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ORIGINAL ARTICLE

Genome-wide screening and characterization of *phospholipase A* (*PLA*)-like genes in sorghum (*Sorghum bicolor* L.)

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Abstract

Main conclusion **The characterisation of *PLA* genes in the sorghum genome using in-silico methods revealed their essential roles in cellular processes, providing a foundation for further detailed studies.**

Abstract *Sorghum bicolor* (L.) Moench is the fifth most cultivated crop worldwide, and it is used in many ways, but it has always gained less popularity due to the yield, pest, and environmental constraints. Improving genetic background and developing better varieties is crucial for better sorghum production in semi-arid tropical regions. This study focuses on the phospholipase A (*PLA*) family within sorghum, comprehensively characterising *PLA* genes and their expression across different tissues. The investigation identified 32 *PLA* genes in the sorghum genome, offering insights into their chromosomal localization, molecular weight, isoelectric point, and subcellular distribution through bioinformatics tools. *PLA*-like family genes are classified into three groups, namely patatin-related phospholipase A (pPLA), phospholipase A1 (PLA_1), and phospholipase A2 (PLA_2). In-silico chromosome localization studies revealed that these genes are unevenly distributed in the sorghum genome. *Cis*-motif analysis revealed the presence of several developmental, tissue and hormone-specific elements in the promoter regions of the *PLA* genes. Expression studies in different tissues such as leaf, root, seedling, mature seed, immature seed, anther, and pollen showed differential expression patterns. Taken together, genome-wide analysis studies of *PLA* genes provide a better understanding and critical role of this gene family considering the metabolic processes involved in plant growth, defence and stress response.

Keywords Bioinformatics tools · *Cis*-motif · Chromosomal localization · Gene expression studies · In-silico · Metabolic process · Sorghum genome

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Abbreviations

PLA	Phospholipase A
pPLA	Patatin-related phospholipase A
PLA_1	Phospholipase A1
PLA_2	Phospholipase A2

Introduction

Sorghum, a widely cultivated crop belonging to the grass family Poaceae, serves multiple purposes, particularly as a vital resource for livestock. As a cereal crop and a source of nourishment for humans and domesticated animals and poultry, sorghum holds a crucial position in India's agricultural landscape (Hariprasanna and Rakshit 2016). Ranking as the country's fifth most important cereal crop and third most significant overall, it is a primary food grain for over 750 million people in semi-arid regions (Tsusaka et al. 2015).

Various essential cellular processes, such as carbon partitioning, cell elongation, defense response, seedling establishment, and plant growth, are associated with liberating fatty acids from membranes and storing lipids in plants. The enzymatic activities of phospholipases, a complex and vital class of enzymes, play a significant role in hydrolyzing phospholipids into free fatty acids (FFAs), phosphatidic acid (PA), diacylglycerol (DAG), lysophospholipids, and soluble head groups. Phospholipases also perform numerous physiological functions within plant (Ali et al. 2022).

Phospholipases are crucial in various biological processes, including intracellular signal transmission, phospholipid metabolism, and cell membrane maintenance. These enzymes are divided into three main groups: PLA, which hydrolyze phospholipids to produce lysophospholipids and free fatty acids; phospholipase C (PLC), facilitating hydrolysis of phospholipids to produce diacylglycerol (DAG) and a phosphorylated head group, and phospholipase D (PLD) helps in the hydrolysis of glycerophospholipids into phosphatidic acid (PA) and a free head group, such as choline. Within the PLA group, three subtypes exist pPLA, PLA_1, and PLA_2, which differ in their specific phospholipid cleavage positions. PLA_1 acts on the sn-1 position, PLA_2 on the sn-2 position, and pPLA exhibits activity in both positions (Chen et al. 2013).

The first pPLA subtype in the *PLA* gene family is the largest class of phospsolipases facilitating hydrolysis of phospholipids and other glycerolipids at both the sn-1 and sn-2 positions. It plays a crucial role in lipid hydrolysis, with Ca^{2+} catalytic sites and a patatin domain (Chen et al. 2013). This gene family, essential in cellular processes like cell growth, signal transduction, lipid metabolism, and stress response, is classified into four groups: pPLAI, pPLAII, pPLAIII, and a fourth class including SDP1, SDP1-like, and ATGL-like (Scherer 2002; Eastmond 2006). pPLAs influence signaling molecules, contributing to diverse cellular activities such as defense signals, anther dehiscence, cell elongation, and gravitropism (Chen et al. 2013). The pPLA-I class of phospholipases regulates basal but not pathogen or wound-induced jasmonic acid production (Yang et al. 2007). Notably, *pPLA-IIa* overexpression in Arabidopsis increases plant cell death and enhances resistance to pathogen attacks (Ackermann et al. 1994). Knockout studies of *pPLA-IIc* in Arabidopsis reveal its essential role in the root response to phosphate deficiency (Rietz et al. 2010). Furthermore, *pPLA-IIId* overexpression results in a *STURDY* mutant phenotype characterized by a rigid inflorescence stem, thick leaves, short siliques, large seeds, round flowers, and delayed growth (Huang et al. 2001). Pollen-specific pPLAs induce haploidy, and this phenomenon is functionally conserved in monocots, including foxtail millet, maize, and wheat (Gilles et al. 2017; Kelliher et al. 2017; Liu et al. 2017, 2020; Yao et al. 2018; Cheng et al. 2021).

The second, PLA_1 subtype within the *PLA* gene family is characterized by a highly conserved GXSXG motif and is calcium-independent, with molecular masses ranging from 45 to 50 kDa (Chen et al. 2013). PLA_1 is further classified into three classes, I, II, and III, based on specific sequences at the N-terminal and similarities in the catalytic region. In Arabidopsis, these are distributed among chloroplasts, plastids, cytosols, and mitochondria (Chen et al. 2013). Arabidopsis contains group I, II, and III PLA_1, along with phosphatidic acid-specific PLA_1 (PA-PLA1) and lecithin: cholesterol acyltransferase-like PLA_1 (LCAT-PLA1) (Chen et al. 2011). In rice, there are group I, II PLA_1, and PA-PLA1 (Singh et al. 2012). PLA_1 transcripts are detected in almost all organs of plants, but the expression levels of the individual isoform have been changed according to the tissue specificity (Seo et al. 2008). PLA_1 genes play crucial roles in various biological processes, including the production of jasmonic acid, plant growth and development, senescence, ultraviolet B (UV-B) defense signaling, and shoot gravitropism (Ishiguro et al. 2001; Kato et al. 2002; Lo et al. 2004; Seo et al. 2008, 2011; Hyun et al. 2008; Ellinger et al. 2010).

The third subtype of the *PLA* gene family, the PLA_2 superfamily in animals, is broadly classified into five main families: secretory PLA_2s ($sPLA_2$), cytosolic PLA_2s ($cPLA_2$), Ca^{2+}-independent PLA_2s ($iPLA_2$s), platelet-activating factor acetyl hydrolases (PAF-AHs), and lysosomal PLA_2s (Schaloske and Dennis 2006). Apart from Arabidopsis, PLA_2 have also been characterized in tobacco (Fujikawa et al. 2012), soybean (Mariani et al. 2012) and wheat (Verlotta et al. 2013). In plants, only low molecular weight secretory PLA_2s have been identified, characterized by molecular masses ranging from 13 to 18 kDa and a PA2c domain (Lee et al. 2005). The genomes of rice and Arabidopsis each have three and four secretory PLA_2 paralogs, respectively, linked to responses to both biotic and abiotic stress. These enzymes play crucial roles in various physiological processes, including auxin control, cell elongation, gravitropism, plant growth and development, and cellular signalling (Mariani and Fidelio 2019; Takáč et al. 2019).

