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SA conceptualized the study, performed formal analysis, data curation, and wrote the original draft. PJ contributed to formal analysis and visualization. OA was responsible for data interpretation. SUK and LUK provided supervision, project administration, review, and edited the final manuscript.

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Characterization of the *AcatSOS* Gene Family Under Salinity Stress in *Areca catechu*

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Abstract:

The salt-overly sensitive (*SOS*) gene is a signalling gene that helps plants to respond to high salinity stress. It is activated when cells are exposed to high levels of sodium ions, leading to changes in gene expression. The *SOS* genes are responsible for salt stress, however, the *SOS* gene in Areca palm has not been studied, despite being present in various plant species. We identified 10 *AcatSOS* genes, dispersed across 5 chromosomes in the genome of *Areca catechu*. Using phylogenetic analysis of *AcatSOS, OsSOS* and *AtSOS* genes, we divided the tree into 5 subfamilies: Group 1, Group 2, Group 3, Group 4, and Group 5. We identified each gene in the *AcatSOS* family based on its physicochemical properties. The plastid and mitochondria contain most of the localization signals. Furthermore, the promoter sequences of the *AcatSOS* genes contained numerous cis-acting areas and light-responsive elements. Areca palm genome has segmental and tandem duplications, providing evidence of a major driving force in the evolution of the *AcatSOS* gene family. Areca palms share numerous genes, in terms of orthologous relationships, with other monocotyledonous plants because of their evolutionary affinity and related heritage. In silico expression identified that *AcatSOS* genes regulate leaf, endocarp, and epicarp development, according to circular heat. *AcatSOS*-4, *AcatSOS*-6, and *AcatSOS*-8 are highly regulated in plants. Genome-wide characterization of *AcatSOS* gene and in-silico expression patterns has revealed that these genes are regulated in response to salt stress. This research will offer a platform to further investigations on the role of *AcatSOS* genes in functioning of Areca palm under salinity stress.

Keywords: *SOS* gene, *Areca catechu*, salinity, phylogenetic analysis, gene expression, abiotic stress.

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INTRODUCTION

Environmental adaptability continuously poses a challenge to plants by disrupting the cellular homeostasis of plants. Salinity is one of the most severe abiotic stresses that affects osmotic balance, ion distribution, nutrient uptake, and cellular metabolism. Sodium chloride concentrations are high in the soil, reducing water to the roots of plants, leading to osmotic stress, and Cl^- accumulates in the soil (Flowers and Colmer, 2008; Munns and Tester, 2008). It also creates oxidative stress caused by salinity, which effect reactive oxygen species and leads to the destruction of proteins, lipids, nucleic acids, and other essential cellular elements (Deinlein *et al.*, 2014; Zhu, 2001). It can cause excessive accumulation of certain molecules, disrupting homeostasis (Kaur *et al*., 2013).

Excessive salt can inhibit enzyme activity, damage metabolism, and impair cell growth and development (Flowers & Colmer, 2008). Previous studies have found that salt stress delays the differentiation and growth of plant organs and tissues through three main mechanisms: ion toxicity, osmotic stress, and nutritional imbalance, which further inhibit whole-plant growth and development (Zhu, 2002). Plant salt tolerance mainly involves three aspects: maintaining cell homeostasis (including ion and osmotic balance), detoxification (such as removal of reactive oxygen species), and growth regulation (such as cell division and stretching) (Zhu, 2001, 2002). When plants are exposed to salt stress, the salt overly sensitive (SOS) pathway is activated. This pathway helps regulate ion homeostasis and improve plant sodium tolerance (Zhu, 2001). The SOS signaling pathway was first discovered in the model plant *Arabidopsis thaliana (Zhu, 2000)*. SOS pathway has been shown to be active in a variety of plants, including rice and tomato (Martínez-Atienza *et al*., 2007; Olias *et al*., 2009). The SOS pathway comprises three genes: *SOS1*, *SOS2*, and *SOS3*. *SOS1* encodes a Na^+/H^+ antiporter that pumps sodium ions out of cells. *SOS2* and *SOS3* do not directly transport Na^+, but instead regulate the ion-transporting activity of *SOS1* (Ji *et al*., 2013). The *SOS* gene family in Arabidopsis includes *SOS2* and *SOS3*, which are located in the cytoplasm. They regulate K^+ and Na^+ homeostasis inside and outside the cell by regulating *SOS1* at the plasma membrane. This can increase the salt tolerance of plants. The *SOS1* gene is a salt-tolerance effector gene that encodes a plasma membrane Na^+/H^+ antiporter. The activity and expression of *SOS1* are controlled by the Ca^{2+} responsive *SOS3-SOS2* genes kinase complex (Shi *et al*., 2000). Some research suggests that the SOS3-SOS2 kinase complex may directly increase Na^+/H^+ exchanger activity, which could regulate sodium flow across the cell membrane (Zhu, 2002). Moreover, AtHKT1, an Arabidopsis Na^+-preferential transporter, primarily controls Na^+ delivery to the roots and shoots (Rus *et al*., 2001). The hypersensitivity of *SOS3* and hkt double mutants to Na^+ was significantly lower than that of *SOS3* single mutants, suggesting that the *SOS3-SOS2* complex may negatively regulate Na^+ delivery. In addition, the activities of H^+-ATPase, H^+PPase, and Na^+/H^+ reverse transporters on the vacuolar membrane may also be regulated by the *SOS3-SOS2* complex (Shi *et al*., 2003).

Areca catechu, commonly known as areca palm, is one of the most significant tropical industrial crops (Henderson, 2009). *Areca catechu* (*2n = 32*), is also called areca nut or betel nut, is a member of the genus Areca in the family *Arecaceae* (Alam *et al.*, 2024). It is a tropical plant commonly grown in the tropics and used in both industry and agriculture. *A. catechu* genome is 2.59 Gb in size, which consist of 31,571 protein-coding genes, of which 92.92% were functionally annotated. The genome also contained many transposable elements, which are mobile genetic elements that can insert themselves into the genome. The areca genome is enriched in genes involved in the biosynthesis of flavonoids, anthocyanins, and monoterpenoids. These compounds contribute to the characteristic taste and aroma of areca nuts. Sequencing the areca genome has provided a valuable resource for researchers studying the biology and genetics of this

important plant. The genome can be used to identify genes that are involved in the development, growth, and reproduction of areca nuts. It can also be used to develop new varieties of areca nuts with improved traits, such as resistance to diseases and pests (Alam *et al.*, 2025).

