Research Article

Computational identification and annotation of salt stress responsive genes in *Solanum melongena* L.

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Abstract

Solanum melongena L. (Eggplant) is an important plant of family Solanaceae. It possesses pronounced nutritional value and is a rich source of minerals and vitamins. As a medicinal plant it is used for the cure of numerous diseases. Nasunin, a main component in anthocyanin pigment of eggplant shows free radical scavenging and iron chelating activities. Salinity is the main abiotic stress in agriculture all over the world. The effects of salinity on plants include ion imbalance, oxidative harm, hyperosmotic stress, reactive oxygen species production, fall of leaf water potential, reduced stomatal conductance and inhibition of photosynthesis. This study is an attempt to identify and annotate the potential orthologues of salt stress responsive genes in eggplant by using a variety of bioinformatics tools (BLASTn, ORF finder, CD search and Clustal W). Briefly a total of 20 well annotated, salt responsive stress genes (CSD1, HVA22A, RCI3, SOS4, CDPK1, P5CS1, SAT32, STZ, SOS1, SZF1, ORYSJ, SODM_ORYSJ, G3PC1_ORYSJ, Os PIP1-3, OSISAP1, OsBADH1, Q40710_ORYSJ, ACA5_ORYSJ, SALT_ORYSJ, SAP11_ORYSJ), 10 each from *Arabidopsis thaliana* (The model dicot plant) and *Oryza sativa* (The model monocot plant) were selected through literature survey and their ORF sequences were subjected to BLASTn against the publically available 98,089 ESTs of eggplant. Consequently all 20 genes were predicted in eggplant and classified as highly similar (showing upto 90% query coverage), somewhat similar (showing upto 60% query coverage) and less similar (showing upto 30% query coverage) orthologues. Furthermore the salt stress responsive conserved domains were identified in 18 genes by using CD search tool. Phylogenetic trees were also constructed for some of the identified genes by using Clustal W. EST based identification of these genes confirms the expression of these genes in eggplant which may be helpful to comprehend its acclimation processes.

Keywords: Conserved domains; Salt stress; *Solanum melongena*

Introduction

Eggplant (*Solanum melongena* L.) is important vegetable as it is an affluent source of minerals and vitamins [1]. Grown typically for its fruit, eggplant is designated for the treatment of several ailments like

diabetes, arthritis, asthma, bronchitis [2], toothache, cardiac debility, neuralgias etc [3]. Nasunin and Pheophytin pigments of eggplant have free radical hunting and anti-mutagenic activity [4]. Salt stress is one of the main abiotic stresses faced by plants.

Plants identify the stress signals and pass them to the cellular apparatus and then the adaptation is fulfilled by the regulation of gene expression [5]. Expressed sequence tags (ESTs) produced from cDNA libraries are the best source to find out stress associated genes [6]. Bioinformatics approaches are the renowned ways of research for nucleic acid sequence analysis to discover new genes [7]. The current research is planned at examination of salt stress genes in *S. melongena* by using a variety of bioinformatics tools like BLAST (Basic Local Alignment Search Tool), ORF finder (Open Reading Frame), CD search (Conserved Domain) and Clustal W. Identification of salt stress responsive genes will be a good input for understanding the salt stress responsive method in eggplant.

Materials and methods

A total of 20 well annotated, salt stress responsive genes (CSD1, HVA22A, RCI3, SOS4, CDPK1, P5CS1, SAT32, STZ, SOS1, SZF1, ORYSJ, SODM_ORYSJ, G3PC1_ORYSJ, Os PIP1-3, OSISAP1, OsBADH1, Q40710_ORYSJ, ACA5_ORYSJ, SALT_ORYSJ, SAP11_ORYSJ) were selected from the model plants i.e. *Arabidopsis thaliana* and *Oryza sativa* (10 genes of each) through literature survey [8-21] and an attempt was made to identify the potential orthologues of these genes in eggplant. The main steps of the methodology are discussed below.

Fetching of the sequences of the selected model genes and their ORFs

Gene Sequences of model plants were fetched using NCBI (National Centre for Biotechnology and Information) and saved in FASTA format. To get the exact coding region of the genes, FASTA sequence of each gene was subjected to ORF finder available at NCBI. Longest reading frames for each of the genes were predicted. The nucleotide sequences of the longest ORFs

were then used to find their orthologues in eggplant.

