A novel phytoene synthase paralog from halophytic *Salicornia* confers salinity tolerance in plants

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Abstract: *Salicornia* seems to be a suitable euhalophyte for the study of salt stress resilience mechanism and exploitation of salinity-tolerant genes for crop yield improvement. In this study, we cloned a novel SePSY1 gene from an extremely salt-tolerant glycophyte, *Salicornia europaea*. The sequence analysis revealed that the newly isolated gene is a paralog of PSY, suggesting a gene duplication event in *Salicornia*. The newly isolated gene encodes a protein of 419 amino acids. The C-terminus containing a Trans-IPPS-H domain with catalytic and binding regions exhibits considerable conservation in various species, while the N-terminus remained divergent. The structural analysis suggested a catalytic role of the C-terminus in the first step of carotenoid biosynthesis, whereas N-terminus is involved in the transport and localization of protein. Gene expression using real-time RT-PCR revealed enhanced transcript signals for salt-treated plants. Transgenic *Arabidopsis* plants overexpressing SePSY1 could withstand 200 mM salt stress in comparison with the wild-type counterpart. Thus, SePSY1 confers salinity stress tolerance in the heterologous system. Strength of gene expression is positively correlated with severity of phenotypes. Our results reveal that the SePSY1 gene can be a potential target for improving salinity tolerance in crop plants that are susceptible to this stress.

Key words: Halophyte, *Salicornia europaea*, phytoene synthase, salinity, gene expression

1. Introduction

Among abiotic stresses, salinity and water scarcity adversely affect plant development, growth, and eventually crop productivity. Due to salinity, plants undergo osmotic and ionic imbalances that lead to impairment of vital cellular functions (James et al., 2011). Salinity along with osmotic and ionic stresses causes oxidative imbalances because of the formation of reactive oxygen species (ROS), which are harmful to macromolecules, nucleic acids, proteins, and lipids, thus affecting the physiological functions of plants (Zhu, 2002; Meriga et al., 2004; Mishra et al., 2011). Oxidative defense mechanisms have evolved to reduce the toxic effects of oxidative radicals, which may be either enzymatic or nonenzymatic (Gill and Tuteja, 2010). Among the nonenzymatic antioxidants carotenoids are yellow, orange, and red pigments synthesized and accumulated in plastids and involved in effective elimination of ROS along with light harvesting in photosynthesis (Tao et al., 2007). The first step in the biosynthesis of carotenoids involves condensation of two molecules of geranyl geranyl diphosphate (GGDP), which are catalyzed by phytoene synthase (PSY) producing the first compound, known as phytoene. The gene encoding the phytoene synthase has been isolated and characterized from different monocots and dicots. The number of paralogs of the PSY family varies between species, ranging from a single copy in *Arabidopsis* (AtPSY, At5g17230) to six members in *Brassica napus* (López-Emparán et al., 2014). These various copies of a gene are the outcome of duplication events.

A number of studies have been conducted on different plant species to improve carotenoid contents by genetic modification of carotenoid biosynthesis pathways (Sandmann, 2001), but research work on improvement of salt tolerance in plants, especially wild halophytes, through genetic manipulation is scarce.

*Salicornia europaea*, belonging to the family Amaranthaceae, is a succulent euhalophyte growing widely in coastal and saline marshes. It is an extremely salt-tolerant plant as it can grow in a wide range of NaCl concentrations, and accumulates Na⁺ up to 50% of dry weight in its shoots without salt glands and salt bladders (Ushakova et al., 2005), suggesting that there are special mechanisms in S.
europaea to deal with high salt concentrations and damage caused by ROS. Previously a PSY gene was cloned from *S. europaea* but details about its complete functional characterization and gene expression patterns remained elusive. Therefore, isolation, cloning, and functional characterization of the PSY gene of *S. europaea* is of vital importance to understand its role in carotenoid synthesis, which is involved in improving salt tolerance by scavenging of ROS and protecting plants from oxidative damage. In the present study, we isolated and cloned a new diverged paralog of SePSY1 and transformed it into *Arabidopsis*. The data revealed that this gene confers salinity tolerance in heterologous hosts though enhancements of transcript expression under salinity stress applications.