In this study, we have performed genome-wide analysis and in silico expression of the *SOS* genes in the Areca palm genome. In total, 10 *AcatSOS* members were identified and mapped to 5 chromosomes. Phylogenetic relationships were established with previously reported *AcatSOS* genes from rice (*Oryza sativa* L.), and *Arabidopsis thaliana*. The *SOS* gene was found to be expressed in various tissues and at different developmental stages, and under salt stress. This study provides a foundation for future research on the function of *AcatSOS* genes in Areca palm. By understanding the function of these genes, new strategies can be developed to improve the salt tolerance of this important crop.

MATERIALS AND METHODS

Identification of *AcatSOS* genes in Areca palm Genome

The genome of the Areca palm was downloaded from the Areca palm genome database, http://arecaceae-gdb.com/#/ (accessed on 22 June, 2023). The NCBI website was used to find all recently available research data on *AcatSOS* genes, https://www.ncbi.nlm.nih.gov/protein (accessed on 22 June, 2023) and Phytozome, https://phytozome-next.jgi.doe.gov/ (accessed on 22 June, 2023) (Goodstein *et al*., 2012). The InterPro tool, https://www.ebi.ac.uk/interpro/ (accessed on 22 June, 2023), was required to determine the *AcatSOS genes* domains (Supplementary File S1). Previous research revealed the described sequence data for tomatoes (*Solanum lycopersicum*), corn (*Zea Mays* L.) and *Arabidopsis thaliana*. For finding the *AcatSOS* physiological and chemical characteristics Expasy ProtParam Tool, https://web.expasy.org/protparam/ (accessed on 23 June, 2023) was used to compute the protein's molecular weight, pI (isoelectric point), and GRAVY (grand average of hydropathicity).

Identification of *AcatSOS* gene Domains

The CDD NCBI tool https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (accessed on 23 June, 2023) was used to detect conserved domains in all 10 *AcatSOS* genes sequences, Using TBTools (Chen *et al*., 2020) the collected information was exploited to illustrate the *AcatSOS* domain's structure. https://www.genome.jp/tools/motif/ (accessed on 24 June, 2023).

Phylogenetic Analysis of the *AcatSOS* Family Using Multiple Alignments

From Phytozome-13, all *AcatSOS* genes sequences for rice (*O. sativa*) and Arabidopsis (*A. thaliana*) were retrieved (https://phytozome-next.jgi.doe.gov (accessed on 24 June, 2023) (Goodstein *et al*., 2012). The "Areca palm genome-website" was used to extract Areca palm protein sequences. For the molecular evolutionary genetic study, Clustal was used to align all 30 genes sequences (MEGA-11) (Tamura *et al*., 2021). Phylogenetic analysis was performed on the aligned gene sequences using the standard statistical tree test and 1000 bootstrap repetitions. iTOL software (Letunic & Bork, 2019) was used to design and visualize the tree (Supplementary File S2).

Chromosomal Distribution and Circos Analysis of *AcatSOS* genes on the Areca palm

The Areca palm genomic library was used to collect information of all 10 *AcatSOS* genes from areca database http://arecaceae-gdb.com/#/ (accessed on 25 June, 2023). With TBTools, the chromosome length was determined “FASTA stats” (Chen *et al*., 2020). Then, by using PhenoGram software chromosome ideogram was generated, http://visualization.ritchielab.org/phenograms/plot (accessed on 25 June 2023). We used the

output image to map all *AcatSOS* genes on the chromosome. A multiple collinearity scan toolbox was used to examine the duplication analysis (MCScanX) having an e-value of 1×10^{-5}. We selected *AcatSOS* gene-related duplication events and displayed them using Circos, with a connecting line linking the potential duplicated genes (Supplementary File S3).

Characterization of Gene Structure, Conserved Motif, and Subcellular Localization

The gene structure of *AcatSOS* was determined by using GFF file of Areca palm downloaded from http://arecaceae-gdb.com/#/ (accessed on 25 June, 2023) and run in TBTools (Chen *et al*., 2020).The MEME suite program was used to analyze the 10 *AcatSOS* genes and identify conserved motifs. We used the default parameters to identify up to 10 motifs at https://meme-suite.org/meme/tools/meme (accessed on 25 June, 2023). Subcellular localization of *AcatSOS* was predicted by Wolf PSORT (https://www.genscript.com/wolf-psort.html (accessed on 25 June, 2023) and CELLO v.2.5 (http://cello.life.nctu.edu.tw/) (accessed on 25 June, 2023) (Supplementary File S4).

In silico Genes Expression of *AcatSOS* genes

The Areca palm genomic library was used to determine the *AcatSOS* gene family's expression profile http://arecaceae-gdb.com/#/tools/efp-gene (accessed on 25 June, 2023). We used different plant tissues to assess the expression levels of all 10 *AcatSOS* genes in root, stem, leaf, fruit, endocarp, mesocarp, and epicarp. All data were obtained from the eFP-Gene tool in the Areca palm database.

Gene Correlation networks of *AcatSOS* genes

The data on all 10 *AcatSOS* genes interactions with other genes were obtained from the Gene correlation network http://arecaceae-gdb.com/#/tools/cor-network (accessed on 25 June, 2023). All of the discovered and interacting genes' data were utilized to construct and visualize the network using the Cytoscape software (Shannon *et al*., 2003).

Analysis of Promoter Regions, KEGG Enrichment and Go Enrichment in *AcatSOS* genes

All the promoter sequences of *AcatSOS* genes file (3000 bp upstream of the start codon) were obtained from Areca palm database http://arecaceae-gdb.com/#/tools/sequence-fetch (accessed on 26 June, 2023). TBTool was used to draw various elements in promoter region from the Areca palm genome. The cis-acting regulatory elements were retrieved from the PlantCARE library (Lescot *et al*., 2002) (Supplementary File S5).

All 10 *AcatSOS* genes were subjected to KEGG pathway and GO term enrichment analysis using the enrichment tool of the Areca palm database http://arecaceae-gdb.com/#/tools/kegg-enrichment (accessed on 26 June, 2023).

RESULTS

Characterization of the *AcatSOS* Family in Areca palm by Genome-Wide Analysis

Genome-wide analysis of the Areca palm genome identified 10 *AcatSOS* genes, classified as *AcatSOS*-1 to *AcatSOS*-10. The CDS length is the number of nucleotides in the gene's coding sequence, and the protein length is the number of amino acids in the protein that the gene encodes. The protein molecular weight is the calculated weight of the protein, and the pI is the isoelectric point, which is the pH at which the protein has no net charge. The GRAVY score measures the gene's predicted stability, with higher scores indicating more stable proteins. The range of protein length and molecular weight, from 195 (Acat_8g000210) to 654 AA (Acat_10g003600), and molecular weight ranged from 21060 Da (Acat_8g000210) to 71482.68 Da (Acat_10g003600). GRAVY values ranging

from 0.403 (Acat_8g000210) to -0.244 (Acat_1g019830). All *AcatSOS* genes comprised varying degrees of pI values, ranging from 4.77 (Acat_1g009860) to 9.16 (Acat_10g003600), with an average value of 6.466 (Table 1).

