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GENOME-WIDE IDENTIFICATION AND ANALYSIS OF SPX GENE FAMILY MEMBERS IN *SOLANUM TUBEROSUM* AND INSIGHTS INTO THEIR POTENTIAL FUNCTIONS

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Abstract

SPX family, named for the presence of conserved Syg1/Pho81/XPR1 (SPX) domain, is one of the most important gene families involved in phosphate ions (Pi) homeostasis and signaling transduction in plants. In this study, we identified 14 SPX family genes in *Solanum tuberosum* at the genome-wide level. These genes were distributed across 7 of the 12 chromosomes. Based on additional domains in the SPX family structure, the SPX family were classified into four SPX subfamilies, SPX, SPX-ERD1/XPR1/SYG1(SPX-EXS), SPX- SPX-Really Interesting New Gene (SPX-RING), and a newly identified SPX-EXS-DNL subfamily characterized by a short C-terminal motif D(N/H). The majority of SPXs were localized to the plasma membrane. Phylogenetic analysis of SPX family genes in *Solanum tuberosum*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa*, and *Zea mays* categorized these genes into 7 groups. The duplication events show that *SPX* genes in *Solanum tuberosum* were mainly produced by whole-genome duplication (WGD)/segmental duplications (SD). The synteny analysis demonstrated that the *SPX* gene family of *Solanum tuberosum* exhibited a closer phylogenetic relationship to *Solanum lycopersicum* compared to *Arabidopsis thaliana*. Additionally, expression profiling of *Solanum tuberosum* SPX family genes showed diverse tissue-specific patterns and varying degrees of responsiveness to both biotic and abiotic stresses. And twelve *Solanum tuberosum* SPX family members could interact and form an association network. Furthermore, one gene (PGSC0003DMG402022752) from the SPX subfamily was found to exhibit the highest expression levels in the floral organs, and one member (PGSC0003DMG400017163) of the SPX-EXS subfamily was highly expressed under heat treatment (35°C). These findings implies that the two genes may play a vital role in floral organ development and heat stress response, respectively. This study offers a basis for future elucidation of the functions of *Solanum tuberosum* SPX family genes, especially in phosphorus utilization.

Key words: *Solanum tuberosum*; Phosphate ions (Pi) nutrition; SPX; Genome-wide; Gene family.

Abbreviations: SPX: SYG1, PHO81, and XPR1; Pi: Phosphate ions; SPX-EXS: SPX-ERD1/XPR1/SYG1; SPX-RING: SPX-Really Interesting New Gene; SPX-EXS-DNL: SPX- EXS-a short C-terminal motif of D(N/H); WGD: Whole-genome duplication; SD: Segmental duplications; SPX-MFS: SPX-Major Facilitator Superfamily; P: Phosphorus; MW: Molecular weight; pI: Isoelectric point; NJ: Neighbour-joining; BAP:6 Benzylaminopurine; ABA: Abscisic acid; IAA: Indole-3-acetic acid; GA3: Indole-3-acetic acid; BTH: Acibenzolar-S-methyl; BABA: DL-β-amino-n-butyric acid; FPKM: Fragments per kilobase per million mapped reads; ORF: Open reading frame; aa: Amino acids; CDS: Coding sequence; TD: tandem duplications; chr: chromosome.

Introduction

The collection of multiple related genes forms a gene family, which are similar in sequence, structures, and biological functions (Baloch *et al*., 2022). The SPX gene family is one such family, members of which are essential for maintaining phosphate ion (Pi) homeostasis and facilitating signaling transduction in both microorganisms and plants (Li *et al*., 2001; Hürlimann *et al*., 2009). SPX proteins are so named due to the presence of conserved Syg1/Pho81/XPR1 (SPX) domain, which is typically found at the N-terminus of the protein and functions either independently or in concert with other domains located at the C-terminus (Peng *et al*., 2007; Duan *et al*., 2008; Secco *et al*., 2012; Liu *et al*., 2016). Based on the characteristics of other domains at the C-terminus, the SPX proteins are divided into four subfamilies in plant, including SPX subfamily, SPX-Major Facility Superfamily (MFS) subfamily, SPX-ERD1/XPR1/SYG1 (EXS) subfamily, and SPX-Really Interesting New Gene (RING) subfamily (Secco *et al*., 2012). To date, the four subfamilies of SPX proteins have been found in *Arabidopsis thaliana*, rice, *Brassica napus*, wheat, maize, and *Solanum lycopersicum* (Secco *et al*., 2012; Du *et al*., 2017; Kumar *et al*., 2019; Xiao *et al*., 2021; Li *et al*., 2021). Not all plants contain the four subfamilies of SPX proteins, and *Phyllostachys edulis* only contains three subfamilies, including the SPX subfamily, the SPX-EXS subfamily and the SPX-MFS subfamily, but lacks the SPX-RING subfamily (Luo *et al*., 2023).

The four subfamilies of SPX proteins play crucial roles in the regulation of phosphate homeostasis in plants (Secco *et al*., 2012). The SPX subfamily acts as positive or negative regulators during plant adaptation to phosphate starvation (Duan *et al*., 2008; Wang *et al*., 2009; Liu *et al*., 2010; Zhang *et al*., 2016). The SPX-EXS subfamily plays a broad role in the transfer of phosphate to the vascular cylinder, facilitating long-distance Pi transfer (Wang *et al*., 2004; Wang *et al*., 2008; Secco *et al*., 2010; Zhao *et al*., 2019). The SPX-MFS subfamily contributes to the import of Pi into vacuoles or the export of Pi from the vacuole (Wang *et al*., 2015; Liu *et al*., 2016; Liu *et al*., 2016). The SPX-RING subfamily was related to nitrogen limitation responses and holds pivotal roles in Pi responses (Peng *et al*., 2007; Kant *et al*., 2011; Yang *et al*., 2017; Yue *et al*., 2017; Zhong *et al*., 2017).

Solanum tuberosum is considered an important tuber food crop and contributing to food security in the world (Anon., 2011). To produce high yields, crop must acquire essential minerals include the macronutrients and the micronutrients (Chen & Liao, 2017). *Solanum tuberosum* as a crop, it is also need acquire these essential minerals to produce high yields. Phosphorus (P) is an essential

macronutrient that is crucial for crop production (Chen & Liao, 2017). *Solanum tuberosum* production needs large amount of phosphorus. SPX family is essential for maintaining Pi homeostasis during the process of plant growth and development. However, SPX family has not been reported in *Solanum tuberosum*. Accordingly, it is imperative to conduct identification and comprehensive characterization of SPX family across the entire genome of *Solanum tuberosum*.