2. Materials and methods

2.1. Plant material

The seeds of *Salicornia europaea* L. were obtained from the coastal area of Sindh Province in Pakistan during the PARC *Salicornia* project. Under suitable growth conditions seeds were germinated in pots containing soil in the glasshouse of the National Institute for Genomics and Advanced Biotechnology, NARC, Islamabad, Pakistan. Three-month-old seedlings were shifted to hydroponics containing Hoagland solution and treated with 0 M, 0.25 M, 0.5 M, 0.75 M, and 1 M concentrations of NaCl for 48 h. Spikes of all control and treated plants were harvested and stored at –80 °C until DNA/RNA extraction was performed.

2.2. Molecular cloning of the SePSY1 gene

The nucleotide coding sequence of the PSY gene (Accession No. AY789515.1) was retrieved from the National Center for Biotechnology Information (NCBI) database. SePSY1 gene-specific primers were manually designed for gene isolation, expression, and vector construction by gateway cloning strategy (Table). Total RNA from spikes of control and NaCl-treated plants was extracted by using an RNA extraction kit following the manufacturers’ instructions (Ambion Life Technology). Next 1 µg/µL of total RNA of control and NaCl-treated plants was used for construction of cDNA libraries by employing a Fermentas RevertAid M-MuLV Reverse Transcriptase first strand cDNA kit (#1621). First strand cDNA was used as template to amplify the full length coding sequence of the PSY1 gene by using rTaq polymerase (TaKaRa, Tokyo, Japan; Code R001) in PCR. The PCR was carried out using the following profile: 95 °C for 5 min, followed by 94 °C for 30 s, 54 °C for 40 s, and 68 °C for 1 min for 40 cycles. The PCR products were resolved on ethidium bromide stained 1% agarose gel and photographed. The purified amplicons were cloned in pTZ57R/T vector (Thermo Scientific Insta TA clone #K1214) following the manufacturers’ instructions and sequenced. The newly isolated sequence of the SePSY1 gene was submitted to NCBI-GenBank under the accession no. KR732931.

2.3. Construction of expression vector and generation of transformants

To generate the p35S::SePSY1-mYFP construct, the full length coding sequence was amplified from the already cloned SePSY1 gene in pTZ57R/T plasmid with gene specific primers having additional attB sites at the 5’ end. These fragments were recombined with pDONR 201 (Invitrogen) by BP recombination and later with destination vector pXCSG-mYFP (Feys et al., 2005). The resultant expression plasmid p35S::SePSY1-mYFP driven under 35S promoter was transferred into *Agrobacterium*.
tumefaciens GV3101 (PMP90RK) strain. Arabidopsis thaliana ecotype Columbia 0 (Col-0) at the flowering stage was transformed by the floral dipping method described by Clough and Bent (1998). Transgenic plants were screened by spraying 250 mg/L BASTA twice a week. To develop homozygous lines for SePSY1, transgenic plants were screened for the subsequent two generations.

2.4. Southern blot analysis
Southern analysis was done to validate the genotypes of Arabidopsis transgenic lines stated in this study. For this purpose the 3’ terminal variable fragment of the SePSY1 gene was selected as a probe sequence from Salicornia. Furthermore, hybridization was performed for wild-type Arabidopsis plants. Genomic DNA (10 µg) was digested with KpnI and NotI enzymes according to the manufacturer’s instructions (Fermentas). Size-separation of fragments was done on 0.8% agarose gel and Hybond-N+ membrane was used for blotting. Preparation of the radiolabeled probe, blot, hybridization, and processing of Southern blot was done according to Khan et al. (2009).