A comprehensive genome-wide analysis of the *PLA* gene family has been conducted for Arabidopsis (Chen et al. 2011) and rice (Singh et al. 2012) model crops. Comprehensive identification of this important family of genes has been very useful in exploring a broad range of catalytic properties and biological functions. In this study, we have identified the *PLA*-like gene family in the sorghum genome, which is mainly classified into three main classes: PLA, PLA_1, and PLA_2. Genome-wide analysis of these genes will help establish a solid foundation for characterizing these genes in sorghum. This comprehensive approach will facilitate the thorough analysis of individual functions across different classes.

Materials and methods

Plant material

The sorghum cultivar ICSR 14001 (Parbhani Shakti) was used for expression studies. Different developmental tissues representing the leaf, seedling, root, anther, pollen, immature seed and mature seed were collected from the life cycle of sorghum. The plants for all experiments were grown in glasshouse conditions under natural daylight oscillations, with day/night average temperatures of approximately 28/22 °C and relative humidity of 70/90%.

Identification of *PLA*-like genes in *Sorghum bicolor*

The PLA-like protein sequences of Arabidopsis and rice were compiled from the Phytozome database (www.phytozome.net/). Every sequence was individually compared with functional annotations by browsing the sorghum genome database (https://phytozome.jgi.doe.gov/pz/portal.html) and NCBI BLASTp, resulting in the identification of *PLA* genes in sorghum. The unclassified *PLA* genes were classified into different isoforms by comparing the phylogenetic relationship of their putative protein sequences with clearly classified *PLA* genes from the phytozome server (http://www.phytozome.net/search.php?show=blast). The protein sequence length, molecular weight (MW), and theoretical isoelectric point (pI), three significant physicochemical characteristics of the discovered proteins, were examined using the Expert Protein Analysis System (EXPASY) (https://web.expasy.org/compute_pi/) (Gasteiger et al. 2005). The protein subcellular localization was predicted using the online tool WoLF PSORT, available at https://wolfpsort.hgc.jp/ (Horton et al. 2007).

Multiple sequence alignment and phylogenetic tree analysis of *PLA*-like genes

ClustalW software was used to perform multiple sequence alignment of all PLA-like proteins. The neighbor-end joining method (NJ) created the phylogenetic tree with 1000 bootstrap replications using MEGA11 software (Tamura et al. 2021). The tree was modified further by iTOL (https://itol.embl.de) (Letunic and Borp 2021). The consensus of protein sequences was visualized by Geneious Prime®2023.2.1 software.

Gene structure, domain and motif identification of SbPLA protein

Gene structures of the sorghum *PLAs* were mapped using TBtools software (Chen et al. 2020), and the domains were identified using NCBI CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Wang et al. 2023). The conserved motifs in the sorghum *PLA* genes family were identified using MEME (http://meme-suite.org/) (Bailey et al. 2015), with the maximum number of motifs to be discovered set at 5.

Chromosomal localization, syntenic analysis and protein–protein interactions

The position of the putative *PLAs* in sorghum was procured from the phytozome database, and genes were mapped with their relative distance on the chromosome using TBtools software. The Multiple Collinearity Scan toolkit (MCScanX) in TBtools with default parameters enabled the gene duplication study in *Sorghum bicolor*, *Oryza sativa*, and *Zea mays*. Protein–protein interaction and pathway analysis were done using the online tool STRING (https://string-db.org/) (Szklarczyk et al. 2015).

Cis-acting elements analysis of *SbPLA*-like genes

To elucidate the possible *cis*-acting elements that are responsible for gene expression in response to various factors. 1500-bp of genomic DNA sequences upstream from the initiation codon (ATG) of the *SbPLA* genes were extracted and analyzed. The PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot 2002) database was adopted to identify the *cis*-acting elements in the promoter regions.

RNA isolation and quantitative real time-PCR

100 mg of sorghum tissue was used for RNA isolation using Qiagen RNeasy Mini Kit (Germantown, MD, USA). The RNA quality and quantity were analyzed using 1.4% formaldehyde agarose gel electrophoresis and a Qubit RNA BR assay kit (Thermo Fisher Scientific, Waltham, MA, USA). 1500 ng RNA was used for cDNA synthesis using Superscript III (Invitrogen, Carlsbad, CA, USA) and as a template after diluting it with nuclease-free water (1:10). qPCR was carried out in 96-well optical reaction plates, and PCR reaction was performed in a total volume of 10 µL containing 0.5 µM of each primer (1.5 µL), cDNA (1.0 µL), and Sensi Master Mix (2X) and dH_2O were added up to 2.7 µL. The PCR primers were designed using Primer3 and had a GC content of 40–60%, a Tm > 50 °C, a primer length of 20–25 nucleotides, and an expected product size of 90–180 bp. The

qPCR reactions were carried out by following the standard thermal profile: 95 °C for 10 s and then 40 cycles of 15 s at 95 °C, 15 s at 61 °C with fluorescent signal recording, and 15 s at 72 °C. After the 40th cycle, amplicon dissociation curves were measured by heating at 58 to 95 °C with fluorescence measured within 20 min. All qPCR data were obtained from three biological replicates with three technical replicates. Normalized expression was calculated with qBase^{+} software (Schmidt and Delaney 2010) with reference genes *eukaryotic initiation factor4α* (*SbEIF4α*) and *protein phosphatase2A* (*SbPP2A*) (Sudhakar Reddy et al. 2016).

Results

Identification of *PLA*-like genes in *Sorghum bicolor*

Based on sequence homology, several phospholipase-like genes were identified from the sorghum genome. Consequently, 32 full-length protein-coding *PLA*-like genes were identified in the sorghum genome. In the sorghum genome, these *PLA*-like genes, categorized into pPLAs, PLA_1, and $sPLA_2$ subtypes, are represented by 21, 7, and 4 members. Identified 32 sorghum *PLA* genes were characterised based on the presence of the number of introns, pI, chromosome localization, number of amino acids, molecular weight, domain localization, cellular localization, and number of transmembrane domains. These identified PLA proteins showed the presence of amino acids ranging from 154 to 1338. Molecular weights ranged from 16.1 to 147.4 kDa, and the pI ranged from 5.55 to 10.07. Subcellular localization prediction results showed that *SbPLA*-like genes are mostly distributed in organelles such as chloroplast, nucleus, and cytoplasm (Table 1).

Multiple sequence alignment and phylogenetic tree analysis of *PLA*-like genes

Multiple sequence alignments across various sorghum PLA classes have verified the presence of highly conserved regulatory and catalytically crucial motifs. In all PLA_1 members, a notably conserved GXSXG motif was identified. Corresponding to earlier findings, pPLA members exhibited the distinctive esterase box GTSTG and the anion-binding DGGGXRG motif. Similarly, $sPLA_2$s displayed a well-defined PA2c domain with a highly conserved Ca^{2+} binding loop marked by the YGKYCGxxxxGC motif and the catalytic site LDACCxxHDxCV (Fig. S1).