In this study, Genome-wide identification and analysis of SPX family was first performed in *Solanum tuberosum*. We present the detailed study of the SPX family in *Solanum tuberosum*. We performed systematic analyses of their gene structures, phylogenetic relationships, motif composition, chromosomal distributions, duplication events and synteny prediction, expression patterns, and interaction network. These results will offer valuable insights into the potential functional roles of SPX family members in *Solanum tuberosum*. Furthermore, they serve as a robust foundation for future investigations aimed at elucidating the functional mechanisms of these proteins, particularly in the context of phosphorus utilization.

Materials and Methods

Identification of SPX family members in *Solanum tuberosum*: To identify and characterize potential members of the SPX family in the genome of *Solanum tuberosum*, we employed a targeted search approach utilizing SPX as the search term. This search was conducted across two reputable databases: UniProt, a comprehensive resource for protein sequence and functional information, and Spud DB Potato Genomics Resources, a specialized repository for potato genomics data. And then, all potential members of the SPX family in *Solanum tuberosum* were retrieved from the UniProt databases and the Spud DB Potato Genomics Resources. Finally, we submitted all the non-redundant potential protein sequences to Expasy-PROSITE (de Castro *et al*., 2006), the SMART website (Letunic & Bork, 2018) and NCBI Batch CD-search Tool (Lu *et al*., 2020) to identify SPX domain. The members of potential SPX family that did not contain the SPX conserved domain were removed. The same identification method was used to search for SPX family members in *Arabidopsis thaliana* and *Solanum lycopersicum*. The potential SPX family members of *Arabidopsis thaliana*, *Solanum lycopersicums*, *Oryza sativa*, and *Zea mays* were downloaded from TAIR database, SGN databases, Rice Genome Annotation Project database, and MaizeGDB.

SPX family proteins physicochemical property, chromosomal location, gene structure and motif analysis: We utilized the ExPaSy platform to determine their molecular weights (MW) and isoelectric points (pI). This analysis was performed following the established protocols by Gasteiger *et al*., (2005). Additionally, to further characterize these proteins, we employed EnsemblPlants to investigate their additional physicochemical properties (i.e., Location, Strand, ORF (aa) and CDS (bp)). To gain a deeper understanding of the SPX family proteins, we performed a series of comprehensive analyses. First, we utilized WoLF PSORT (Protein Subcellular Localization Prediction) to predict the subcellular localization of these proteins. Next, we constructed the chromosomal distribution of SPX family genes using MG2C (Chao *et al*., 2021). Furthermore, we conducted gene structural analysis of the SPX family using GSDS (Hu *et al*., 2015). Lastly, to identify conserved motifs within the SPX family proteins, we employed the online MEME (Multiple Em for Motif Elicitation) software v5.5.3 (Li *et al*., 2022). Using stringent settings (20 maximum motifs, motif width ranging from 6 to 50 residues).

Phylogenetic tree construction and the domains analysis of the SPX family protein genes: To investigate the evolutionary relationships among the SPX family proteins across different species, we constructed an inter-species phylogenetic tree. This analysis encompassed protein genes from *Solanum tuberosum*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa*, and *Zea mays*. The tree was generated using the MEGA 11.0 software (Tamura *et al*., 2021), employing the neighbor-joining (NJ) method with 1000 bootstrap replicates. Subsequently, we uploaded the constructed inter-species phylogenetic tree to the online utility ITOL (Letunic *et al*., 2021) to generate a high-quality tree illustration. Furthermore, a phylogenetic tree was constructed for SPX family protein genes from *Solanum tuberosum* using the aforementioned method. To comprehensively identify the protein domains within the SPX family proteins, we harnessed the power of two complementary tools: ExPaSy (Sigrist *et al*., 2013) and HMMER (Finn *et al*., 2015). Furthermore, to visually represent these identified domains, we utilized MyDomains-Image Creator (Hulo *et al*., 2008) to generate high-quality domain figures.

SPX family genes duplication events in *Solanum tuberosum*, and synteny analysis with other plants: To analyze SPX family gene duplication events in *Solanum tuberosum* and to explore their syntenic relationships with other plant species, we sourced genome files from various reputable databases. Specifically, the genome of *Solanum tuberosum* was retrieved from Spud DB Potato Genomics Resources. For comparison, we also obtained the genome files of *Solanum lycopersicum* from the SGN databases and those of *Arabidopsis thaliana* from TAIR. To analyze the duplication events within the SPX family genes in *Solanum tuberosum*, we employed the MCScanX tool (Wang *et al*., 2012). Furthermore, to visualize the collinear relationships between the SPX family genes in *Solanum tuberosum* and other selected plant species, we utilized the Circos and TBtools software (Wang *et al*., 2012; Krzywinski *et al*., 2009; Chen *et al*., 2020). To gain a comprehensive understanding of the syntenic relationships among the SPX family genes in *Solanum tuberosum*, *Solanum lycopersicum*, and *Arabidopsis thaliana*, we performed a thorough synteny analysis. This analysis was facilitated by the Dual Synteny Plot feature integrated within MCScanX (Wang *et al*., 2012) and TBtools software (Chen *et al*., 2020).

Expression analysis of *Solanum tuberosum* SPX family genes: RNA-Seq gene expression data were retrieved from Spud DB Potato Genomics Resources (Anon., 2011) to analyze the expression levels of SPX family genes in *Solanum tuberosum* different tissues and in entire plants subjected to diverse treatments including abiotic and biotic stresses. The expression analysis encompassed a total of 15 tissues, which

included roots, shoots, leaves, petioles, stolons, whole flowers, sepals, petals, stamens, carpels, mature fruits, immature fruits, mesocarp & endocarp, tubers (tubers 1 and tubers 2) and callus. The whole *Solanum tuberosum* plants abiotic stresses were treated for 24 h with salinity (150 mM NaCl), drought (260 µM mannitol), as well as hormone treatments like BAP (6 benzylaminopurine) (10 µM), ABA (abscisic acid) (50 µM), IAA (indole-3-acetic acid) (10 µM), GA3 (indole-3-acetic acid) (50 µM) and heat (35°C) (Anon., 2011). To induce biotic stresses, leaves was treated with *Phytophthora infestans* (Pi isolate US8: Pi02-007) and two chemical inducers, acibenzolar-S-methyl (BTH) and DL-β-amino-n-butyric acid (BABA) for 24, 36 and 72h. Subsequently, mixed samples of leaves treated for 24, 36, and 72h were employed to collect the RNA-Seq data (Anon., 2011). To delve into the expression patterns of the SPX family genes, we employed the FPKM (fragments per kilobase per million mapped reads) metric, which provides a quantitative assessment of gene expression levels. These FPKM values were subsequently visualized in the form of heatmaps using the TBtools-II software (version No.1.120) (Chen *et al*., 2020).

SPX family genes interaction network analysis: To gain insights into the potential protein-protein interactions (PPIs) within the SPX family in *Solanum tuberosum*, we utilized the STRING database (Szklarczyk *et al*., 2023). Subsequently, we employed Cytoscape (version No.3.9.0) software (Shannon *et al*., 2003) to visualize and analyze the predicted PPI network.