2.5. Salt tolerance assays in transgenic Arabidopsis
In order to analyze the salt tolerance potential of transgenic plants, we conducted experiments to observe the effect of NaCl on seed germination, primary root length, and chlorophyll contents of both wild-type and T3 transgenic Arabidopsis lines. For this purpose, sterilization of Arabidopsis seeds was performed with ethanol (70%) for 1 min followed by 50% Clorox bleach for 10 min and rinsing six times with sterilized distilled water. After sterilization approximately 200 seeds were stratified on petri plates containing ½ MS (Murashige and Skoog, 1962) solid medium supplemented (0 mM, 50 mM, 200 mM) in three replicates and plates were kept under optimum conditions, i.e. temp- 22 °C and 16 h/8 h light/dark photoperiod. After sowing, the rate of germination was scored until day 10. For measuring the primary root length 5-day-old seedlings of wild-type and overexpressing lines were shifted onto a MS salts mixture supplemented with NaCl at 0 mM, 50 mM, 80 mM, 120 mM, and 150 mM concentration. Root length of seedlings grown in normal and stress conditions was measured in triplicate after day 7 of seeding shift.

For measurement of chlorophyll contents, 15-day-old seedlings of wild-type and 35S::SePSY1 overexpressing lines were separated separately to simple MS medium without salt as well as MS medium containing 50 mM 80 mM, 120 mM, and 150 mM of NaCl. After 7 days leaves of all the samples were collected from wild-type and transgenic lines. Total chlorophyll contents were extracted using ethanol in triplicate and the absorbance measured with a spectrophotometer. Data were recorded in means of triplicates with ±SD. In addition, the salt tolerance level of wild-type and transgenic lines was assessed by watering 3-week-old plants with NaCl solution of 50 mM, 100 mM, 150 mM, and 200 mM concentrations. Plants were irrigated with salty solution twice a week. Finally photographs were taken to observe the phenotypes of treated plants.

2.6. Gene expression using real-time RT-PCR
The estimation of relative transcript abundance of the SePSY1 gene in control and 250 mM, 500 mM, 750 mM, and 1000 mM NaCl-treated Salicornia plants was done by real-time RT-PCR. The Applied Biosystems StepOnePlus Real-Time PCR system was employed for this assay. The real-time PCR was carried out by adding 2x Maxima SYBR Green (Fermentas), gene-specific primers, and template cDNA. To normalize the reaction, the 18S rRNA gene was used as endogenous reference. A no-template control (NTC) was used in the assay and the reactions were performed in triplicate. The amplification profile used comprised initial polymerase hot start activation 95 °C for 10 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 45 s. The fluorescence data were recorded after each cycle.

2.7. Sequence analysis
A homology search was performed using nucleotide BLAST in the NCBI (http://www.ncbi.nlm.nih.gov/blast/) database. Multiple nucleotide and protein sequence alignments were generated with ClustalW in BioEdit software. It is demonstrated that a phylogenetic network could better reveal evolutionary relationships than a phylogenetic tree-like structure (Huson and Bryant, 2006). Hence, a neighbor-net network reconstruction analysis (Bryant and Moultron, 2004) was executed in SplitTree4 software (Huson and Bryant, 2006). The Chlorop 1.1 Server (http://www.cbs.dtu.dk/services/ChloroP/) was employed for prediction of chloroplastic transit peptide. For prediction of the transmembrane region of protein, MEMSAT3 and MEMSAT-SVM software were used. The primary structure of protein motif were predicted by GenomeNet Database Resources, sequence motif search MOTIF, www.genome.jp. ExPASy ProtParam tool (Gasteiger et al., 2005) was employed to determine the physical and chemical characteristics of PSY1 protein. Protein localization was determined by WoLFPSORT (Horton et al., 2007). The secondary structure was determined by PSipred server (McGuffin et al., 2000). MODELLAR 9.10 homology modeling program was used for predicting three-dimensional (3D) structures. The 3AE0 selected a correct template from the protein data bank (PDB). The predicted 3D structure was pictured by Chimera 1.6.