The study of *PLA* gene evolution in sorghum, Arabidopsis, and rice identified distinct subgroups in each species. Phylogenetic analysis divided these genes into three subtypes: pPLAs, PLA_1, and $sPLA_2$, based on their evolutionary relationships between Arabidopsis and rice (Fig. 1). Additionally, sorghum *PLA*-like genes were further classified into subgroups according to their functional domains.

Gene structure, domain and motif identification of SbPLA protein

Coding and genomic sequences were extracted to analyse the intron–exon structure. The position of introns and exons was analysed in all 32 candidate *SbPLA* genes. All genes showed the presence of none to 8 introns in their structure except *SbpPLA-I* (Fig. 2c). The *SbpPLA-I* gene showed the presence of 18 introns in the gene organization. The position and presence of introns were different in all subtypes. While the length of each exon was similar for most members in each subfamily, some deviations were also noted. Intron exon length varied widely within all 32 *SbPLA* genes. In a nutshell, *SbPLA* showed a complex structural organization with varied lengths of introns and exons.

To study the functions of different SbPLA-like proteins, their protein domains were analyzed according to the subtype. The SbpPLA subtype contains the patatin domain. The patatin domain was found in all SbpPLAs except SbpPLA-IIIc, SbpPLA-IIIf, SbpPLA-IIIe and SbpPLA-IIIg. In the SbsPLA2 subtype, a phospholipase_A2_1 domain was identified in all except SbsPLA2b. The SbPLA1 subtype showed the presence of lipase_3 domain (Fig. 2b). To understand the structural features of SbPLA proteins, we also examined the motif of the PLA proteins according to their subtypes. The genes contained in the evolutionary tree subtype SbpPLA subdivided into four categories based on the number of motifs like SbpPLA-I, SbpPLA-II, SbpPLA-III, and SbSDP1 contained one, five, two and none, respectively (Fig. S2a). The second type, the SbPLA1 class, contained five conserved motifs in most of the sequences, except SbPLA1-IIa and SbPLA1-Ia which showed four motifs in their protein structure (Fig. S2b). The third subtype, SbsPLA2, contained motifs ranging from three to five (Fig. S2c).

Chromosomal localization and syntenic analysis of *SbPLA*-like genes

In silico chromosome localization prediction showed that the *SbPLA* genes were distributed throughout the sorghum genome except for chromosomes 6 and 9 (Fig. 3). All chromosomes carried at least 1 (chromosome 4 and 8) and a maximum of 8 (chromosome 1) of *SbPLA* genes. *SbpPLA* genes are distributed in all chromosomes except chromosomes 4, 6 and 9. The *SbPLA1* gene subtype was found to be distributed in chromosomes 2, 3, 7 and 10. Meanwhile, *SbsPLA2* genes were distributed in chromosomes 1, 4, and 5. The MCscanX results also contributed to detecting syntenic and collinear relationships of sorghum with other crop species. We constructed collinearity syntenic maps of sorghum

Table 1 In-silico characterization of the candidate genes

Annotated name	Gene ID	Introns	Number of amino acid	MW (kDa)	pI	Instability index	Aliphatic index	Gravity	Cellular localization
pPLA									
SbpPLA-I	Sobic.002G320900	32	1338	147.4	5.82	54.93	95.55	− 0.097	Nucleus
SbpPLA-IIa	Sobic.002G228700	5	482	52.4	8.86	31.27	93.79	− 0.095	Cytoplasm
SbpPLA-IIb	Sobic.007G158600	3	435	46.8	6.27	30.26	90.14	− 0.199	Chloroplast
SbpPLA-IIc	Sobic.007G158700	4	487	53.3	5.58	41.73	86.08	− 0.144	Chloroplast
SbpPLA-IId	Sobic.007G158800	8	401	44	6.11	29.73	88.1	− 0.215	Chloroplast
SbpPLA-IIe	Sobic.005G186100	2	410	44.5	6.27	26.75	88.1	− 0.146	Chloroplast
SbpPLA-IIf	Sobic.005G186200	2	405	43.9	6.08	25.11	89.43	− 0.136	Chloroplast
SbpPLA-IIg	Sobic.005G186400	3	413	44.8	5.88	33.1	88.18	− 0.151	Cytoplasm
SbpPLA-IIh	Sobic.003G389400	4	333	45.4	9.34	34.21	77.36	− 0.326	Chloroplast
SbpPLA-IIQ	Sobic.001G348600	3	437	47.4	9.34	42.62	85.79	− 0.136	Chloroplast
SbpPLA-IIk	Sobic.002G377600	4	403	43.7	5.76	32.06	84.07	− 0.218	Cytoplasm
SbpPLA-IIm	Sobic.007G158500	4	437	46.6	6.47	35.35	90.85	− 0.129	Chloroplast
SbpPLA-IIIa	Sobic.001G433900	1	460	48.9	6.11	38.78	74.46	− 0.245	Nucleus
SbpPLA-IIIb	Sobic.001G067500	1	449	46.6	9.37	38.89	81.92	− 0.080	Chloroplast
SbpPLA-IIIc	Sobic.002G031800	2	492	50.3	10.07	51.47	77.97	− 0.112	Cytoplasm
SbpPLA-IIId	Sobic.010G228100	1	471	48.2	9.02	46.32	80.42	− 0.013	Chloroplast
SbpPLA-IIIe	Sobic.001G157700	0	461	47	9.06	44.69	80.87	0.036	Chloroplast
SbpPLA-IIIf	Sobic.008G166050	2	484	49.2	9.33	46.55	76.26	− 0.071	Chloroplast
SbpPLA-IIIg	Sobic.001G157900	0	445	45.6	8.76	42.42	78.74	0.015	Chloroplast
SbSDP1	Sobic.003G304200	3	907	100	6.12	53.25	86.96	− 0.306	Nucleus
SbSDP1-L	Sobic.001G041900	7	841	92.9	6.3	52.78	87.24	− 0.230	plastid
PLA1									
SbPLA1-Ia	Sobic.007G036900	0	509	53.9	9.58	54.6	87.31	− 0.12	Chloroplast
SbPLA1-Ib	Sobic.010G047800	0	547	60.5	6.11	42.68	74.9	− 0.4	Chloroplast
SbPLA1-Ic1	Sobic.003G432600	1	526	58.1	6.54	39.35	78.97	− 0.327	Chloroplast
SbPLA1-IIa	Sobic.002G177200	4	277	30	7.06	36.07	101.12	0.16	Nucleus
SbPLA1-IIb	Sobic.003G239500	1	440	46.9	6.27	40.35	82.8	− 0.188	Chloroplast
SbPLA1-IIc	Sobic.003G239200	2	408	45.5	5.98	41.27	79.85	− 0.445	Cytoplasm
SbPLA1-IId	Sobic.003G274700	0	473	51	5.55	38.64	86.83	− 0.149	Chloroplast
sPLA2s									
SbsPLA2a	Sobic.001G120400	3	155	16.5	5.76	64.79	85.61	0.098	golgi
SbsPLA2b	Sobic.005G145100	3	169	18	5.75	64.57	84.97	− 0.026	ER
SbsPLA2c	Sobic.004G357800	2	157	16.7	8.72	53.86	85.1	0.221	ER
SbsPLA2d	Sobic.001G429900	3	154	16.1	6.03	46.59	90.58	0.144	Extra

The selected candidate genes are characterized by the total number of introns, number of amino acids, molecular weight (MW), isoelectric point (pI), instability index, aliphatic index, gravity, and cellular localization

and the two other crops, *Oryza sativa* and *Zea mays*. The *PLA* gene pairs within the significant collinear blocks (zero e-value) were identified in sorghum-rice (22 pairs) and sorghum–maize (23 pairs) (Fig. 4).