Table 1. Physiochemical properties of *AcatSOS* genes in the Areca palm (*Areca catechu*. L).

Transcript ID	***Rename ID***	**Chr No**	**Start Position**	**End Position**	**A.A Length**	**M w (Da)**	**pI**	**GRAVY**
Acat_1g009860	*AcatSOS*-1	1	90846470	90857128	212	23929.8	4.77	0.565
Acat_1g019830	*AcatSOS*-2	1	163000000	163147651	203	22114.79	5.62	0.801
Acat_1g017970	*AcatSOS*-3	1	125883653	126000000	557	61807.54	8.65	0.559
Acat_6g002750	*AcatSOS*-4	6	65473952	65522354	516	56962.24	6.76	0.52
Acat_8g000210	*AcatSOS*-5	8	2484041	2521105	195	21060.2	4.97	0.403
Acat_8g002990	*AcatSOS*-6	8	68924847	68949552	458	50069.78	8.19	0.548
Acat_10g013220	*AcatSOS*-7	10	45521591	45530482	288	32120.47	5.58	0.648
Acat_10g003600	*AcatSOS*-8	10	12033769	12048297	654	71482.68	9.16	0.463
Acat_10g009410	*AcatSOS*-9	10	32014283	32040464	538	58608.1	5.79	0.477
Acat_13g008520	*AcatSOS*-10	13	26536273	26561123	305	33406.2	5.17	0.676

pI; isoelectric point, **GRAVY**; grand average of hydropathy, **A.A;** Amino acid length, **M w;** Molecular weight.

Additionally, domain-based analyses of all 10 *AcatSOS* genes were performed using an NCBI domain search. The domain structure was demonstrated using the obtained information in TBTools. The Na-H Exchanger was present on 10 chosen sequence data chosen, according to this domain-based study (Figure 1).

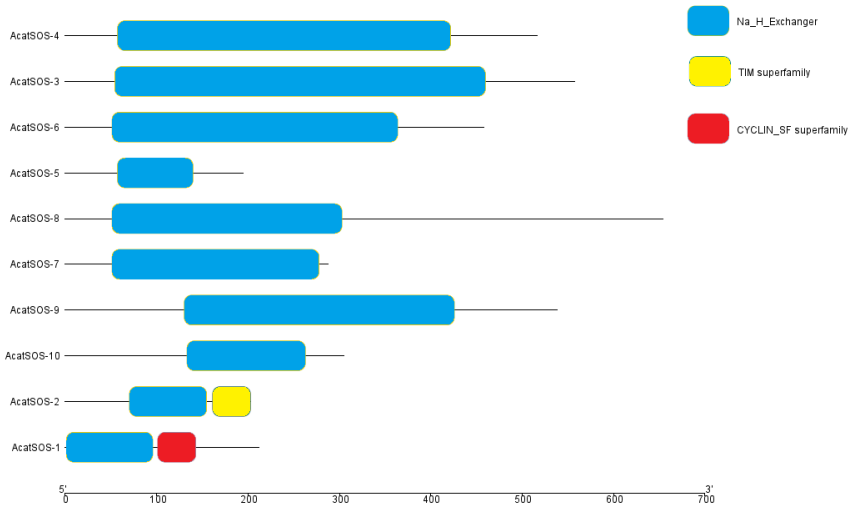

Fig. 1. Domains of *AcatSOS* genes ranged from (*AcatSOS-1* to *AcatSOS-10).* Green color represents Na-H Exchanger, Yellow show Na-H Exchanger superfamily and Black line show protein sequences that are not conserved.

Analysis of Phylogenetic tree of *AcatSOS* Gene

A phylogenetic tree was constructed from rice (*Oryza sativa* L.), Arabidopsis thaliana, and Areca catechu. Using the Phytozome database and published AcatSOS genes sequences from three distinct species, we obtained all AcatSOS genes from each species' genome. We constructed the phylogenetic tree from 30 protein sequences. Based on previous studies, we divided the comparative phylogenetic tree into 5 subfamilies: Group 1, Group 2, Group 3, Group 4, and Group 5 (Figure 2).

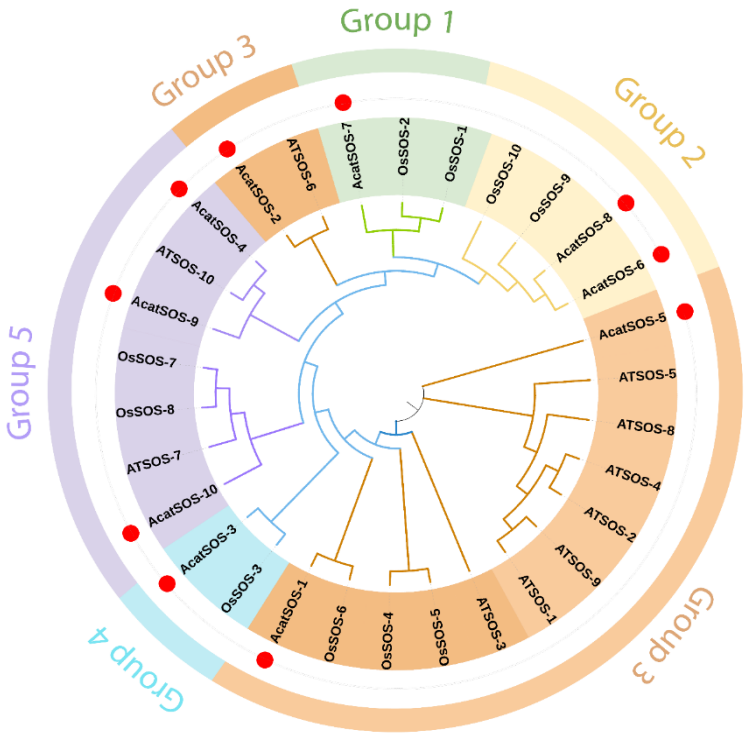

Fig. 2. Comparative phylogenetic analysis of *AcatSOS* genes along with rice and Arabidopsis. It is classified based on conserved motifs. Each cluster is differentiated through various colors: 1; Purple, 2; Blue, 3; Orange, 4; Yellow and 5; Green.

Analysis of *AcatSOS* genes Structure and Motifs

We identified *AcatSOS* gene structures by comparing the intron and exon structures of each gene (Figure 3). Most of the 10 *AcatSOS* genes showed introns and exons and mostly had an exon pattern that reflected the phylogeny and evolution of the gene family.