Results

Identification of SPX family members in *Solanum tuberosum*: Based on UniProt databases, Spud DB Potato Genomics Resources, Expasy-PROSITE, SMART website and NCBI Batch CD-search Tool, in all, 14 *SPXs* were identified in *Solanum tuberosum*. The gene ID, location on chromosome, strand, protein length (ORF) (aa), coding sequence (CDS) lengths (bp), molecular weights (MWs) (KDa), isoelectric point (pI) and subcellular localization are listed in Table 1. Analysis of the SPX family genes revealed a range of variation in their coding sequence (CDS) lengths, spanning from 789 bp (PGSC0003DMG400001340) to 2373 bp (PGSC0003DMG400026017). These CDS lengths translated into proteins with amino acid (aa) counts ranging from 262 to 790 aa, highlighting the diversity within the SPX family. Furthermore, the molecular weights (MWs) of the SPX proteins exhibited significant variation, from 29.66 KDa to 91.65 KDa, reflecting their distinct structural properties. The isoelectric points (pI) of these proteins ranged from 4.76 to 9.38, indicating diverse physicochemical characteristics. Notably, half of the 14 *SPX* genes were located on the forward (plus) strand, while the other half resided on the reverse (minus) strand, suggesting no obvious strand bias in their genomic organization. Seven SPXs were located to plasma membrane, five SPXs targeted to nucleus, and the only 2 SPXs were located to cytoplasm.

Table 1. Detailed information on physical parameters of *Solanum tuberosum* SPX family genes.

Gene ID	Location	Strand	ORF (aa)	CDS (bp)	MW (KDa)	pI	Subcellular localization
PGSC0003DMG400001340	Chr1:70183000-70185203	Plus	262	789	29.66	7.63	Cytoplasm
PGSC0003DMG400001430	Chr2:46027379-46034412	Minus	294	885	33.5	4.76	Nucleus
PGSC0003DMG400002890	Chr2:45999331-46003399	Plus	766	2301	88.48	9.38	Plasma membrane
PGSC0003DMG400011399	Chr2:25500408-25508317	Minus	307	924	34.35	4.86	Nucleus
PGSC0003DMG400012523	Chr5:1623135-1632749	Minus	667	2004	77.12	9.08	Plasma membrane
PGSC0003DMG400015850	Chr5:53768-59724	Plus	780	2343	91.65	9.24	Plasma membrane
PGSC0003DMG400017163	Chr8:124092-133704	Plus	694	2085	77.86	5.89	Plasma membrane
PGSC0003DMG402022752	Chr8:15890911-15892602	Minus	266	801	30.9	6.87	Nucleus
PGSC0003DMG400022866	Chr8:53883630:53891903	Minus	697	2094	78.42	5.71	Plasma membrane
PGSC0003DMG400025127	Chr8:43137116-43141690	Plus	732	2199	85.29	8.92	Plasma membrane
PGSC0003DMG400025135	Chr9:52575368-52580483	Minus	333	1002	37.92	8.95	Nucleus
PGSC0003DMG400026017	Chr9:56661754-56671464	Minus	790	2373	90.92	9.19	Plasma membrane
PGSC0003DMG400028396	Chr11:31974040-31979861	Plus	336	1011	38.5	8.45	Nucleus
PGSC0003DMG400041272	Chr12:2943690-2947071	Plus	292	879	33.54	6.16	Cytoplasm

Phylogenetic analysis of the SPX family genes in the *Solanum tuberosum* and other species: To elucidate the potential evolutionary relationships among the SPX family genes across a diverse array of plant species, we embarked on a comprehensive phylogenetic analysis. Utilizing MEGA (version 11.0), we constructed a neighbor-joining (NJ) phylogenetic tree that encompassed a total of 50 SPX genes from five representative plant species: *Solanum tuberosum*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa*, and *Zea mays* (Fig. 1). The NJ phylogenetic tree analysis showed that the *SPX* genes were categorized into seven groups (class I, II, III, IV, V, VI, and VII), with class IV containing the highest members of *SPXs* (19 *SPX*s) and class III had the fewest members (7 *SPX*s). Our NJ phylogenetic tree analysis revealed a compelling organization of the SPX gene family into seven distinct classes (I, II, III, IV, V, VI, and VII). Notably, class IV emerged as the most populous group, encompassing 19 *SPX* genes. In contrast, class III comprised the fewest members, with only 7 *SPX* genes. Class I contained 14 *SPX* members, including 2 *SPX*s from *Solanum tuberosum*, 2 *SPX*s from *Arabidopsis thaliana*, 3 *SPX*s from *Solanum lycopersicum*, 3 *SPX*s from *Oryza sativa*, and 4 *SPX*s from *Zea mays*. Class II contained 17 *SPX* members, including 3 *SPX*s from *Solanum tuberosum*, 9 *SPX*s from *Arabidopsis thaliana*, and 5 *SPX*s from *Solanum lycopersicum*. Class III was the least group and contained 7 *SPX* members, including 2 *SPX*s from *Solanum tuberosum*, 1 *SPX* from *Arabidopsis thaliana*, 2 *SPX*s from *Solanum lycopersicum*, 1 *SPX* from *Oryza sativa*, and 1 *SPX* from *Zea mays*. Class IV was the largest group and contained 19 *SPX* members, including 3 *SPX*s from

Solanum tuberosum, 3 *SPX*s from *Arabidopsis thaliana*, 3 *SPX*s from *Solanum lycopersicum*, 5 *SPX*s from *Oryza sativa*, and 5 *SPX*s from *Zea mays*. Class V contained 9 *SPX* members, including 2 *SPX*s from *Solanum tuberosum*, 2 *SPX*s from *Arabidopsis thaliana*, 2 *SPX*s from *Solanum lycopersicum*, 2 *SPX*s from *Oryza sativa*, and 1 *SPX* from *Zea mays*. Class VI was composed of 9 *SPX* members from dicotyledonous, including 2 *SPX*s from *Solanum tuberosum*, 3 *SPX*s from *Arabidopsis thaliana*, and 4 *SPX*s from *Solanum lycopersicum*. Class VII was composed of 9 *SPX* members from monocotyledonous, including 4 *SPX*s from *Oryza sativa* and 5 *SPX*s from *Zea mays*. Within *Solanum tuberosum*, the 14 SPX family genes were systematically categorized into six distinct classes: class I, class II, class III, class IV, class V, and class VI.