In silico analysis of *SbPLA* promoter regions

To identify putative *cis*-acting elements in the promoter region of *SbPLA*-like genes, genomic sequences approximately 1500 bp upstream from the translational start site were extracted and analysed using the PlantCARE database. The analysis mainly focussed on abiotic stress, phytohormone responsiveness and development-related *cis*-elements, as they are key players in signalling different stresses and biological processes. The abiotic stress-related *cis*-acting elements identified in promoters of the *PLA* genes included LTR, cis-acting element involved in low-temperature responsiveness, STRE, stress-responsive elements and WUN-motif, wounding-related. The phytohormone-responsive *cis*-acting elements included P-box, TCA, CGTCA motif: *cis*-acting element involved in the MeJA-responsiveness, TGACGA motif, ABRE: cis-acting element involved in the abscisic acid responsiveness, TATC box and TGA element: auxin-responsive element. The development-related *cis*-acting elements included GCN4 motif, RY element, MBS (MYB binding site involved in drought-inducibility), circadian (*cis*-acting regulatory element involved in circadian control),

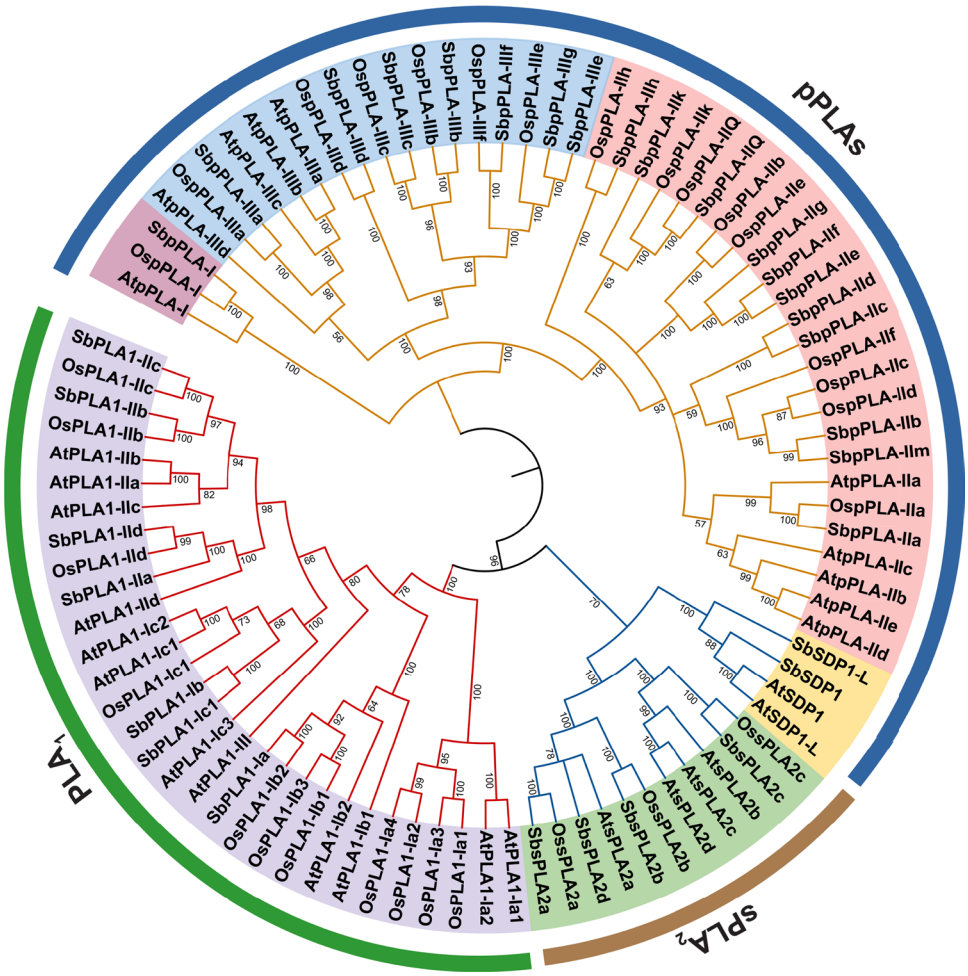

Fig. 1 Phylogenetic tree of PLAs from *Sorghum bicolor*, *Arabidopsis thaliana* and *Oryza sativa*. Multiple sequence alignments were performed using amino acid sequences from the PLA class of genes from Arabidopsis, rice and sorghum. Default parameters for ClustalW in Mega 11.0 were used to perform the alignment. The alignment was then used to construct a phylogenetic tree in MEGA 11.0 by the neighbour-joining method with 1000 bootstrap replications. The tree was classified into three different subtypes (pPLAs, sPLA$_2$ and PLA$_1$) based on the clades and is indicated by different colour

CCGTCC box and CCGTCC motif. Almost all promoter regions showed the presence of CGTCA motif, TGCGA motif, STRE, and ABRE motif. From this, it can be concluded that all genes mostly contain developmental-specific and hormone-responsive *cis*-acting elements (Fig. 5).

In silico protein–protein interaction analysis

A STRING database was used to analyse protein–protein interactions. This prediction analysis showed that the *SbPLA* genes are involved in various pathways such as linoleic acid metabolism, arachidonic acid metabolism, Ether lipid metabolism, alpha-linolenic acid metabolism, and glycerophospholipid metabolism. The reported functions of this class of candidate genes are lipid degradation, lipid metabolism, and hydrolases. Figure 6 suggests that CM000760.3, CM000766.3, CM000767.3, CM000763.3, and LOC8065835 putative lipoxygenase 5 regulate the genes' major number of the SbPLA class. If considering only the SbPLA family, SbSDP1-L and SbsPLA2a are related to a maximum number of proteins in the PLA family (Fig. 6).