The Motif of *AcatSOS*-1 to *AcatSOS*-10 was constructed using the MEME software; each *AcatSOS* gene was checked for homologous motifs. We found some motifs in almost all *AcatSOS* genes. However, certain genes do not consistently include motifs (Figure 4). Most *AcatSOS* genes in the group had nearly identical motifs. These findings showed the close evolutionary connections among the same protein family and classified *AcatSOS* genes on the basis of conserved motifs and divided them into 5 subfamilies.

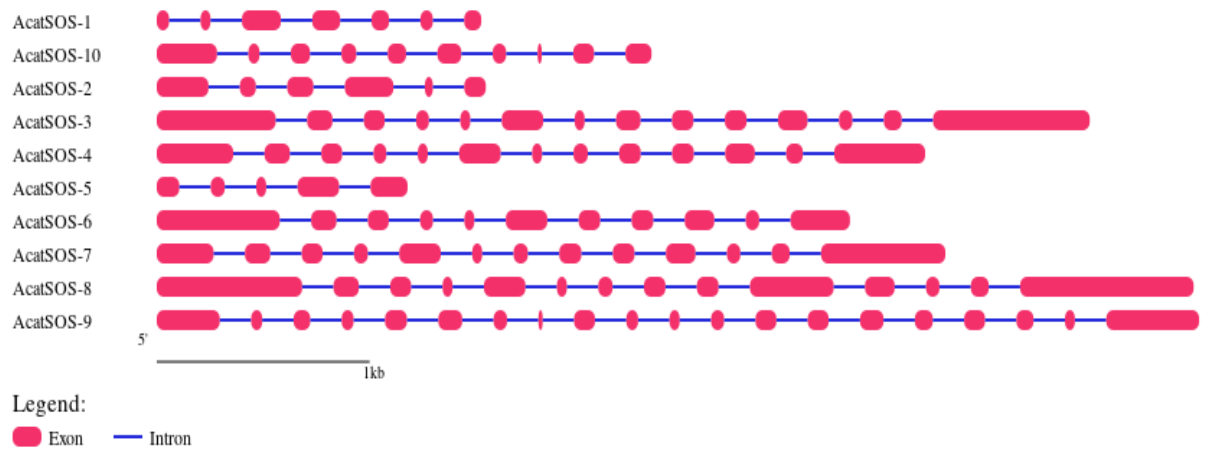

Fig. 3. Gene structure of *AcatSOS*-1 to *AcatSOS*-10. **Red** color shows the exon structure. Blue color shows introns in the gene structure.

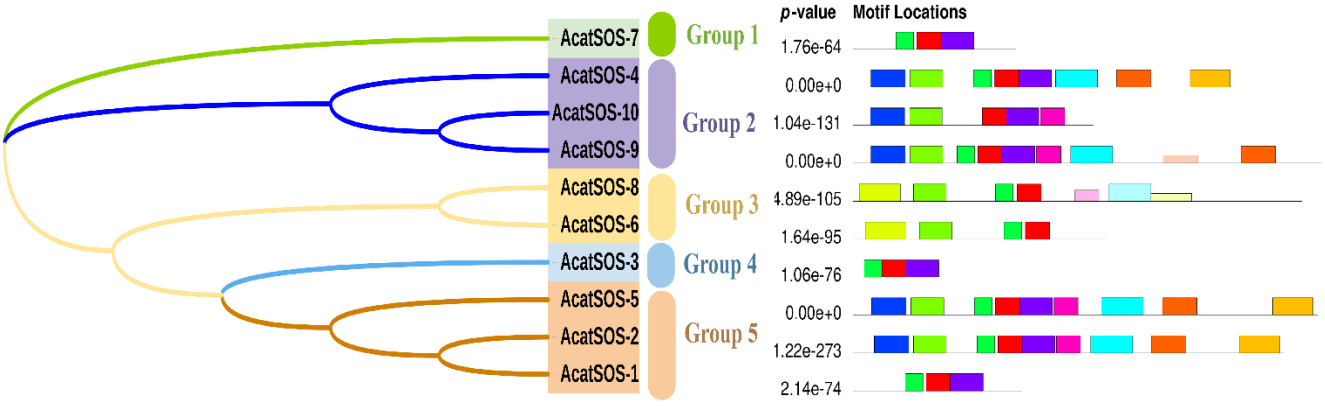

Fig. 4. Phylogenetic and Motif analysis of *AcatSOS* genes. Group 1 and Group 4 contain 1 gene, Group 2 and Group 5 contain 3 genes and Group 3 contain 2 genes.

Chromosomal Distribution and Circos Analysis of the *AcatSOS* genes

In the Areca palm genome, the *AcatSOS* genes were structurally placed on 5 chromosomes (chr). Using the data from the Areca palm genome database, each Areca palm species was given a specific *AcatSOS* gene http://arecaceae-gdb.com/#/ (accessed on 1 June 2023). The chr length and position of each gene in the Areca palm genome are displayed in 5 chromosomes (Figure 5).

On Areca palm chromosomes, there were 10 *AcatSOS* genes that spread irregularly in the chromosome. We examined whether gene duplication events occurred in the genome of the Areca palm by circos analysis (Figure 6).

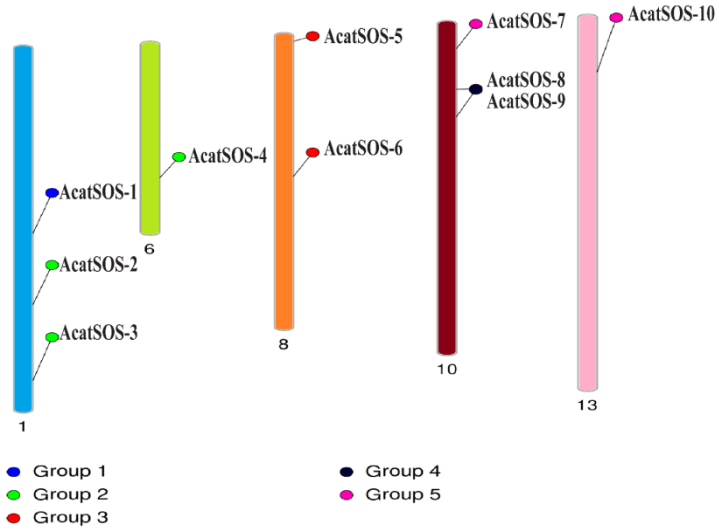

Fig. 5. Chromosomal distribution of *AcatSOS* genes on 5 Areca palm chromosomes. Different chromosome are differently colored.