Domain, conserved motif, and gene structure analysis of SPX family members in *Solanum tuberosum*: All the 14 SPX family members contain SPX domain at the N-terminal (Fig. 2). We only found three subfamilies of SPX proteins in *Solanum tuberosum*, including SPX subfamily, SPX-EXS subfamily, and SPX-RING (Fig. 2). The SPX subfamily was composed of seven members (PGSC0003DMG400001340, PGSC0003DMG400 002890, PGSC0003DMG400012523, PGSC0003 DMG400015850, PGSC0003DMG402022752, PGSC 0003DMG400025135 and PGSC0003DMG4000 26017), SPX-EXS subfamily contained four members (PGSC0003DMG400001430, PGSC0003DMG4000 17163, PGSC0003DMG400028396 and PGSC0003DMG 400041272), and SPX-RING subfamily had two members (PGSC0003DMG400011399, PGSC0003DMG4000 22866) (Fig. 2). We didn't find the SPX-MFS subfamily in *Solanum tuberosum*, but we found a new subfamily named SPX-EXS-DNL subfamily (PGSC0003DMG4000 25127) (Fig. 2). The SPX-EXS-DNL subfamily was designated as 'DNL' based on the presence of a characteristic short C-terminal motif featuring a D(N/H) amino acid sequence. This subfamily is distinguished by its unique structural organization, which encompasses not only the conserved N-terminal SPX and C-terminal EXS domains but also harbors an additional DNL domain immediately downstream of the EXS domain. This unique domain arrangement suggests potential functional specializations within the SPX-EXS-DNL subfamily, warranting further investigation into its biological roles and regulatory mechanisms.

The phylogenetic analysis of *SPX* family members in *Solanum tuberosum* revealed a clear segregation into six distinct clades: Clade I, Clade II, Clade III, Clade IV, Clade V, and Clade VI (Fig. 3a). This clade-based organization underscores the evolutionary divergence and potential functional differentiation within the SPX gene family in *Solanum tuberosum*. Our analysis revealed a more nuanced organization within the *SPX* family, with the *SPX* subfamily comprising Clade I, Clade II, and Clade III. In contrast, Clade IV, Clade V, and Clade VI were affiliated with the *SPX-RING*, *SPX-EXS*, and *SPX-EXS-DNL* subfamilies, respectively. Notably, Figure 3 demonstrates striking similarities in gene structure (Fig. 3b) and conserved motif composition (Fig. 3c) between Clade I and Clade II of the SPX subfamily. These observations suggest a shared evolutionary history and potentially conserved functions for these closely related clades, underscoring the value of subclassification within the broader SPX gene family. All members of both Clade I and Clade II consistently exhibited a three-exon gene structure (Fig. 3b). Moreover, a comprehensive analysis of their conserved motif composition revealed the ubiquitous presence of motifs 1, 3, 4, 8, 13, and 14 (Fig. 3c). Clade III of the SPX subfamily had some differences from Clade I and Clade II, all the two members of which had ten exons (Fig. 3b) and all contained the same motifs including motifs 1, 2, 3, 4, 11, 12,14, 15,17, 18, 19, and 20 (Fig. 3c). SPX-RING subfamily was composed of Clade IV containing the two members, all which possessed six exons (Fig. 3b) and all contained the same motifs including motifs 1, 14 and 16 (Fig. 3c). The SPX-EXS subfamily, specifically represented by Clade V, comprises four members that exhibit a remarkable degree of complexity in their gene structure and conserved motif composition (Fig. 3). One member (PGSC0003DMG 400017163) of Clade V had fifteen exons (Fig. 3b) and contained motifs 1, 2, 3, 4, 5, 6, 7, 9, 10, and 14 (Fig. 3c). The other three members (PGSC0003DMG400001430, PGSC0003DMG 400028396, and PGSC0003DMG4000 41272) of Clade V all had thirteen exons (Fig. 3b) but possessed different conserved motif compositions (Fig. 3c). One member (PGSC0003DMG400001430) of the other three members contained motifs1, 2, 3, 4, 5, 6, 7, 9, and10, another member (PGSC0003DMG400028396) contained motifs 1, 2, 3, 4, 5, 6, 7, 9, 10, and 14, and the last member (PGSC0003DMG400041272) contained motifs 1, 2, 3, 4, 5, 6, 7, 9, and 14 (Fig. 3c). Clade VI was composed of only one SPX-EXS-DNL subfamily member, which possessed thirteen exons, and contained 5 motifs including motifs 1, 2, 5, 7, 10 (Fig. 3c).

Analysis of chromosome distribution and duplication event of the SPX family genes in the *Solanum tuberosum*: The identified 14 *SPX* genes were distributed to 7 of the 12 *Solanum tuberosum* chromosomes (Fig. 4). The chromosome 1, chromosome 11, and chromosome 12, each harbor one *SPX* gene. The chromosome 5 and chromosome 9 each contain two *SPX* genes. The chromosome 2 contained three *SPX* genes and the chromosome 8 contained four *SPX* genes (Fig. 4).

To investigate gene duplication events within the *SPX* family in *Solanum tuberosum*, MCScanX was employed to analyze these events, and a collinear diagram was subsequently generated using the Circos and TBtools software. As shown in this analysis results, 5 pairs of whole-genome duplication (WGD)/segmental duplications (SD) were found on *Solanum tuberosum* chromosomes, but no pairs of tandem duplications (TD) were found on *Solanum tuberosum* chromosomes (Fig. 5). Collectively, our findings indicate that the *SPX* gene family in *Solanum tuberosum* may have undergone significant expansion primarily through whole-genome duplication (WGD) and segmental duplication (SD) events. This conclusion underscores the pivotal role of these duplication mechanisms in shaping the evolutionary landscape of the *SPX* gene family in *Solanum tuberosum*, with potential implications for the functional diversification and adaptive evolution of these genes.