Expression profile of *SbpPLA* genes in various tissues of sorghum

The expression levels of *SbpPLA* candidate genes in various tissues of sorghum were analyzed by qPCR. Seedling, root, post anther, pollen, mature seed, leaf, and immature seed were considered to examine the expression level of the *SbpPLA* candidate genes. The qPCR results showed that the expression pattern of the *pPLA* gene family is quite diverse. *SbpPLA-I, SbpPLA-IIb*, *SbpPLA-IIc*, *SbpPLA-IId*, *SbpPLA-IIe*, *SbpPLA-IIf*, *SbpPLA-IIg*, *SbpPLA-IIh*, and *SbpPLA-IIm* showed low to moderate expression levels in immature seed, leaf, mature seed and pollen whereas downregulation occurred in post anther, root and seedling stages. However, *SbpPLA-IIa* showed a moderate level of expression in pollen and post anther stages, while *SbpPLA-IIQ* and *SbpPLA-IIk* showed a moderate level expression in pollen. *SbpPLA-IIIb* and *SbpPLA-IIIe* were moderately expressed at the mature seed stage. On the other hand, expression levels of the *SbpPLAIII* class of genes such as *SbpPLA-IIIb*, *SbpPLA-IIId*, *SbpPLA-IIIe*, *SbpPLA-IIIf*, *SbpPLA-IIIg* showed an upregulation in roots and seedling stage of the plant (Fig. 7). This

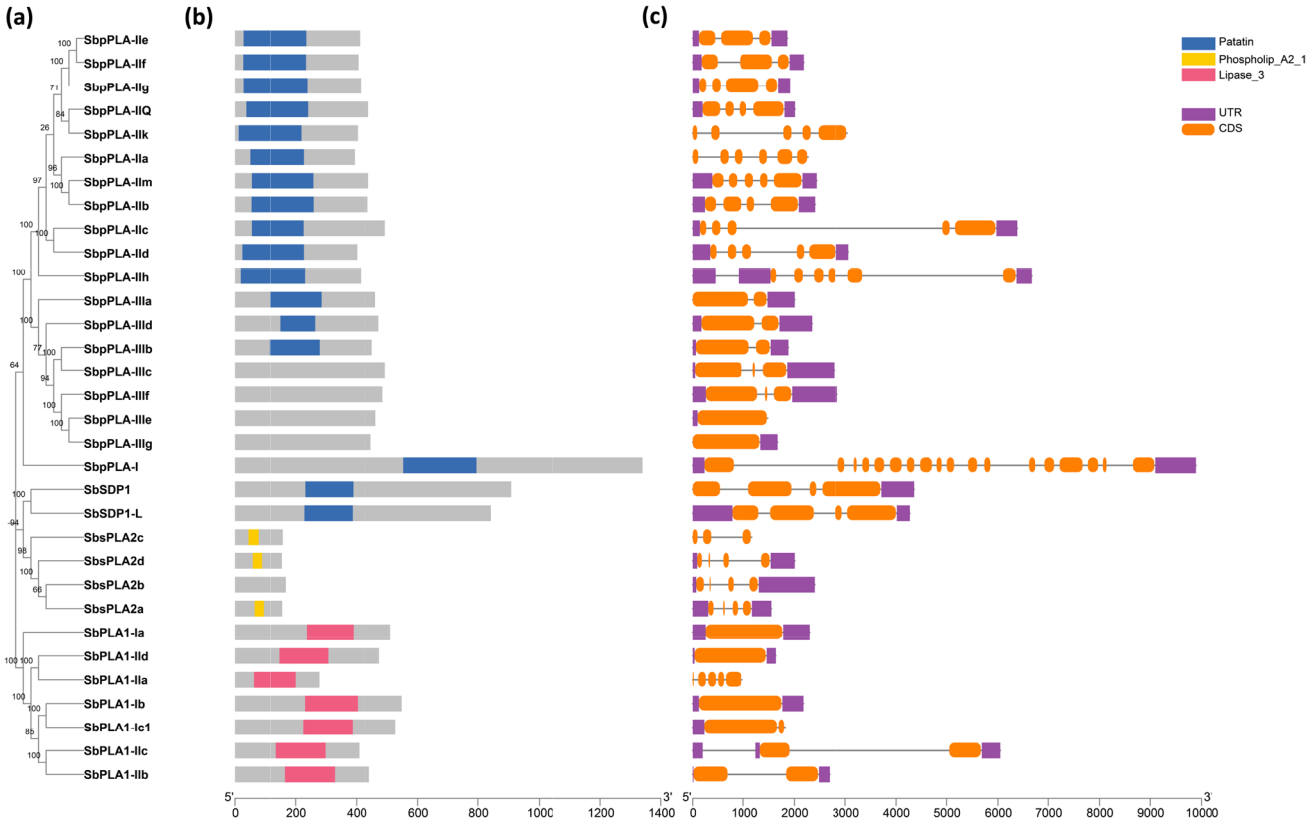

Fig. 2 Phylogenetic relationships, conserved protein domain and gene structure of *PLA* genes in *Sorghum bicolor*. **a** Phylogenetic tree of 32 amino acid sequences *SbPLA* gene family. **b** Protein conserved domain in *PLA*-like genes of sorghum. Blue boxes represent the presence of patatin domain, yellow boxes represent phospholipase_A2_1 domain, pink boxes represent lipase_3 domains while the grey boxes represents the rest of the protein sequences except the conserved domain in the respective SbPLA protein sequences. **c** The exon–intron structure of *SbPLA*-like genes. The purple boxes represent untranslated region (UTR), the orange boxes indicate exons and black lines indicate introns

signifies the role of the *SbPLA* gene family in vital tissue functioning. Overall, these qPCR results depict diverse transcript abundance at different stages of the tissue, proving a solid backbone in regulating growth, defence and stress regulation mechanisms.

Discussion

Proteins of the phospholipase family are categorized into four types:PLAs, PLBs, PLCs, and PLDs, distinguished by their diverse enzymatic functions (Wang 2001). PLAs and PLBs remove hydrophobic fatty acid tails from the glycerol of phospholipids, whereas PLCs and PLDs eliminate hydrophilic phosphate bands or side chains from protein structures.

The exhaustive exploration of the sorghum genome through various methodologies has resulted in the identification of 32 *SbPLA* genes. Similar to Arabidopsis and rice, three distinct groups of PLAs were discerned. Rice exhibited 31 *PLAs* (Singh et al. 2012), while Arabidopsis had 29 (Chen et al. 2011). According to the evolutionary relation between SbPLA with rice and Arabidopsis, *PLA* genes are distributed based on homologous protein similarity and into clusters. Phylogenetic analysis suggests the division of SbPLA proteins into three groups, namely pPLA, PLA_1, and $sPLA_2$, consistent with the classification proposed in Arabidopsis, which showed 13, 12, and 4 members, respectively (Chen et al. 2011), and rice, which exhibited 16, 11, and 3 members, respectively (Singh et al. 2012). Similarly, these proteins are distributed across these classes in sorghum, comprising 21, 7, and 4 members, respectively (Fig. 1).

