pathways from different plant models were referenced for genome mining of proline, glycine betaine, ROS, ABA and SOS. Genes were searches by two parallel approaches, a) search by name for already annotated genes and b) search by similarity, using previously published genes from model plants). Finally, the completion of a pathway was determined according to the number of genes found by either name or sequence, divided by the total number of the published genes of a certain pathway from a model plant.

RESULTS AND DISCUSSION

One of salinity tolerance mechanisms in plants is to reduce the osmotic effect, ion toxicity and nutritional imbalance by overproducing compatible solutes such as proline, glycine betaine, polyol, sugars etc. in plant cells and activate antioxidant and salt-overly-sensitive systems (Ashraf, 1994; Ashraf and Harris, 2004; Garcia-Sanches and Syvertsen, 2006; Hossain and Dietz, 2016). This can trigger an influx of water or reduce the ions efflux in root cells (Kavi-Kishor *et al.*, 2005). Accordingly, the results of mining *Citrus sinensis* genome by search on possible salinity-related pathways and its completion ratio were illustrated in Fig. (1) and Table (1). The genes of the proline pathway were found to be 100% complete in orange genome through the two methods of search: by gene name search, where 75% of genes were found and gene sequencing of *A. thaliana*, where 25% of genes were found. Also, the search of glycine betaine (GB) and ABA pathway genes recovered the same trend as proline. But the complete pathways (100%) were obtained by gene name search. These results explained that the organic osmolytes, such as proline and glycine betaine play an important role in maintaining citrus cell volume under salinity stress condition and the vital role of ABA in regulation of many plants physiological processes. Tuteja (2007) found those genes to be important to each of *A. thaliana*, *Oryza sativa* and *Zea mays* for improved salinity tolerance.

On the other hand, the salt overly sensitive (SOS) signaling pathway maintains ion homeostasis in plant under salt stress by regulating the entry of sodium ion to the root cells through inhibition of its entry or reducing its concentration (Yang *et al.*, 2015). However, genes of SOS pathway were not complete in *Citrus sinensis* genome as only 83.33% of SOS pathway genes were found by the usage of gene sequence of *A. thaliana* in search. The *SOS1* gene was not found in orange genome although it plays an important role in plant cells under salinity stress. It is able to maintain low concentration of sodium ion in plant by transport it out of root cells to soil solution (Ji *et al.*, 2013). While, the search process on antioxidant defense systems against (*ROS*) genes were only 64.28% by gene name search in *Citrus sinensis* genome in addition to 28.57% by gene sequence search (being 92.85% as a total) of *Oryza sativa, Zea mays* and *Olea europaea*. The significant effect of *SOS* and *antioxidant* genes in orange under salinity stress are to enhance plant ability to tolerate saline stress, through reducing the level of sodium influx and oxidative stress has been advocated by Cheng *et al.* (2004) and Hossain and Dietz (2016). However, it is possible that SNPs have occurred in each of *SOS1* genes which belongs to SOS pathway and *XOD* gene which belongs to ROS pathway in *Citrus sinensis* genome or could be substituted by other genes. However, this region has not been identified or definied in the *Citrus sinensis* genome yet.

Finally, by the utilization of genome mining we found that genes of each of proline, glycine betaine (GB) and ABA pathways were previously known and were annotated in the *Citrus sinensis* genome. These findings confirmed that these pathways play basic and active role in Citrus under salinity stress. But, *SOS1* gene in SOS pathway and *XOD* gene in ROS pathway are not identified yet. It has been suggested that: i) some alternative genes can play their functions, ii) or the pathways can undertake their roles under salinity stress without their presence, iii) or further function annotation to some regions in *Citrus sinensis* genome is required. Therefore, the present an *in-silico* study of mining the genome of *Citrus sinensis* provided substantial evidence toward understanding some key components of these pathways involved in salinity tolerance. However, discovery and development of genes which ameliorate the adverse impacts of salinity on citrus plant cells for further finetuned study.