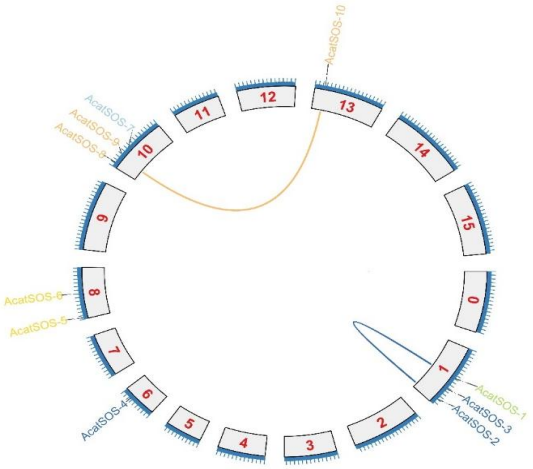

Fig. 6. Circos analysis of *AcatSOS* genes. Events of segmental and tandem duplication are shown by colored lines.

Gene Localization of *AcatSOS* genes

In gene localization we predict the function of *AcatSOS* genes in cell organelles, Nucleus, Chloroplast, Peroxisome, Mitochondria etc. From the result we concluded that most of the genes are involved in plastid and mitochondria (Figure 7).

Synteny Assessment of *AcatSOS* genes

Numerous conserved regions/genes on the Areca plant's chromosome number were discovered using synteny analysis. We have analysed the synteny assessment of *Oryza sativa* and *Arabidopsis thaliana* with *Areca catechu* (Figure 8).

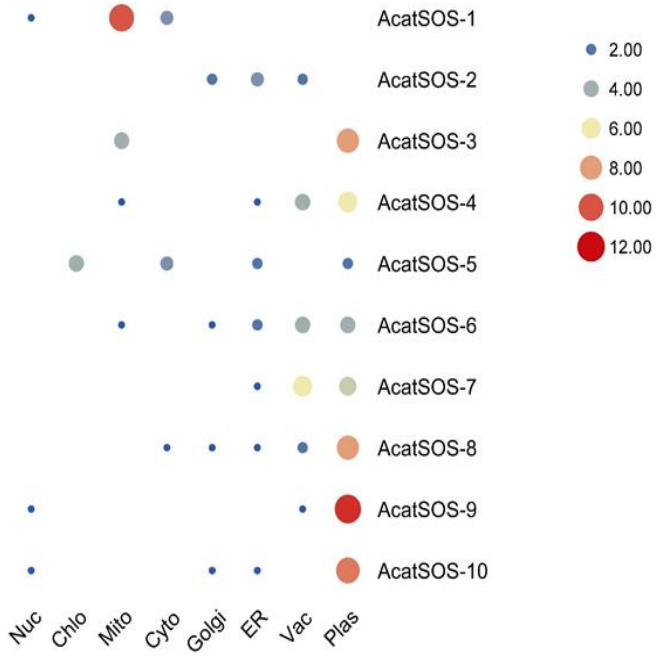

Fig. 7. Gene Localization of *AcatSOS* genes. The color bar with Red signifies a high concentration of proteins, Blue represents small value, and Yellow shows an intermediate intensity.

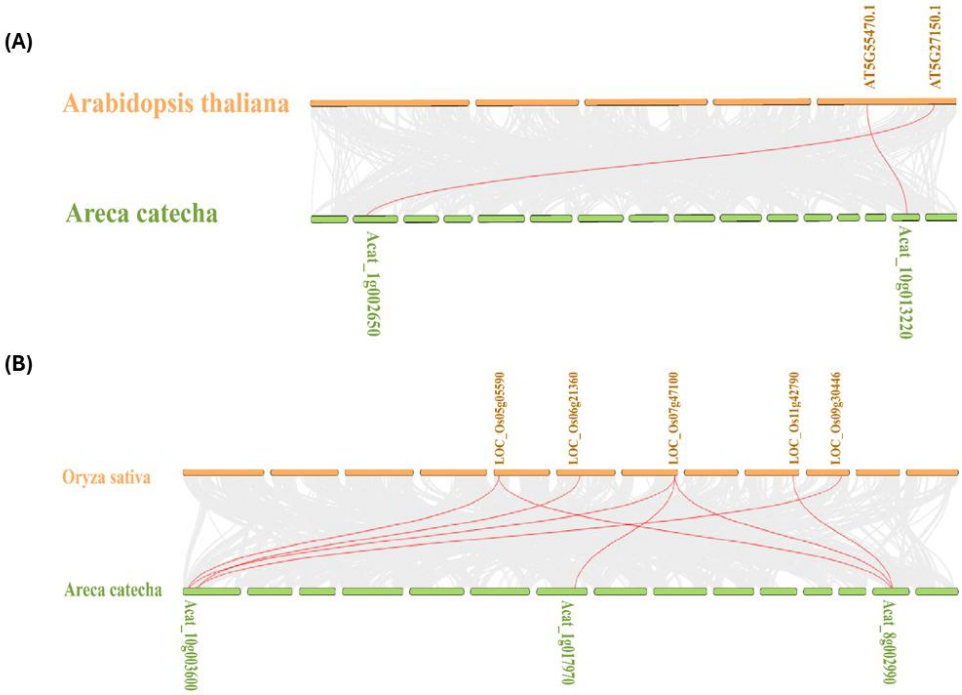

Fig. 8. Synteny analysis of (A) *Arabidopsis thaliana* and *Areca catechu* (B) *Oryza sativa* and *Areca catechu.* Orange color shows *Oryza sativa* and *Arabidopsis thaliana*, and green color shows *Areca catechu*. It was illustrated through TBtools using the MCScanX tool.

Correlation of *AcatSOS* genes

We have analyzed 10 *AcatSOS* genes through Gene Correlation Network from the Areca palm database and designed in Cytoscape, which shows interactions among genes. *AcatSOS* genes show strong interactions that enhance gene stability (Figure 9).

Characterization of Cis-acting Elements in *AcatSOS* Promoter Sequence

All 10 *AcatSOS* genes sequences were analyzed to determine the biological roles (release of stress hormones, development and growth) of the *AcatSOS* genes (3000 bp upstream of the start codon) using the PlantCARE search resource for cis-element analysis. The *AcatSOS* gene family of the areca plant had nine genes with increased expression that displayed a variety of cis-acting regulatory factors (Figure 10). These cis-acting elements were categorized into nine groups: AURE; auxin responsive elements, LIRE; light responsive elements. AARE; abscisic acid responsive elements, MejARE; meja responsive elements, DSRE; defence and stress response, GIRE; giberline responsive elements, LTRE; low temperature elements, SARE; salicyclic acid responsive elements and MYB binding elements.

KEGG Enrichment and Go Enrichment Analysis of *AcatSOS* genes

KEGG (Kyoto Encyclopedia of Genes and Genomes) is used to identify the enriched pathways in a given set of genes. The results show that *AcatSOS* genes are mostly involved in photosynthesis proteins. The x-axis represents the gene ratio, and the Y-axis represents the count of *AcatSOS* genes (Figure 11).