Results from *in silico* tools for subcellular localization revealed the variable distribution of *SbPLA* genes in chloroplasts, cytoplasm, nucleus, and other organelles. Notably, the *SbpPLA* genes followed the localization pattern observed in Arabidopsis (Chen et al. 2011) and rice (Singh et al. 2012). Mostly all the *SbpPLA* genes were found in chloroplasts (Table 1). The plant PLA_1 class exhibits a molecular weight range of approximately 45–60 kDa (Matos and Pham-Thi 2009). Similarly, the SbPLA1 class consists of higher

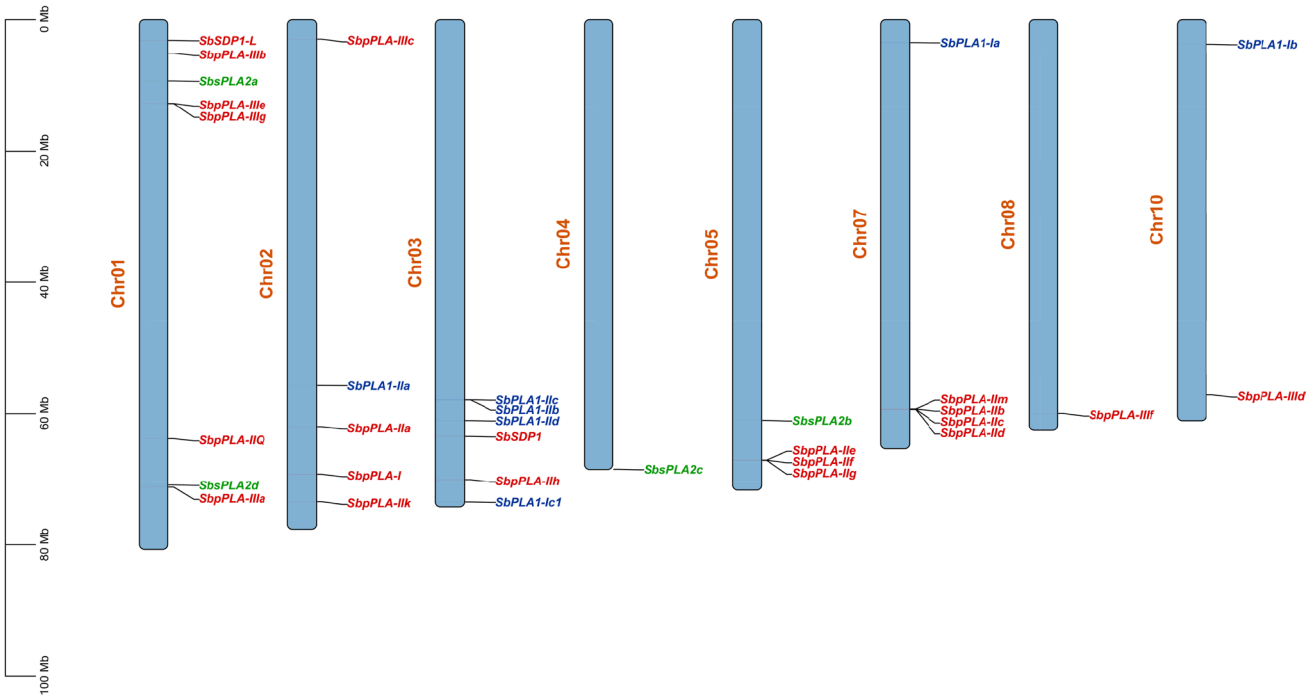

Fig. 3 The distributions of *SbPLA*-like genes of *Sorghum bicolor* across ten chromosomes. *SbPLA* genes are widely distributed in the sorghum genome and found on all chromosomes. The chromosome number is located beside each bar. The length of chromosomes is displayed in millions of bases (Mb) on the scale bar on the left side of the figure

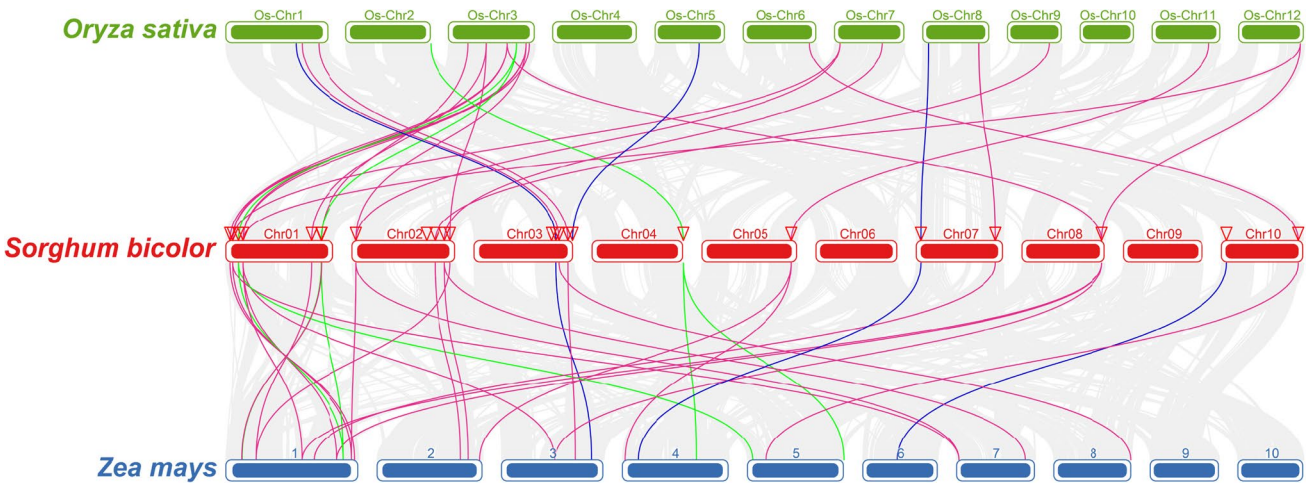

Fig. 4 Synteny analysis of *PLA* genes between *Sorghum bicolor*, *Oryza sativa* and *Zea mays*. The collinear gene pairs were identified using MCscanX. The grey color background shows a collinear box between *Sorghum bicolor* with *Oryza sativa* and *Zea mays*. An inverted triangle shows the position of homologous genes on the chromosome. Various *PLA* gene pairs corresponded according to their classes in different colored lines: pink, pPLAs; blue, PLA_1; green, $sPLA_2$.

molecular weights, ranging from about 45.5 to 60.5 kDa, except the SbPLA1-IIa protein (Table 1).

Domain analysis allowed the recognition of specific characteristics within different PLA classes, such as the presence of the 'patatin' domain in the pPLA class, the 'lipase3' domain in the PLA_1 class, and the 'PA2c' domain in the $sPLA_2$ class. These distinct domains differentiate various classes of PLAs in sorghum, as observed in Arabidopsis and rice (Ishiguro et al. 2001; Holk et al. 2002; Lee et al. 2005; Singh et al. 2012) (Fig. 2b).

In terms of intron–exon structure, SbPLA1 members in subtype 1 (except SbPLA1-Ic1) were found to be intronless,