Homology search

BLAST is one of the most excellent tools for homology search [22]. The available 98,089 ESTs (dbEST release 130101, 01 January 2013) of eggplant were computationally screened to search 20 genes. To find out existence of gene, the ORF sequences of every model plant gene were subjected to BLASTn against *S. melongena*. The best matched EST (on the basis of query coverage and percentage identity) were selected and their sequences were saved in FASTA format. The selected ESTs were categorized as highly similar (showing upto 90% query coverage), somewhat similar (showing upto 60% query coverage) and less similar (showing upto 30% query coverage) orthologues.

Finding conserved domains

Conserved domain database (CDD) [23] is another important tool of NCBI. Model plant genes and best matched ESTs of *S. melongena* were subjected to CDD to detect the occurrence and position of conserved domains which perform their function in salinity. With this method conserved domains of the model plant genes were compared with the conserved domains of best matched ESTs of *S. melongena*.

Phylogenetic analysis of genes

Advanced version of Clustal-w called MUSCLE, was used to conduct phylogenetic analysis of some genes. Cladograms were constructed for some of the predicted genes in eggplant and other plants.

Results

Identification of new conserved salt stress genes in eggplant

All the selected (20) salt stress responsive genes were Identified in *S. melongena* through insilico analysis of 98,089 ESTs of *S. melongena*. Homology search of the selected model genes in *S. melongena*

resulted up to 99% query coverage. The identified homologous genes were classified as highly similar, somewhat similar and less similar i.e showing up to 90%, 60% and 30% query coverage respectively. Seven genes (CSD1, HVA22A, RCI3, ORYSJ, SODM_ORYSJ, G3PC1_ORYSJ and Os PIP1-3) were highly similar, five genes (SOS4, CDPK1, P5CS1, OSISAP1 and OsBADH1) were somewhat similar and eight genes (SAT32, STZ, SOS1, SZF1, Q40710_ORYSJ, ACA5_ORYSJ, SALT_ORYSJ and SAP11_ORYSJ) were less similar (Figure1 & 2).

Identification of conserved domains in predicted genes

The conserved domains examination of selected plant genes in *S. melongena*

demonstrated that on 18 genes similar conserved domains were also present in *S. melongena* irrespective of query coverage and identity, excluding two genes (SALT_ORYSJ and SAP11_ORYSJ). (Table 1 & 2).

Phylogenetic analysis of genes

Eight of the salt stress responsive genes (CSD1, HVA22A, SODM_ORYSJ, SODM_ORYSJ, RCI3, SOS4, ORYSJ and G3PC1_ORYSJ) were randomly selected for phylogenetic analysis. Cladograms were constructed by comparing three homolog genes, i.e one from selected model plant, second from *S. melongena* (subject plant) and third one from dicot (in case of *A. thaliana*) and monocot (in case of *O. sativa*). The results are shown in (Figures 3-10).

Figure 1. Homology of *S. melongena* with the Model genes from *A.thaliana*

Figure 2. Homology of *S. melongena* with the model genes from *O. sativa*

Table 1. Comparison of existence and type of conserved domain families between *A. thaliana* and *S. melongena*

S #	Gene name	Conserved domain family	
		<i>A. thaliana</i>	<i>S. melongena</i>
1	CSD1	Cu_Zn superoxide dismutase super-family	Cu_Zn superoxide dismutase super-family
2	HVA22A	TB2_DP1_HVA22 super family	TB2_DP1_HVA22 super family
3	RCI3	plant peroxidase like super family	plant peroxidase like super family
4	SOS4	ribokinase_pfkB_like super family	ribokinase_pfkB_like super family
5	CDPK1	PKc_like super family	PKc_like super family
6	P5CS1	AAK_super family	AAK_super family
7	SAT32	IFRD super family	IFRD super family
8	STZ	zf-C2H2_6 super family	zf-C2H2_6 super family
9	SOS1	Asp-Al_Ex super family	Asp-Al_Ex super family
10	SZF1	ZnF_C3H1 super family	ZnF_C3H1 super family