3. Results
3.1. The newly isolated SePSY1 from Salicornia is a novel gene sequence
A full length CDS of SePSY1 was amplified from S. europaea by RT-PCR (Figure 1a). A BLAST search in the GenBank
Both the sequences share 94% identity for a nucleotide length of 1259 bp. As the newly isolated sequence lacks deletions or insertions, the size of gene (1259 bp) and protein (419 amino acids) remained conserved. The newly isolated SePSY1 gene contains 71 nucleotide substitutions and there are 20 amino acids that are different between the two sequences (Supplementary Figure 1; Figure 1b). Substitution mutations are not located in a specific domain but are randomly distributed over the entire length of the sequence. These substitutions might not affect the protein functions as we found no change in open reading frame or tri-dimensional protein structure. Hence these mutations fit into the nonsynonymous type.

Multiple protein alignment revealed that the Trans_IPPS_H domain present in the C-terminus is conserved in all aligned homologs. The SePSY1 contains a single Trans-Isoprenyl Diphosphate Synthases (Trans_IPPS_H) domain. Only nonsynonymous substitution mutations of 12 amino acids were observed in the Trans_IPPS_H domain and Aspartate-rich region 1 (DELVD), Aspartate-rich region 2 (DVGED), and two squalene-phytoene synthase motifs were also located in this domain (Figure 1b). Structural analyses indicate that there is a great variability in the N-termini of all the homologs (Supplementary Figure 2). This might indicate that this region of the enzyme is not involved in catalytic activity. Furthermore, the variability in the N-terminus of the PSY
gene possibly underlies variations that might be implicated in functional divergence.

3.2. SePSY1 originated through gene duplication in Salicornia

Evolutionary history could be better revealed by phylogenetic networks than a phylogenetic tree. Hence the evolutionary history of PSY genes of Salicornia was determined by neighbor-net network analysis (Figure 2). Nucleotide coding sequences of the PSY gene family from different monocots and dicots plant species were retrieved from the NCBI database: Salicornia europaea (SePSY-AY789515), Arabidopsis thaliana (AtPSY-AAA32836), Manihota esculenta (MePSY1-ACY42666, MePSY2-ACY42670), Solanum lycopersicum (SIPSY1-P08196, SIPSY2-ABV68559, SIPSY3 XP004228928), Oryza sativa (OsPSY1-AAS18307, OsPSY2-AAK07735, OsPSY3-ABC75828), Zea mays (ZmPSY1-P49085, ZmPSY2-AAQ9183, ZmPSY3-ABC75827). Both the PSY genes of Salicornia clustered together, which may indicate a gene duplication event or very varied alleles exist in this species. Significant variations in these PSY paralogs evidently suggested the duplication or diversification of PSY in Salicornia analogous to PSY paralogs in other plant species. Since Salicornia is a diploid, this duplication is most probably the consequence of gene duplication rather than genome duplication. Moreover, the smaller branch length might hint at a recent doubling event.

3.3. SePSY1 contains various structural attributes

The ExPASy ProtParam tool was used to determine the physical and chemical characteristics of the newly isolated SePSY1 protein. The SePSY1 protein has a molecular weight of 47.1 kD, a 9.03 theoretical isoelectric point (pI), and 88.95 aliphatic index. It consists of 53 negatively charged (Asp+Glu) and 61 (Arg+Lys) positively charged amino acids residues with several hydrophilic and hydrophobic regions; Leu (L) 45 high in no forming (10.7%) of all amino acid composition followed by Ala (A) 39, 9.3%. The instability index II is computed as 50.39, which classifies the protein as unstable with a considered methionine N-terminus. Aliphatic index is 88.95. WoLFPSORT predicted that SePSY1 is possibly located in the chloroplast.

The Trans-Isoprenyl Diphosphate Synthases (Trans_IPPS) conserved domain was detected through the conserved domain database (CDD) search tool in NCBI. It is predicted that the SePSY1 protein belongs to the superfamily of IPPS and class I terpene cyclases and the Trans-IPPS domain is subdivided into six regions. These regions are substrate binding pocket, Mg2 binding site, active site lid residues, catalytic residue, aspartate-rich region 1, and aspartate-rich region 2 (Supplementary Figure 3a). Aspartate-rich region 1 has 167-DELVD-171 while aspartate-rich region 2 contains 293-DVGED-29. Two squalene-phytoene synthase motifs, 245-YCYYV AGTVGLMSVP-260 and 281-LGIANQLTNILRDVGEDARRGRVYLP-306, were predicted by the GenomeNet Database (Supplementary Figure 3b).