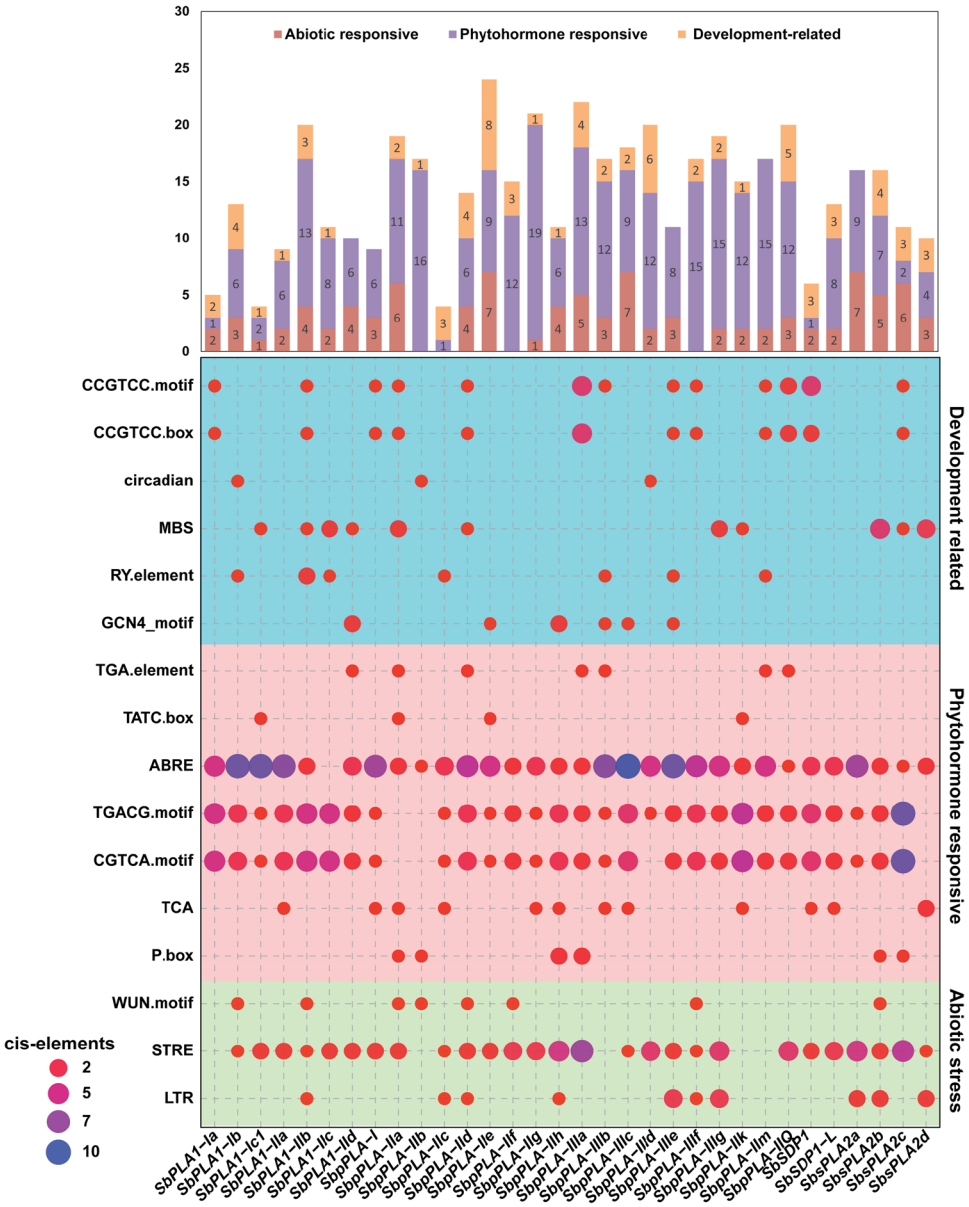

Fig. 5 The distribution of *cis*-acting elements in the promoter sequences of *SbPLA*-like genes in *Sorghum bicolor*. The upper bar graph uses different colours to recognize various cis-acting elements representing the total number of cis-acting elements, such as abiotic responsive, phytohormone responsive, and plant development related. The bubble graph is plotted based on the number of *cis*-acting elements in the promoter region. The bubble size is proportional to the abundance of each *cis*-acting element (1.5 kb upstream of the translation start site)

consistent with studies in Arabidopsis, while subtype 2 members contained 1–2 introns in sorghum, similar to rice. However, pPLAs exhibited differences in intron–exon structure compared to Arabidopsis (Scherer 2002) (Fig. 2c). Chromosomal mapping of *SbPLA* genes showed variable distribution on 10 chromosomes, excluding chromosomes 6 and 9. Similarly, in rice, *PLA* genes are distributed on 11 chromosomes except chromosome 4 (Singh et al. 2012) (Fig. 3).

Previous findings have indicated the significance of the *phospholipase* gene family in early developmental stages, as many candidates exhibit developmental-specific elements in their promoter regions. These elements include MBS, circadian, GCN4 motif, RY element, and CCGTCC-box, as well as hormone-responsive elements such as CGTCA motif, TGCGA motif, STRE, P-box, TCA element, TATC-box, TGA box, and ABRE motif, reflecting diverse functions in plants (Fig. 5). Phospholipases are crucial in developmental and hormone-specific gene functions, contributing to abiotic stress-triggered signalling pathways (Takáč et al. 2019). Moreover, diverse PLA members are present in various plant tissues and overall plant development (Saddhe and Potocky 2023).

The application of STRING analysis on the genes facilitated the construction of a network, offering insights into the potential utility of the candidates. It provided an overview of diverse pathways where the candidates may play a role. The analysis highlighted the predominant involvement of these candidates in lipid degradation, lipid metabolism, and hydrolases. Notably, SbSDP1-L and SbsPLA2a emerged as key