SUMMARY

Citrus is an important horticulture genus which exclusively includes all cultivated edible citrus plants and their rootstocks. The genetic relationship and relatedness among the genus member have been extensively reported. Among other traits, the fluctuation in the salinity tolerance ability of several citrus species in relation to other relatives have been shown. The current study aimed to subject the published *Citrus sinensis* genome as a model to find possible salinity-related pathways and its completion ratio compared to some plant models (e.g. *Arabidopsis thaliana*). Toward a better understanding of how some citrus species can cope the high salinity stress for further application at arid and semiarid regions with a special relevant to high-calcic soil and newly reclaimed lands. The genome mining revealed that all genes of each of proline, glycine betaine (GB) and Abscisic

acid (ABA) pathways were previously annotated and identified in the *Citrus sinensis* genome. The SOS and ROS pathway genes did not appear complete in the mining process. SNPs may occur to each of *SOS1* gene which belongs to the SOS pathway and *XOD* gene which belongs to the ROS pathway in *Citrus sinensis* genome or substituted by other genes either copy or allele, thus this region has not been identified or defined in the *Citrus sinensis* genome yet. Thus, an *in-silico* study of *Citrus sinensis* by genome mining for the identification of active pathways which relate with salinity tolerance, the discovery and development of genes which could decrease the damaging effect of salinity on citrus plant cells needed more research and study.

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Table (1): Search results of salinity stress pathways genes (e.g., proline, glycine betaine, ABA SOS, and ROS) in *Citrus sinensis* genome, each of (No, Yes, and —) refer to, not gene found in genome, gene found in genome and search not done, respectively.

Pathway	No.	Gene code name	Gene full name	Search by gene name	Search by sequence
Proline pathway	1	PLC	Phosphoinositide phospholipase C	No	Yes
	2	MYB2	transcription factor	No	Yes
	3	CaM	Ca^{2+} calmodulin	Yes	—
	4	P5CS	1-Pyrroline-5-carboxylate synthetase	Yes	—
	5	P5CR	Pyrroline-5-carboxylate reductase	Yes	—
	6	bZIP	transcription factor	Yes	—
	7	P5CDH	△1-Pyrroline-5-carboxylate dehydrogenase	Yes	—
	8	PDH	Proline dehydrogenase	Yes	—
Complete pathway percentage				75.0%	25.0%
				100.0%	

Table (1): Cont’

GB pathway	1	CMO	Choline monooxygenase	Yes	—
	2	BADH	Betaine aldehyde dehydrogenase	Yes	—
Complete pathway percentage				100.0%	00.0%
				100.0%	
ABA pathway	1	ZEP	Zeaxanthin epoxidase	Yes	—
	2	MCSU	Molybdenum cofactor sulfurase	Yes	—
	3	AAO	Abscisic aldehyde oxidase	Yes	—
	4	NCED	Nine-*cis*-epoxycarotenoid dioxygenase	Yes	—
Complete pathway percentage				100.0%	00.0%
				100.0%	
SOS pathway	1	SOS1	Salt Overly Sensitive 1	No	No
	2	SOS2	Salt Overly Sensitive 2	No	Yes
	3	SOS3	Salt Overly Sensitive 3	No	Yes
	4	NHX	vacuolar Na$^+$/H$^+$ exchanger	No	Yes
	5	HKT1	Na$^+$/K$^+$ transporters	No	Yes
	6	CAX1	H$^+$/Ca^{2+} antiporter	No	Yes
Complete pathway percentage				0.0%	83.33%
				83.33%	
ROS pathway	1	AOX	Alternative oxidase	No	Yes
	2	GO	Glycolate oxidase	No	Yes
	3	PTOX	Plastid terminal oxidase	Yes	—
	4	RBOH	Respiratory burst oxidase homolog	No	Yes
	5	XOD	Xanthine oxidase	No	No
	6	SOD	Superoxide dismutase	No	Yes
	7	APX	Ascorbate peroxidase	Yes	—
	8	CAT	Catalase	Yes	—
	9	DHAR	Dehydro-ascorbate reductase	Yes	—
	10	GPX	Glutathione peroxidase	Yes	—
	11	GR	Glutathione reductase	Yes	—
	12	GST	Glutathione-S-transferase	Yes	—
	13	MDHAR	Monodehydroascorbate reductase	Yes	—
	14	PRX	Peroxiredoxin	Yes	—
Complete pathway percentage				64.28%	28.57%
				92.85%	