The Sec structure predicted by the PSIPred tool indicated that 419 amino acid peptide of PSY gene contains 212 random coil 50.5%, 207 alpha helix 49.4%, and 6 extended strands 1.43% (Supplementary Figure 4). The tertiary structure of SePSY1 was also constructed by MODELLAR 9.10. Supplementary Figure 5a reveals that the protein is constituted by a single polypeptide chain composed of alpha helix and beta coils like secondary structure but no
strand element was identified by MODELLAR. Chlorop 1.1 Server was used to predict chloroplastic transit cTP sequence, which consisted of 1–65 N-terminal residues. To find the transmembrane region MEMSAT3 and MEMSAT-SVM software were used. The carboxy-terminus is present in the cytoplasm, 246–261 amino acid residues cross the membrane and constitute the transmembrane region, while the N-terminus having 1–40 amino acids signal peptide resides in the extracellular fluid (Supplementary Figure 5b).

3.4. The SePSY1 gene is strongly expressed under salt-stress conditions
Gene expression analysis in native plants indicated that SePSY1 expression appeared to be much stronger in spike tissues of the NaCl-treated plants as compared to control plants (Figure 3a). The expression of transcripts in T1 (0.25 M), T2 (0.5 M), and T3 (0.75 M) salt-treated tissue was significantly higher than in control plants. In contrast, transcript accumulation of SePSY1 in T4 (1 M) NaCl-treated plant tissues was lower than the control, suggesting downregulation of the PSY gene at higher NaCl concentration. The quantitative real-time RT-PCR result of SePSY1 in transgenic Arabidopsis is shown in Figure 3b. It is evident that expression of the SePSY1 gene was higher at 80 mM NaCl concentration but drastically reduced at 50 mM, 100 mM, and 150 mM NaCl concentrations in comparison with control plants. These findings suggest that expression of the SePSY1 gene is not

Figure 3. Expression analysis by real-time PCR (a) Native relative expression levels SePSY1 of S. europaea in the spikes of controlled and 48 h salt-treated plants. (b) Heterogeneous relative expression levels SePSY1 in A. thaliana in leaves of controlled and NaCl treated plants. Bars represent relative expression levels (ΔCT mean ± SD of 3 replicates) of SePSY1 gene normalized by 18S rRNA as an endogenous control.
only induced but upregulated with a moderate level of salt stress administration. It is speculated that upregulation in expression might lead to enhanced level of carotenoids synthesis and increased photosynthesis.

3.5. SePSY1 confers salinity tolerance in transgenic plants in the heterologous system

In order to determine the copy number of SePSY1 in transgenic Arabidopsis thaliana T2 plants, genomic DNA of 6 overexpression lines along with WT control was digested with two restriction enzymes, and Southern hybridization was done (Figure 4). In 3 DNA samples (Oe11, Oe12, Oe16) single hybridization signals were observed. However, in Oe14 and Oe16 multiple signals were detectable, indicating possible double integration of SePSY1. In contrast, no signals were visible in Oe13 and Oe15 along with WT control. Nevertheless, the presence of signals confirmed the stable integration of the foreign gene into the genome of Arabidopsis.