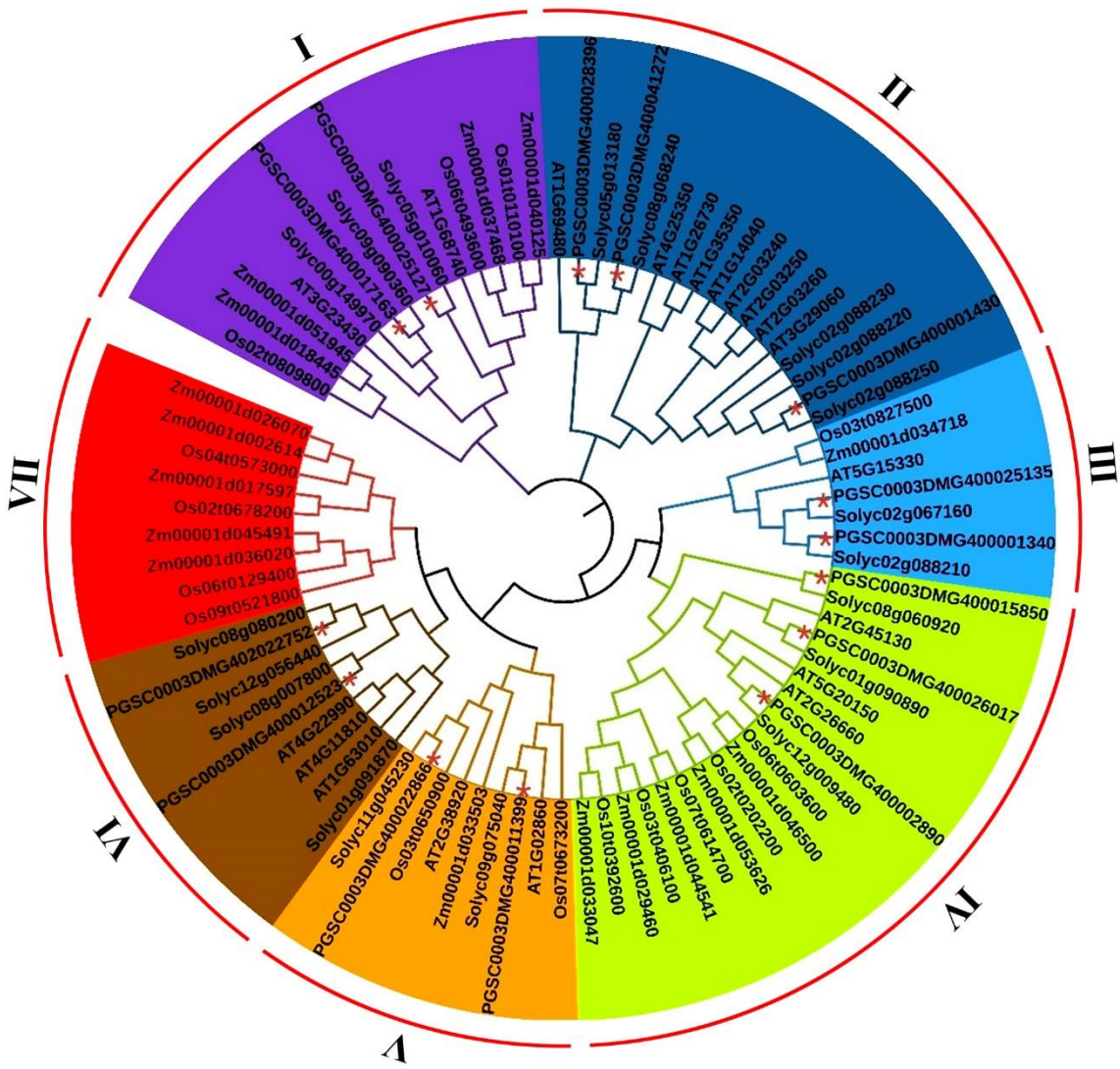

Fig. 1. Phylogenetic analysis of the SPX family genes in *Arabidopsis thaliana* (At), *Solanum lycopersicum* (Solyc), *Solanum tuberosum* (PGSC), *Oryza sativa* (Os), and *Zea mays* (Zm). Neighbor-Joining molecular phylogenetic tree was constructed with 1000 bootstrap replicates. Differently colors indicate different groups of SPX family genes. Red hexagrams indicate *Solanum tuberosum* SPX family genes.

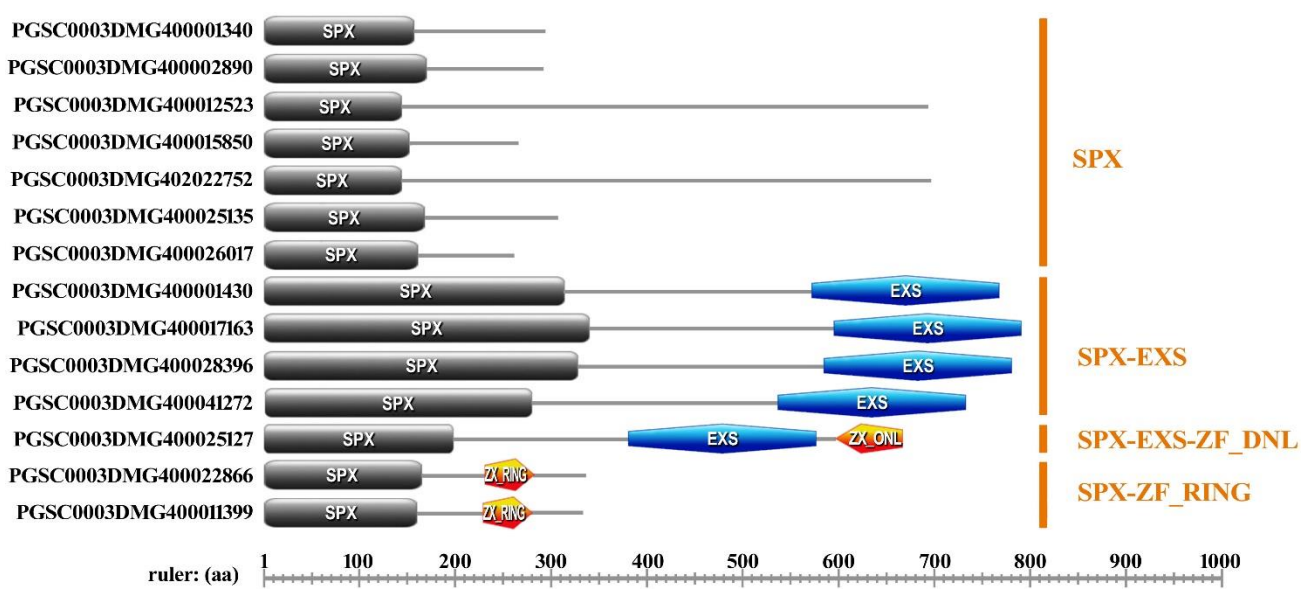

Fig. 2. Domains of the SPX family proteins in the *Solanum tuberosum*. Conserved domains of the SPX family proteins were marked with different colors and shapes. SPX subfamily name is shown on the right side of the figure.