We determined the functions of all 10 *AcatSOS* genes using the Areca database. X-axis shows gene ratio while Y-axis represents count number of genes. From result it is concluded that these genes are mostly involved in protein transmembrane transport (Figure 12).

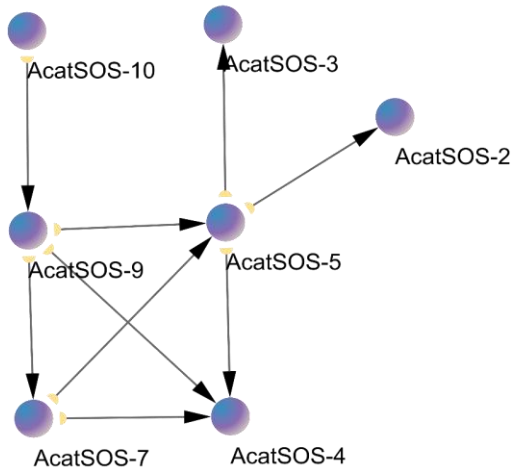

Fig. 9. A gene correlation network of *AcatSOS* genes**. Bluish color** represents Genes, **Black line** show interaction among *AcatSOS* genes, **Black** arrowhead and **Yellow** tail represent start and end of gene interaction.

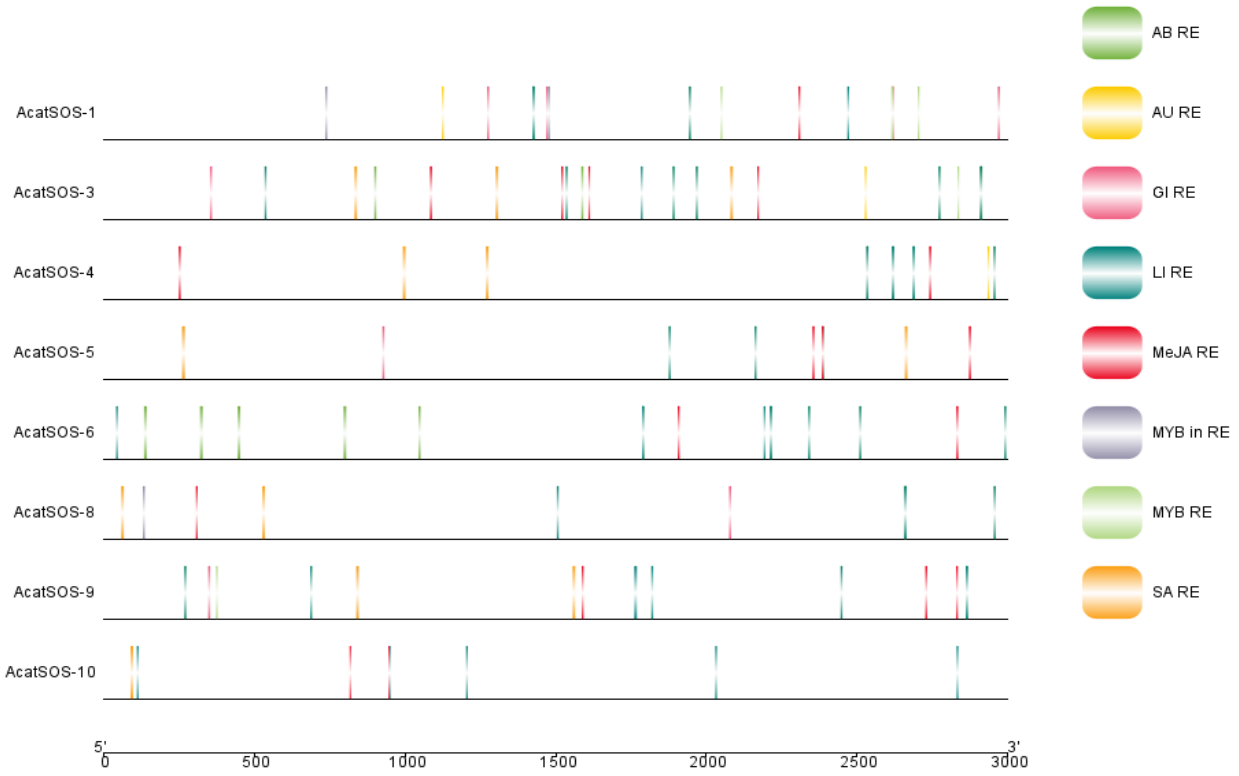

Fig. 4. Promoter regions of 10 *AcatSOS* genes, located 3000 bp upstream of the start codon. Each element has been represented with different colors. Most of the genes that are responsible in promoter regions are light responsive elements.

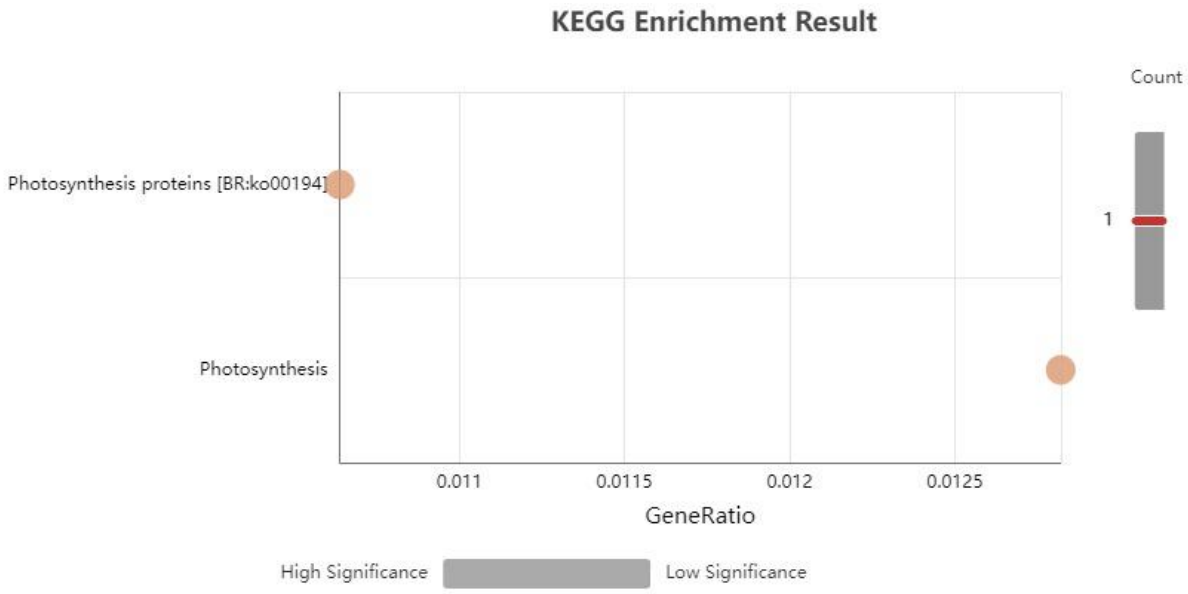

Fig. 5. KEGG enrichment of *AcatSOS* genes in which highest count is 1 and responsible for Photosynthesis protein with >0.0125 gene ratio.