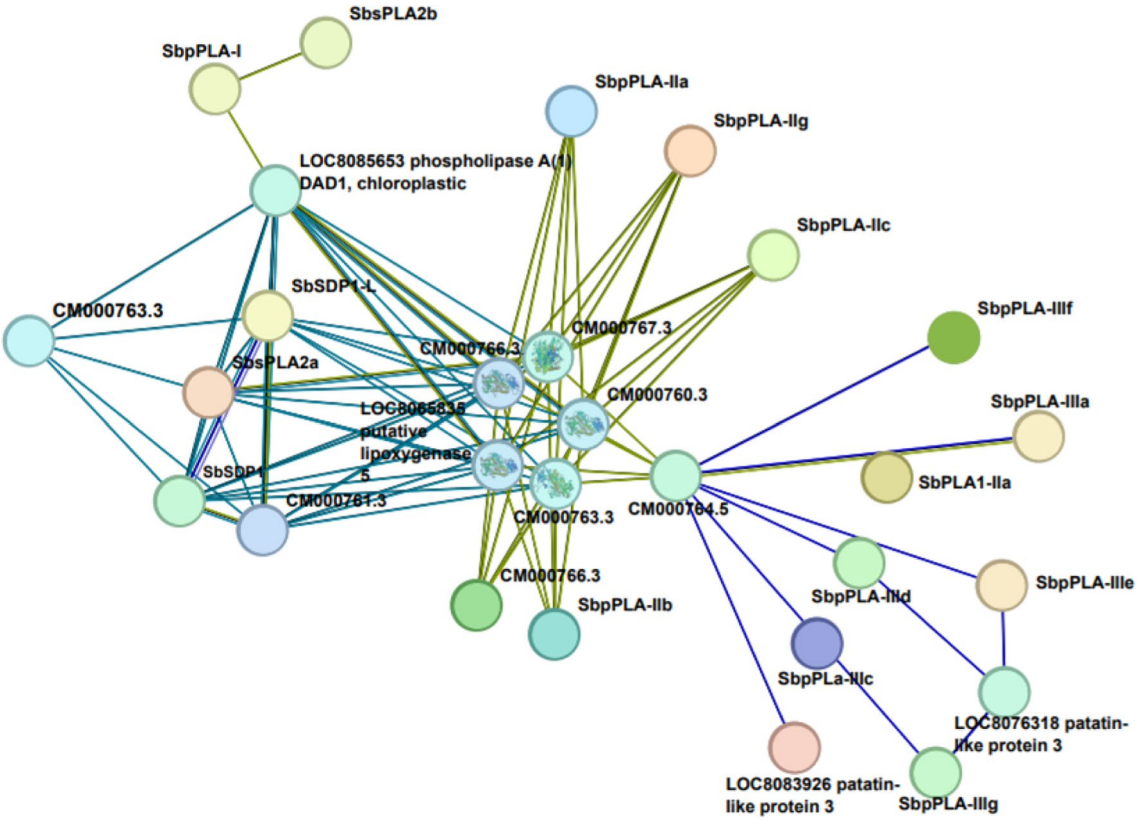

Fig. 6 Protein–protein interaction network of SbPLA proteins. The image represents the protein–protein interaction studies performed on the SbPLA protein sequences. The blue line connecting the proteins indicates the co-occurrence evidence of the proteins connected by that blue line. Coloured nodes represent query proteins and the first shell of interactors. White nodes represent the second shell of interactors. Empty nodes represent the proteins with unknown 3D structures. Filled nodes represent that the 3D structure of a protein is either known or predicted. Known interactions, sky blue nodes—taken from curated databases; magenta nodes—experimentally determined predicted Interactions; green gene, neighbourhood; red gene, fusions; blue gene, co-occurrence. Other interactions are light green nodes, text mining; lavender nodes, protein homology

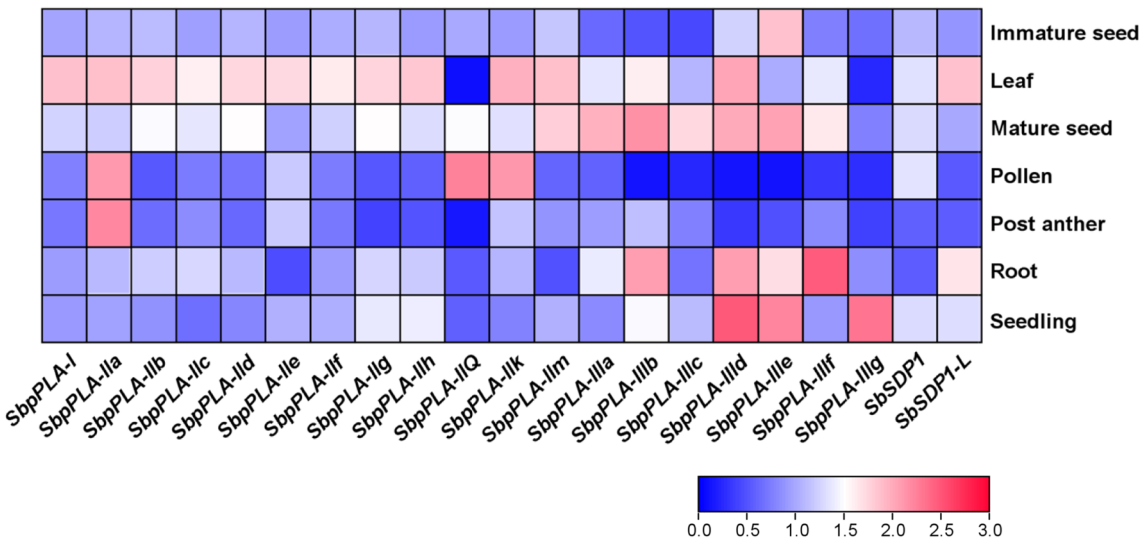

Fig. 7 Heatmap showing the relative expression levels (by qPCR) of the *SbPLA* transcripts in different sorghum tissues. The expression levels were compared among stages such as immature seed, leaf, mature seed, pollen, post anther, root, and seedling. The red color depicts high expression, blue color depicts low expression while the white color depicts moderate levels of the *SbPLA* homologs in sorghum

controllers, participating in various processes and regulating the network of the *PLA* gene family. These proteins belong to the lipolytic acyl hydrolase family, indicating their crucial role in governing membrane function regulation.

The *PLA* gene family is actively involved in various developmental processes of plants, including seed development, root development, and cell elongation (Ali et al. 2022). PLA_2 plays a role in auxin-induced cell elongation in plants (Scherer 2002). Based on previous studies, the pPLA class is involved in different expression studies in developmental tissue in rice (Singh et al. 2012), Arabidopsis (Matos et al. 2008), and cotton (Wei et al. 2023). This study extends this analysis to examine the pPLA class's expression profiling in various sorghum tissues. Our findings reveal the upregulation of SbpPLA-III, particularly in the root and seedling stages, suggesting its potential role in seed development and root elongation processes. In Arabidopsis, certain members of pPLA have been reported to be up-regulated under drought stress conditions, influencing root development via auxin and phosphate deficiency pathways (Matos et al. 2008). Conversely, the expression patterns of *SbpPLA-IIa*, *SbpPLA-IIQ*, and *SbpPLA-IIk* exhibit moderate levels of pollen-specific expression (Fig. 7), hinting at their potential involvement in the fertilization process and suggesting further investigation into their role in haploid development. Phospholipase genes specific to pollen are pivotal in haploid induction across various crops. For instance, the maternal haploid induction system in monocots, based on pPLA, is known as *MATRILINEAL/NOT LIKE DAD/ZmPHOSPHOLIPASE-A1* (Kelliher et al. 2017; Liu et al. 2017; Gilles et al. 2017), and has been successfully implemented in maize (Kelliher et al. 2017), rice (Yao et al. 2018), wheat (Liu et al. 2020), and foxtail millet (Cheng et al. 2021). Recent studies have also demonstrated haploid induction in japonica rice and Arabidopsis through the loss of function in pollen-expressed phospholipase *OsMATL2* (*OspPLA-IIα*) gene and gynoecium-expressed *phospholipase AII* (*pPLAIIγ*) genes, respectively (Jang et al. 2023a, b).

Conclusion

In this investigation, we successfully identified and characterized 32 *PLA* genes in *Sorghum bicolor*. Utilizing phylogenetic analysis, the 32 *SbPLAs* were classified into three distinct subtypes: pPLA, PLA_1, and $sPLA_2$. In silico sequence analysis gained comprehensive insights into the *SbPLA* gene family, encompassing gene structure, conserved motifs, chromosomal localization, promoter elements, and protein–protein interactions. Expression analysis revealed the up-regulation of the pPLA subtype in pollen tissue, while most other genes exhibited a moderate expression level in leaves. Identifying pollen-specific genes in sorghum suggests their potential role in haploid induction, shortening the breeding cycle process. The phospholipase genes identified in sorghum present promising avenues for future studies exploring signalling pathways related to biotic-abiotic stress, growth hormones, lipid degradation, lipid metabolism, and hydrolases. These investigations pave the way for detailed molecular characterization and an exploration of the in-planta roles of *PLA* genes in sorghum.

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Author contributions Conceptualization: PSR; Methodology: VS, AS, PSR; software: VS and AS; data analysis: VS and AS; writing—original draft preparation, VS and AS; writing—review and editing, PSR; supervision and funding acquisition: PSR. All authors have read and agreed to the published version of the manuscript.

Data availability Data are contained within the article or supplementary material.

Declarations

Conflict of interest The authors declare no conflict of interest.

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