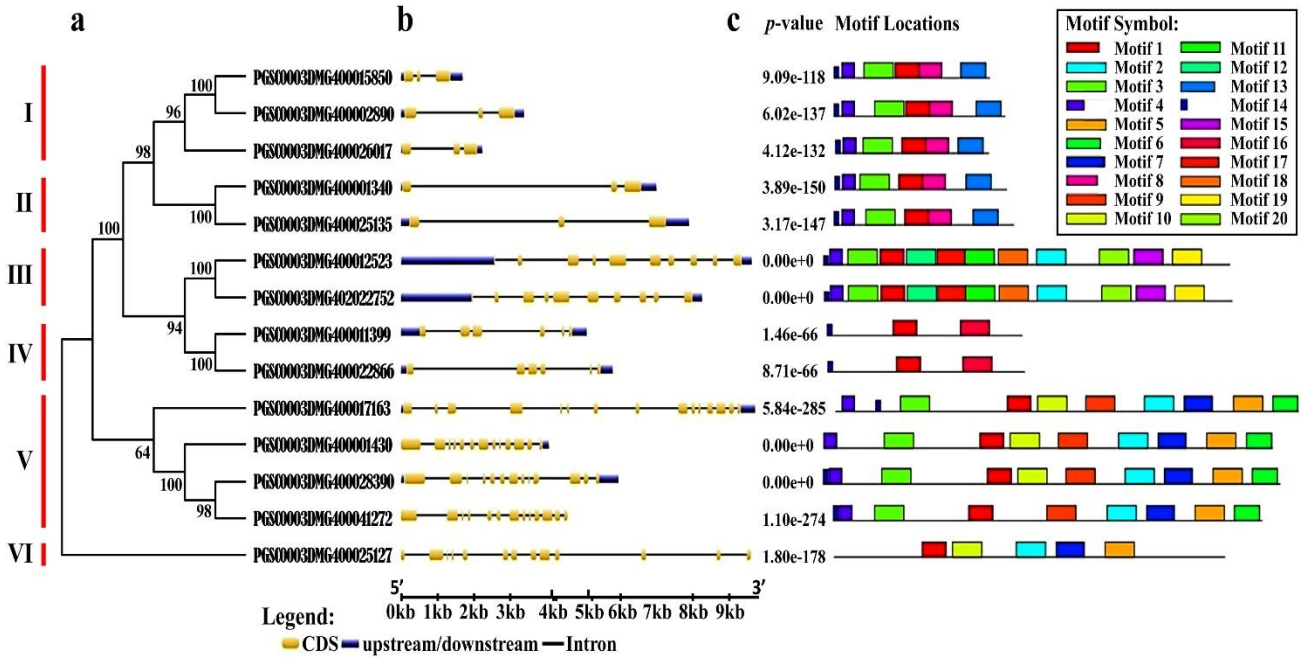

Fig. 3. Phylogenetic relationships, gene structures, and motif composition of the SPX family members. (a) Phylogenetic tree of *Solanum tuberosum* SPX family genes. The NJ algorithm with 1000 bootstrap replicates was used to construct the phylogenetic tree. (b) Structural analysis of *Solanum tuberosum* SPX family genes. The Exons and introns are represented by yellow boxes and black lines, respectively. The blue boxes indicate upstream/downstream. The lengths of exons and introns of each SPX family gene can be estimated using the scale at the bottom. (c) Motif composition of *Solanum tuberosum* SPX family members. The motifs are displayed using different colored boxes numbered 1-20.

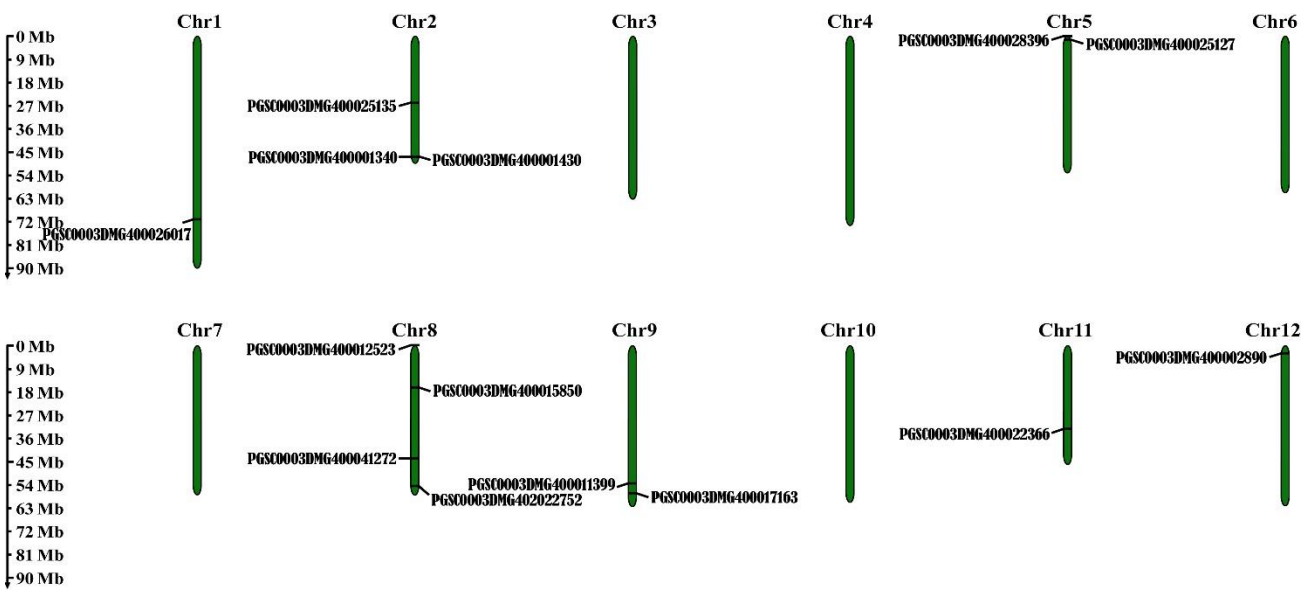

Fig. 4. Distribution of *Solanum tuberosum* SPX family genes on chromosomes. The chromosome number is indicated at the top of each chromosome.

Syntenic analysis between *Solanum tuberosum* SPX family genes with others different plant species: To analyze the syntenic relationship of *SPX* genes in *Solanum tuberosum* with those in other plant species, the synteny analysis of *SPX* genes in *Solanum tuberosum*, *Solanum lycopersicum*, and *Arabidopsis thaliana* was conducted using Dual Synteny Plot for MCScanX and TBtools software (Fig. 6). Totally, 10 *Solanum tuberosum SPX* gene members were found to be syntenic with *SPXs* in *Arabidopsis thaliana*, and 4 *Solanum tuberosum SPX* gene members were not found to be syntenic with *SPXs* in *Arabidopsis thaliana* (Fig. 6). All the identified 14 *SPX* genes in *Solanum tuberosum* were all discovered the syntenic with *SPXs* in *Solanum lycopersicum* (Fig. 6). The above results revealed a closer syntenic relationship between the *SPX* gene family in *Solanum tuberosum* and its counterpart in *Solanum lycopersicum*, as compared to that observed in *Arabidopsis thaliana*. This observation underscores the evolutionary proximity and potential functional conservation of the *SPX* gene family between *Solanum tuberosum* and *Solanum lycopersicum*, highlighting the importance of comparative genomics in unraveling the evolutionary history and functional diversity of plant genes.