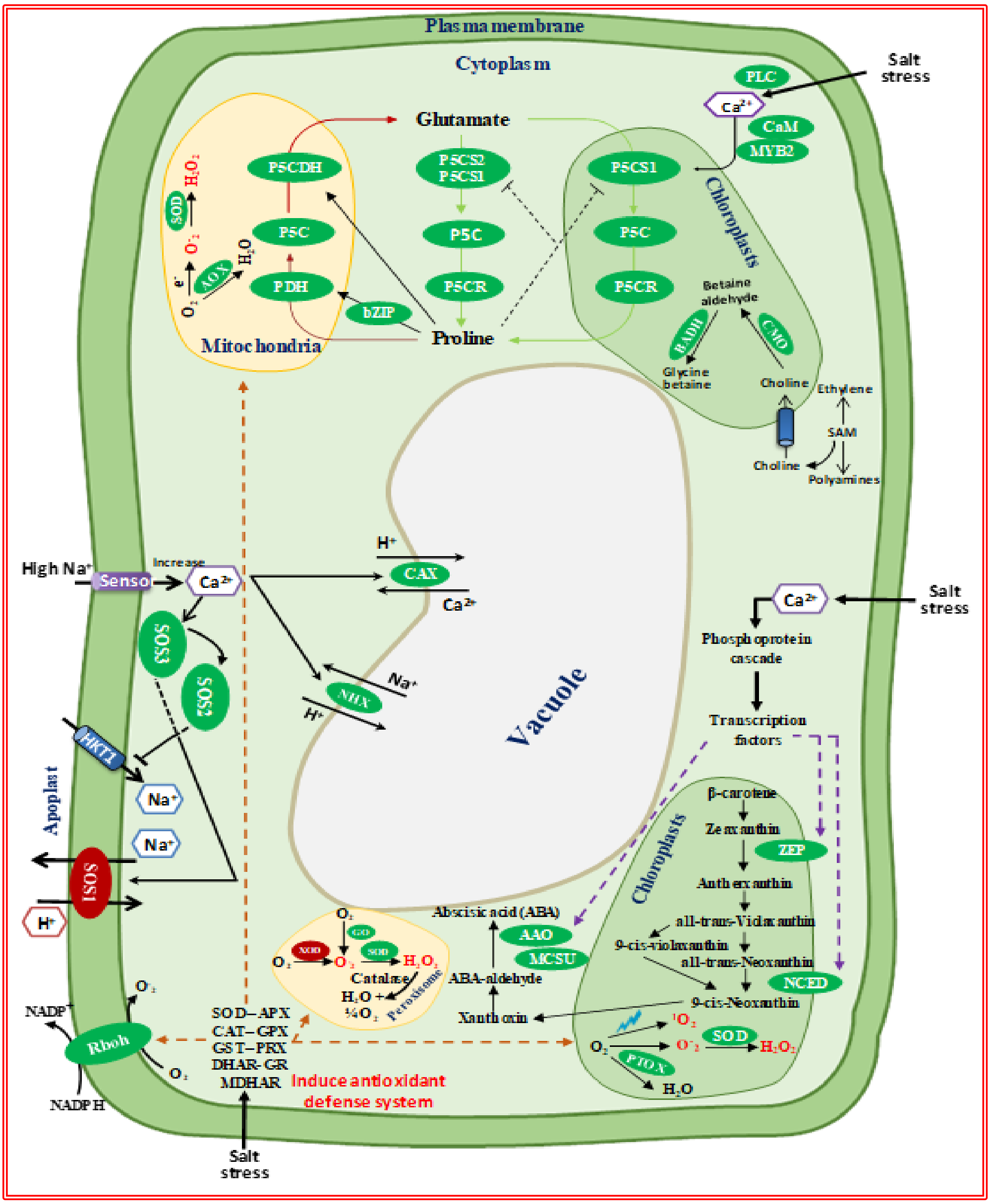

Fig. (1): Search results of salinity stress pathways genes (e.g., proline, glycine betaine, ROS, ABA and SOS) in *Citrus sinensis* genome. Green circle refers to genes that have been detected in orange genome, but red circle refers to genes that have not been detected in orange genome (SOS1 and XOD genes). Adopted from (Chinnusamy *et al*., 2004; Rhodes *et al*., 2004; Tuteja, 2007; Szabados and Savoure, 2009; Hossain and Dietz, 2016).