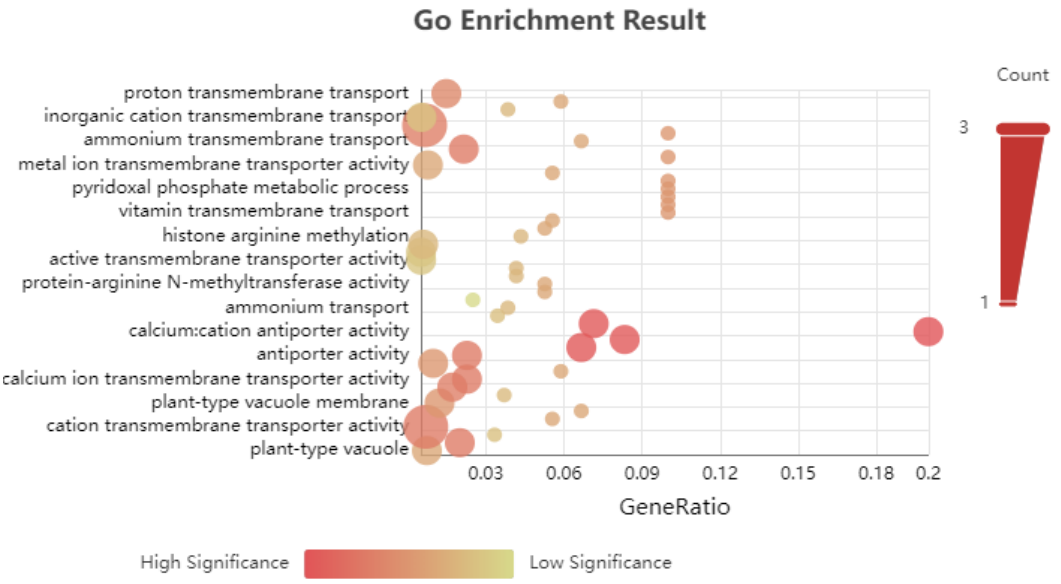

Fig. 6. GO enrichment of *AcatSOS* genes in which highest count is 3 that is responsible for protein transmembrane transport with 0.2 gene ratio. **Red** shows high significance and **Yellow** shows low significance.

In silico Expression Pattern of *AcatSOS* genes

From the Areca palm database, we extracted all the eFP-Gene data of *AcatSOS* genes. All the *AcatSOS* genes were compiled and a circular heatmap was constructed through the TBtools. Among them, 3 genes are highly expressed: *AcatSOS*-4, *AcatSOS*-6, and *AcatSOS*-8, which are involved in leaf, endocarp, and epicarp development (Table 2).

Table 2. Gene expression of *AcatSOS* genes in leaf, root, endocarp, stem, epicarp and mesocarp. Highly expressed genes are represented in **Brown** Highlighted form.

Rename ID	**Mesocarp**	**Endocarp**	**Fruit**	**Leaf**	**Root**	**Epicarp**	**Stem**
AcatSOS-1	NaN	0	NaN	0	NaN	0	NaN
AcatSOS-2	NaN	1.276024333	NaN	0	NaN	1.982273667	NaN
AcatSOS-3	NaN	9.991280667	NaN	5.351949	NaN	104.1729407	NaN
AcatSOS-4	NaN	16.040033	NaN	3.893313	NaN	6.999934	NaN
AcatSOS-5	NaN	0	NaN	0	NaN		NaN
AcatSOS-6	NaN	48.922114	NaN	36.798729	NaN	44.344672	NaN
AcatSOS-7	NaN	0.981822333	NaN	0.033856	NaN	1.460505667	NaN
AcatSOS-8	NaN	27.095471	NaN	14.250592	NaN	28.44155833	NaN
AcatSOS-9	NaN	15.30590267	NaN	16.263288	NaN	27.19187367	NaN
AcatSOS-10	NaN	4.339673	NaN	2.731218333	NaN	2.176102333	NaN

We have revealed the expression pattern in leaf, endocarp, fruit, stem and epicarp. From circular heat map we concluded that most of the *AcatSOS* genes are involved in leaf, endocarp and epicarp (Figures 13,14 and 15).

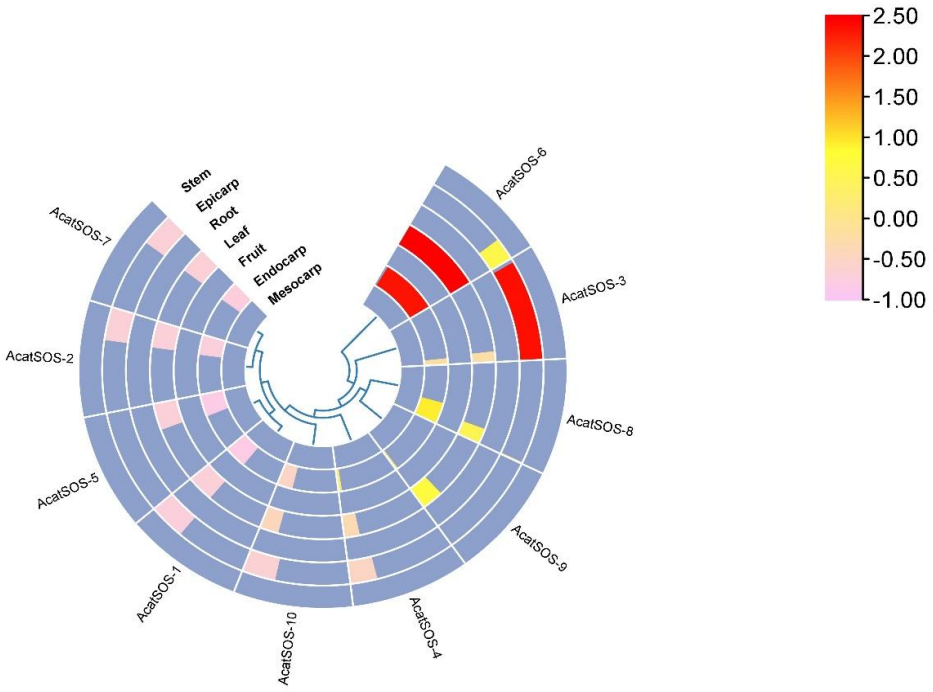

Fig. 7. The circular heat map of *AcatSOS* expression of genes in Areca palm tissues. In this heatmap, 10 columns indicate the genes and 7 rows that reflect the responses of various tissues: Root, Stem, Leaf, Fruit, Endocarp, Mesocarp and Epicarp.