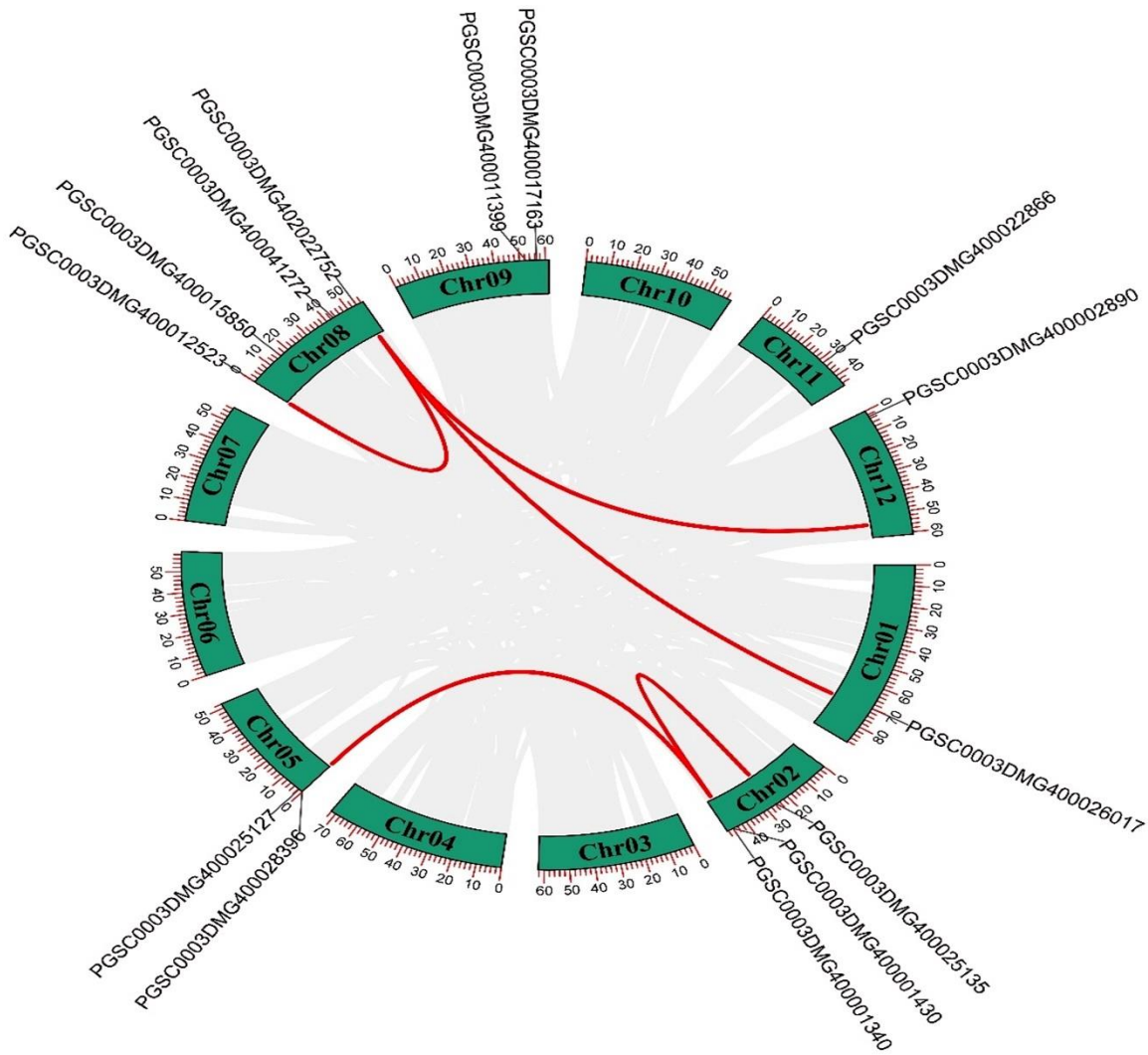

Fig. 5. Synteny analysis of *Solanum tuberosum* SPX family genes in *Solanum tuberosum* chromosomes. Gene duplication events of *Solanum tuberosum* SPX family genes were analyzed by MCScanX. Gray lines in the background indicate the collinear blocks within the *Solanum tuberosum* genome. Red colored lines inside represent *Solanum tuberosum* SPX family genes syntenic blocks in the *Solanum tuberosum* genome.

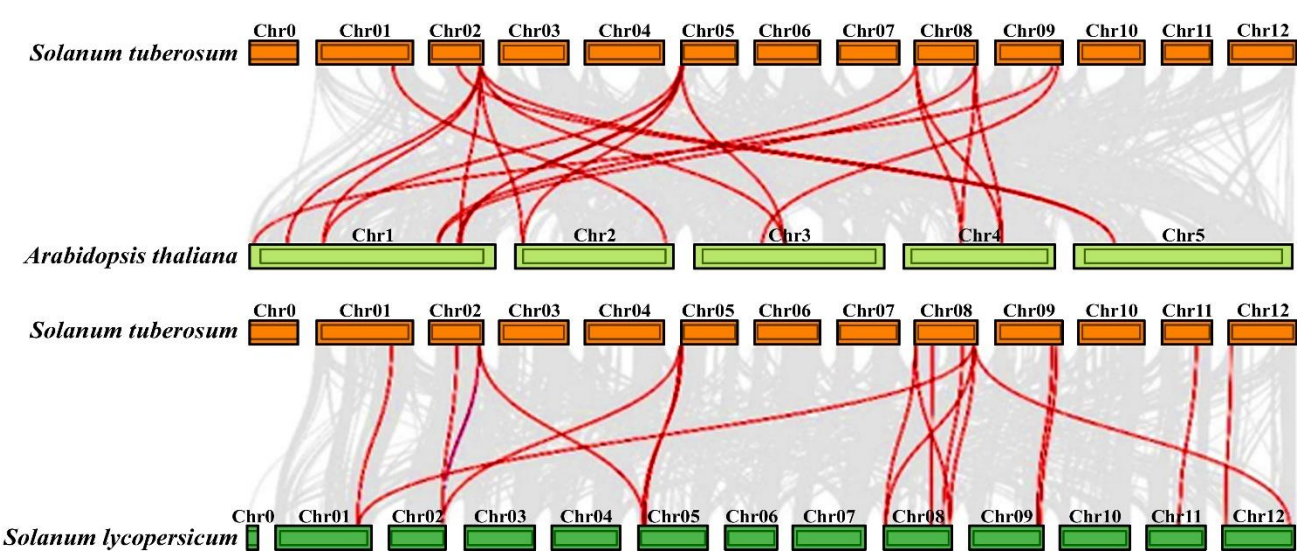

Fig. 6. Synteny analysis of *Solanum tuberosum* SPX family genes with *Arabidopsis thaliana* and *Solanum lycopersicum*. Gray lines in the background indicate the collinear blocks between the *Solanum tuberosum* and *Arabidopsis thaliana*, *Solanum lycopersicum* genomes. Red colored lines inside represent *Solanum tuberosum* SPX family genes syntenic blocks.