Table 2. Comparison of existence and type of conserved domain families between *O. sativa* and *S. melongena*

S #	Gene name	Conserved domain family	
		<i>O. sativa</i>	<i>S. melongena</i>
1	ORYSJ	14-3-3 super family	14-3-3 super family
2	SODM_ORYSJ	Sod_Fe_C and Sod_Fe_N super families	Sod_Fe_C and Sod_Fe_N super families
3	G3PC1_ORYSJ	Gp_dh_C and Gp_dh_N super families	Gp_dh_C and Gp_dh_N super families
4	<i>Os PIP1-3</i>	MIP super family	MIP super family
5	OSISAP1	ZnF_AN1 and zf-A20 super families	ZnF_AN1 and zf-A20 super families
6	OsBADH1	ALDH-SF super family	ALDH-SF super family
7	Q40710_ORYSJ	Glyco_tranf_GTA_type super family	Glyco_tranf_GTA_type super family
8	ACA5_ORYSJ	CaATP_NAI and Cation_ATPase_N super families	CaATP_NAI and Cation_ATPase_N super families
9	SALT_ORYSJ	Jacalin_like super family	thioredoxin_like super family
10	SAP11_ORYSJ	TT_ORF1,ZnF_AN1, zf-A20 like super families	AdoMet_MTases super family

Arabidopsis 0.124179
Coffeaarabica 0.102845
Solanummelongena 0.102845

Figure 3. Cladogram for CSD1 gene from showing closeness of *S.melongena* with *C. arabica* as compared to *A.thaliana*

Figure 4. Cladogram for HVA22A gene showing closeness of *A. thaliana* with *S.melongena* as compared to *S. lycopersicum*

Figure 5. Cladogram for SODM_ORYSJ gene showing closeness of *O.sativa* with *Zea mays* as compared to *S.melongena*

Figure 6. Cladogram for RCI3 gene showing closeness of *A.thaliana* with *S.melongena* as compared to *Malus domestica*

Figure 7. Cladogram for SOS4 gene showing closeness of *V. vinifera* with *S. melongena* as compared to *A. thaliana*

Figure 8. Cladogram for ORYSJ gene showing closeness of *O. sativa* with *S.melongena* as compared to *Zea mays*

Figure 9. Cladogram for G3PC1_ORYSJ gene showing closeness of *O.sativa* with *Zea mays* as compared to *S.melongena*

Figure 10. Cladogram for OSPIP1-3 gene showing closeness of *O.sativa* with *S.melongena* as compared to *W. australiana*

Discussion

Bioinformatics approaches are now commonly used by various researchers to

identify stress related genes and proteins in plants [18, 24-26]. Our study resulted in identification of 20 salt stress responsive

genes in eggplant. Even though some genes didn't show a high query coverage but presence of the similar conserved domains in reference sequences and *S. melongena* sequences indicates the potential role of these domains during salt stress. EST analysis is a significant tool to identify new conserved genes [27-30]. This approach provides direct evidence for miRNA expression that cannot be inferred from genomic sequence surveys [31]. All of the salt stress genes have been predicted in ESTs of *S. melongena* which is confirmation of their expression. Phylogenetic analysis of the selected genes shows that salt stress genes may be conserved within monocots and dicots and some genes from monocots may be more close to that of dicot genes as compared to the same gene of another monocot and vice versa. All our results are in agreement with the work of other researchers [32-39] and these findings can be valuable to properly comprehend the salt stress tolerance mechanism in eggplant.

Conclusion

A total of 20 new conserved salt stress genes were computationally predicted in eggplant by data mining of ESTs. Even though all the selected (20) genes didn't show a high query coverage but presence of the similar conserved domains in reference sequences and *S. melongena* sequences indicates the potential role of these domains in salt stress. These findings will be helpful in studying the gene expression profiling of eggplant.

Authors' contributions

Conceived and designed the experiments: IA Baloch, Performed the experiments: B Sadia, Analyzed the data: B Sadia & W Noor, Contributed materials/ analysis/ tools: ZA Bazai & SS Qambrani, Wrote the paper: B Sadia.

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