The A. thaliana transgenic plants at T3 generation were further validated for response to salt tolerance on different NaCl levels along with wild-type plants. For this purpose germination rate, primary root length (Figures 5a and 5b) and chlorophyll contents of wild-type transgenic plants were determined. The seed germination rate of wild-type and overexpressing transgenic lines was almost the same on simple MS medium without NaCl while on 50 mM NaCl the germination rate was significantly reduced. However, on 200 mM, no germination of wild-type seed was observed and transgenic seeds could germinate with the germination frequency of 48.5% (Figure 5c), indicating that germination of transgenic seeds was less affected by NaCl as compared to the wild-type seeds. The result obtained in the case of primary root length (Figure 5d) demonstrated that there is no significant difference in primary root length of wild-type and transformed seedlings under normal conditions. In both WT and transgenic seedlings a reduction in primary root length was observed at higher stress implication of NaCl, while transgenic seedlings exhibited longer primary roots in comparison with wild-type. A gradual reduction in total chlorophyll contents level was detected with increased dose of salt for both the transgenic and wild-type plants (Figure 5e). Transgenic plants showed higher chlorophyll contents than wild-type under normal and stress conditions. Moreover, transgenic plants exhibited salt tolerance when watered with NaCl solution. Phenotypic variations in growth were also observed. Wild-type plants showed chlorosis and stunted growth at 200 mM NaCl as shown in Supplementary Figure S6, but no phenotypic change was observed in wild-type and transgenic plants under normal conditions. These results unequivocally demonstrate that transgenic plants exhibited better growth than wild-type in a salty environment.

4. Discussion

Elevated level of ROS produced under salt stress causes damage to plants. Carotenoids play a vital role in elimination of ROS by imparting tolerance against oxidative stress. Early breeding and modern genetic engineering approaches mainly focused on improving β-carotene in crop plants to reduce vitamin A deficiency. Golden rice is a glaring example of this promotion (Ye et al., 2000; Paine et al., 2005). Other metabolically manipulated plants for carotenoid improvement are tomato (Fray et al., 1995) canola (Shewmaker et al., 1999), wheat (Cong et al., 2009), potato (Romer and Fraser, 2005), and soybean (Kim et al., 2012). However, knowledge about the role of genes involved in carotenoids biosynthesis for induction of salt
The occurrence of gene duplications in stress-related gene families is not well described though these are important in expression divergence evolutionary fitness of a gene. In the present study, we isolated the SePSY1 gene from *S. europaea*, which seems to be originated through gene duplication. *S. europaea* is distinct from other glycophytic plants in exhibiting independent evolution of the PSY gene in monocots and dicots as indicated by Han et al. (2008). The role of gene and genome duplication in stress is demonstrated by other scientists as well (Tu et al., 2014). Duplication of the PSY gene has already been reported in both monocots and dicots, being more prevalent in monocots. In the present study the existence of two PSY paralogs undermines the occurrence of gene duplication since Salicornia is a diploid (2n = 18) plant. Previously, the PSY paralogs were reported to be recruited in the stress-induced production of abscisic acid (ABA) in roots (Li et al., 2008; Welsch et al., 2008; Arango et al., 2010).
Plastids are carotenoids biosynthesizing organelles located in thylakoid membranes (Andrade-Souza et al., 2011). It was reported that PSY of Arabidopsis and PSY1 of *S. lycopersicum* are associated on the side lines of the thylakoid membrane (Fraser et al., 2000). We predicted transmembrane domain present between 246 and 261 amino acids, a chloroplastic transit cTP sequence at the N-terminus from 1–65 residues having 1–40 signal peptide for targeting to plastids, and determined the protein localization in chloroplast. Further support is provided by multiple alignments of protein sequences, great variability was found at the N-terminus, and this part is not involved in catalytic function and is responsible for plastid localization, which is in accordance with the suggestion of Hsieh and Hsieh (2015). These results might allow us to speculate that SePSY1 protein is transported, located, and bounded in the membranes of plastids (Han et al., 2008). However, for localization studies the transformation of the SePSY1 gene in plants with a fluorescence reporter such as YFP is inevitable.

Protein alignments of SePSY1 revealed variability at the N-terminus region. It was demonstrated by Cheng et al. (2006) that the N-terminus is essential for protein targeting and dimerization, which are crucial for stability and activity of enzyme. Furthermore, the N-terminus diversity may provide specific attributes to different members of PSYs of various species regarding targeting of protein, processing, and formation of functional complex (Kim et al., 2003). In *Dunaliella bardawil* and *D. salina* it was suggested that variation at the N-terminus may be accountable for fine tuning of PSY in elucidating the regulation mechanisms. However, the C-terminus of the PSY protein is special and contains the Trans-IPPS-H domain having aspartate rich region I, II (DELVD) and (DVGED) as conserved motifs as well as a substrate binding domain with a catalytic function (Lao et al., 2011).