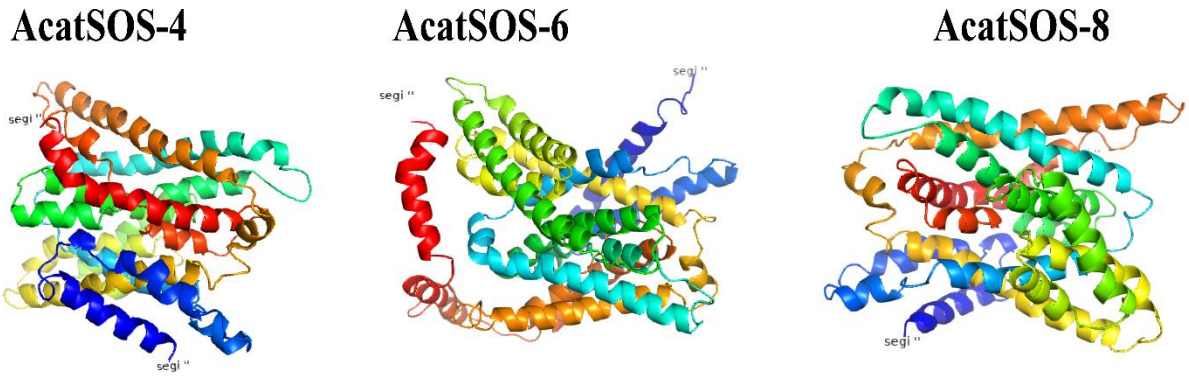

Fig. 8. Homology 3D structure of highly expressed genes. They are mostly similar in shape and help to maintain salt concentration.

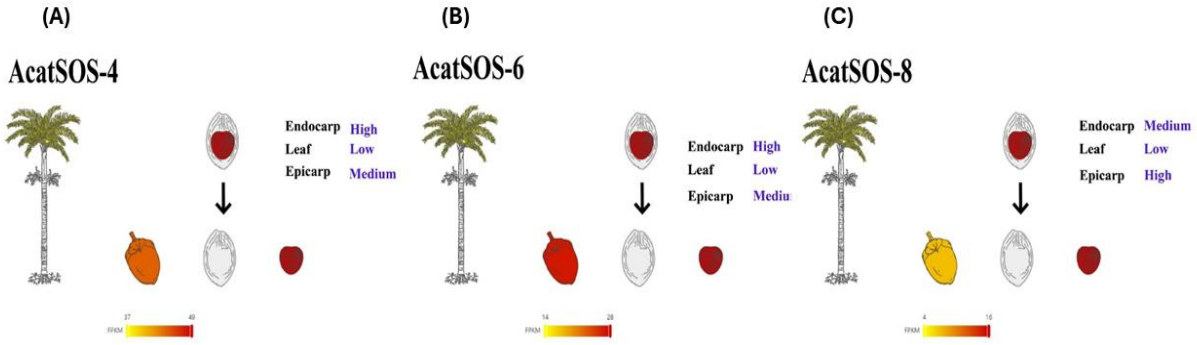

Fig. 15. In silico expression of highly expressed genes in Leaf, Endocarp and Epicarp. **(A)** *AcatSOS*-4, **(B)** *AcatSOS*-6 and **(C)** *AcatSOS*-8 genes expression

DISCUSSION

Areca palm (*Areca catechu* L.) is one of the most commercially significant tropical palm plants. Its monoecious spadix bears flowers at the base (Ding *et al*., 2023). It is a Southeast Asian native known as the areca palm or betel nut palm. The *Areca catechu* nut is a common component in the development of conventional medications as well as a stimulant used recreationally in various cultures. The nut contains a number of bioactive substances, such as alkaloids, tannins, and flavonoids, which have been associated with a number of medicinal and health advantages. The current study identified 10 *AcatSOS* genes, which are scattered over 5 chromosomes in the areca palm genome (Figure 5). The identification of *AcatSOS* genes, along with the physicochemical properties of each genes protein length and molecular weight, start and end points on each chromosome, PI, GRAVY, and predicted sub-cellular localization of each *AcatSOS* family member protein, have all been significant findings of the current study. In a phylogenetic study using sequences from rice, Arabidopsis, and *Areca catechu*, we matched the 10 *AcatSOS* gene sequences with the 30 *SOS* gene sequences. *AcatSOS* genes were divided into 5 subfamilies, according to previous studies Group: 1, 2, 3, 4, and 5 (Figure 2). All genes were assigned to the appropriate *SOS* family according to conserved motifs and gene structure. Exon-intron structure study showed that the gene patterns of several subgroups were very distinct from one another. However, the structure was largely similar (Figure 3). Most *AcatSOS* have between 0 and 2 introns, which is a comparatively low number, and most of its genes have just one or no introns. Earlier studies have linked this low number to the stress response (Sidhu *et al*., 2020).

The results suggest that the function *of AcatSOS* vary significantly across various subgroups. All *AcatSOS* genes between the groups had the same type, and order of motifs. All motif sequences had similarities, according to motif-I. In addition, nearly every group had its own distinctive and largely conserved motifs. Motif 3 is a highly conserved motif (Zhang *et al*., 2019). Gene duplication, including tandem and segmental duplication, is common in angiosperm evolution. This can increase the size of the gene family (Liu *et al*., 2018). Tandem and segmental duplication events are also present in *AcatSOS* at the same time. In the current study, we found 1 pair of segmental duplication events. We observed that *AcatSOS* promoters contain many cis-acting elements, including gibberellin, auxin, ABA, jasmonic acid, and salicylic acid-responsive elements. These genes can be used to investigate the mechanisms that control specific plant traits. Gene expression analysis showed that most genes are involved in leaf,

epicarp, and endocarp development, such as *AcatSOS*-4, *AcatSOS*-6, and *AcatSOS*-8. All these analyses proved the role of *AcatSOS* genes in salinity stress, but still gene expression and further validations will be required.

CONCLUSION

In this study we identified 10 *AcatSOS* genes in the genome of Areca palm (*Areca catechu*) and divided into five sub-groups according to the motifs and gene structure. Several motifs were rare and specific to subgroups. The *AcatSOS* family has mainly evolved via segmental and tandem gene duplications. The results suggest that *AcatSOS* genes are involved in the regulation of various metabolic processes during the growth and development of Areca palm. It is the first study of *AcatSOS* gene family in the tropical fruit, thus providing the preliminary basis for future study of the salt stress tolerance, development, growth and improvement of areca cultivar.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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