Response to stress tolerance and expression of PSY paralogs varies greatly with respect to tissues and crop. In maize the PSY3 is upregulated in roots in response to drought, salt, and exogenous application of ABA (Li et al., 2008). Under salt-stress applications, the upregulation of PSY and other carotenoids synthesizing enzymes was observed in roots of *Arabidopsis*, indicating that the increased accumulation of carotenoids in roots might be due to an increase in the metabolic flux to the carotenoid pathway assisted by higher PSY expression levels (Ruiz-Sola et al., 2014). In rice at 250 mM concentration of salt, the level of PSY3 transcripts was enhanced in roots compared to PSY1 and PSY2 paralogs. For example, the time lines for the induction of PSY3 under salt stress were the same for both the rice and maize PSY3 orthologs but in roots severe response to stress (20-fold) for rice OsPSY3 was observed than ZmPSY3 (6 fold). In leaves OsPSY3 was responsive while ZmPSY3 was not at all (Welsch et al., 2008).

We have attempted to reveal expression of SePSY1 transcripts in *Salicornia* under different salt stress regimes. Upregulation was found at moderate to high salt stress administration while extremely higher salt application downregulated gene expression. The data allow us to speculate that stronger expression of SePSY1 reinforces the activity of antioxidant enzymes that play a crucial role in ROS scavenging activity, thus rescuing plants from salt-stress damage.

**References**


Figure S1. Nucleotide sequence alignment

By using BioEid software ClustalW alignment of newly isolated SePSY1 gene and its homolog SePSY gene was done. Nucleotides in different colors highlight substitutions and in black show similarities.
Figure S2 Multiple alignment of protein, the conserved Trans_IPPS_HH domain found in PSY. Comparison of deduced amino acid sequence of SePSY1 gene with its homologs indicated a functional conserved domain Trans_IPPS_HH, which lays in the C-terminus from 132 aa to 407 aa marked with a green line below. Two Aspartate rich I and II regions (DELVD 167-171, DVGED 293-297) are indicated by red blocks. To maximize sequence homology gaps are introduced and are shown by dashes. Amino acid residues identical in all sequences are highlighted in black; less conserved residues appear in different colors on white background.
Figure S3. Conserved domain and motif search

**a)** Conserved domain in PSY detected by NCBI Conserved Domains Search. There is a conserved domain homologous with the Trans_IPPS_HH in SePSY1 from 128 aa to 395 aa, which consists of six motifs: substrate binding pocket, Mg2+ binding site, active site lid residues, catalytic residues, and two aspartate-rich sites.

**b)** SePSY1 amino acid sequence. Trans-Isoprenyl Diphosphate Synthases (Trans_IPPS) domain 128-395 aa residue are shown in gray background. Motif 1: Squalene and phytoene synthase 245-260, Motif 2: Squalene and phytoene synthase 281-306 are indicated by red bold aa residues.
Figure S4. Secondary structure of SePSY1 predicted by PSIPRED server (McGuffin et al., 2000), sixteen helices are shown in pink rod-like structures, 2 strands are represented by yellow arrows, and nineteen coils are indicated by straight black lines.
Figure S 5 (a) The tertiary structure of SePSY1. Comparative modeling was performed using Modeller 9.10. The structure was visualized by RasMol as ribbons (the secondary structures). The Alpha helix and Beta coils regions of the putative protein are shown with ribbons (green and orange) and bands (green), respectively. (b) A transmembrane region between amino acid 246 and 261 residues was detected by MEMSAT3 and MEMSAT-SVM Program. A 1-40 amino acids signal peptide at the N-terminus is located extracellularly.
Figure S 6. Phenotypic variation in transgenic and wild-type plants.
Under control condition there is no phenotypic difference in transgenic and wild-type plants (A). At 150 mM and 200 mM NaCl stress, wild-type became chlorotic with stunted growth while wild type-plants exhibited better growth.