Expression analysis of *Solanum tuberosum* SPX family genes in different tissues: In total, the comprehensive expression analysis encompassed a total of 15 tissues types, which included roots, shoots, leaves, petioles, stolon, whole flowers, sepals, petals, stamens, carpels, mature fruits, immature fruits, mesocarp & endocarp, tubers (tubers 1 and tubers 2) and callus. To investigate the tissue-specific expression patterns of the SPX family genes in *Solanum tuberosum*, we leveraged RNA-Seq gene expression data sourced from the comprehensive Potato Genomics Resources available at Spud DB. This approach enabled us to conduct a comprehensive analysis of the expression levels of the *SPX* genes across various tissues of the *Solanum tuberosum* plant, thereby providing insights into their potential roles and functional diversification within distinct developmental contexts. Plants primarily acquire phosphate ions from the soil through their roots and subsequently transport Pi to above-ground organs to meet their needs. So, to assess the differential expression profiles of the SPX family genes across various tissues in *Solanum tuberosum*, we employed a comparative approach using root tissue as the reference. This method allowed us to comprehensively understand the tissue-specific expression patterns of *SPX* genes by comparing their expression levels in different tissues relative to those in the roots.

The SPX subfamily was composed of seven members (PGSC0003DMG400001340, PGSC0003DMG400002890, PGSC0003DMG400012523, PGSC0003DMG400015850, PGSC0003DMG402022752, PGSC0003DMG400025135 and PGSC0003DMG400026017), and SPX subfamily genes in *Solanum tuberosum* displayed various expression patterns (Fig. 7a). Four genes (PGSC0003DMG400001340, PGSC 0003DMG400002890, PGSC0003DMG400015850, and PGSC0003DMG400025135) of SPX subfamily genes were increased in shoots, two genes (PGSC0003DMG4000 12523 and PGSC0003DMG402022752) were decreased, and one (PGSC0003DMG400026017) was too low to be detected. One gene was increased in petioles, five genes (PGSC0003DMG400001340, PGSC0003DMG400002890, PGSC0003DMG400015850, PGSC0003DMG402022752, and PGSC0003DMG400025135) were decreased, and one (PGSC0003DMG400026017) was too low to be detected. We found that the expression levels of almost all *SPX* subfamily genes were relatively lower in petioles than in leaves. Three genes (PGSC0003DMG400001340, PGSC 0003DMG400012523, and PGSC0003DMG400026017) exhibited increased expression levels in stolons, four genes (PGSC0003DMG400002890, PGSC0003DMG400015850, PGSC0003DMG402022752, and PGSC0003DMG4000 25135) showed decreased expression levels, and the expression levels of all SPX subfamily genes were relatively higher in stolon than in petioles. Almost all SPX subfamily genes exhibited elevated expression levels in whole flowers, with the exception of one gene (PGSC0003DMG400026017) that was expressed at levels too low to be detected. Similar to the expression in whole flowers, almost all SPX subfamily genes were increased in levels of expression in sepals except one gene (PGSC0003DMG400002890) was decreased. Three gens (PGSC0003DMG400012523, PGSC0003DMG 402022752, and PGSC0003DMG400025135) were increased in levels of expression in petals, three genes (PGSC0003DMG400001340, PGSC0003DMG400002890, and PGSC0003DMG400015850) were decreased, and one (PGSC0003DMG400026017) was too low to be detected. In addition, the expression levels of all SPX subfamily genes were decreased in petals compared with sepals. Four gens (PGSC0003DMG400001340, PGSC0003DMG400012523, PGSC0003DMG402022752, and PGSC0003DMG4000 25135) were increased in levels of expression in stamens, two genes (PGSC0003DMG400002890 and PGSC0003 DMG400015850) were decreased, and one (PGSC0003 DMG400026017) was too low to be detected. Fiver gens (PGSC0003DMG400001340, PGSC0003DMG400012523, PGSC0003 DMG400015850, PGSC0003DMG402022752, and PGSC0003DMG400025135) were increased in levels of expression in carpels, one gene (PGSC0003DMG40000 2890) was decreased, and one (PGSC0003DMG400026017) was too low to be detected. Two gens (PGSC0003DMG 400012523, and PGSC0003DMG402022752) were increased in levels of expression in mature whole fruit, four genes (PGSC0003DMG400001340, PGSC0003DMG 400002890, PGSC0003DMG400015850, and PGSC0003 DMG400025135) were decreased, and one (PGSC0003 DMG400026017) was too low to be detected. Five gens (PGSC0003DMG400001340, PGSC0003DMG 400012523, PGSC0003DMG400015850, PGSC0003 DMG402022752, and PGSC0003DMG400026017) were increased in levels of expression in immature whole fruit, and two genes (PGSC0003DMG400002890 and PGSC0003DMG4000 25135) were decreased. Three gens (PGSC0003DMG 400001340, PGSC0003DMG400012523, and PGSC0003 DMG400026017) were increased in levels of expression in mesocarp & endocarp, and four genes (PGSC0003DMG 400002890, PGSC0003DMG400015850, PGSC0003DMG 402022752, and PGSC0003DMG400025135) were decreased. Almost all SPX subfamily genes were decreased in levels of expression in tubers including tubers1 and tubers 2. Five gens (PGSC0003DMG400001340, PGSC0003 DMG400002890, PGSC0003DMG400015850, PGSC0003 DMG400025135, and PGSC0003DMG400026017) were increased in levels of expression in callus, and two genes (PGSC0003DMG400012523 and PGSC0003DMG4020 22752) were decreased. The SPX subfamily genes in *Solanum tuberosum* displayed diverse expression patterns, suggesting that they might act as important regulators in different tissues to phosphate homeostasis. It was further found that the gene (PGSC0003DMG402022752) mentioned above of the SPX subfamily genes showed the highest levels of expression in sepals, petals, stamens, and carpels, suggesting its possible involvement in the regulation of phosphate homeostasis in floral organs (Fig. 7a).

SPX-EXS subfamily in *Solanum tuberosum* contained four members (PGSC0003DMG400001430, PGSC0003 DMG400017163, PGSC0003DMG400028396 and PGSC 0003DMG400041272). One gene (PGSC0003DMG40000 1430) showed high levels of expression in stolon, tubers (tubers 1 and tubers 2), and callus (Fig. 7a), suggesting a potential involvement in the long-distance Pi transfer or in the transfer of phosphate to the vascular cylinder in these tissues. Another gene (PGSC0003DMG400017163) showed high levels of expression in shoots, petioles, stolon, and whole flowers, stamens, and carpels, suggesting this gene may participate in the long-distance Pi transfer or in the transfer of phosphate to the vascular cylinder in these tissues. The third gene (PGSC0003DMG400028396) was increased in levels of expression in all most tissues except for carpels and tubers 1. The last gene (PGSC0003DMG400041272) was too low to be detected in all most tissues except for mature whole fruit and mesocarp & endocarp.

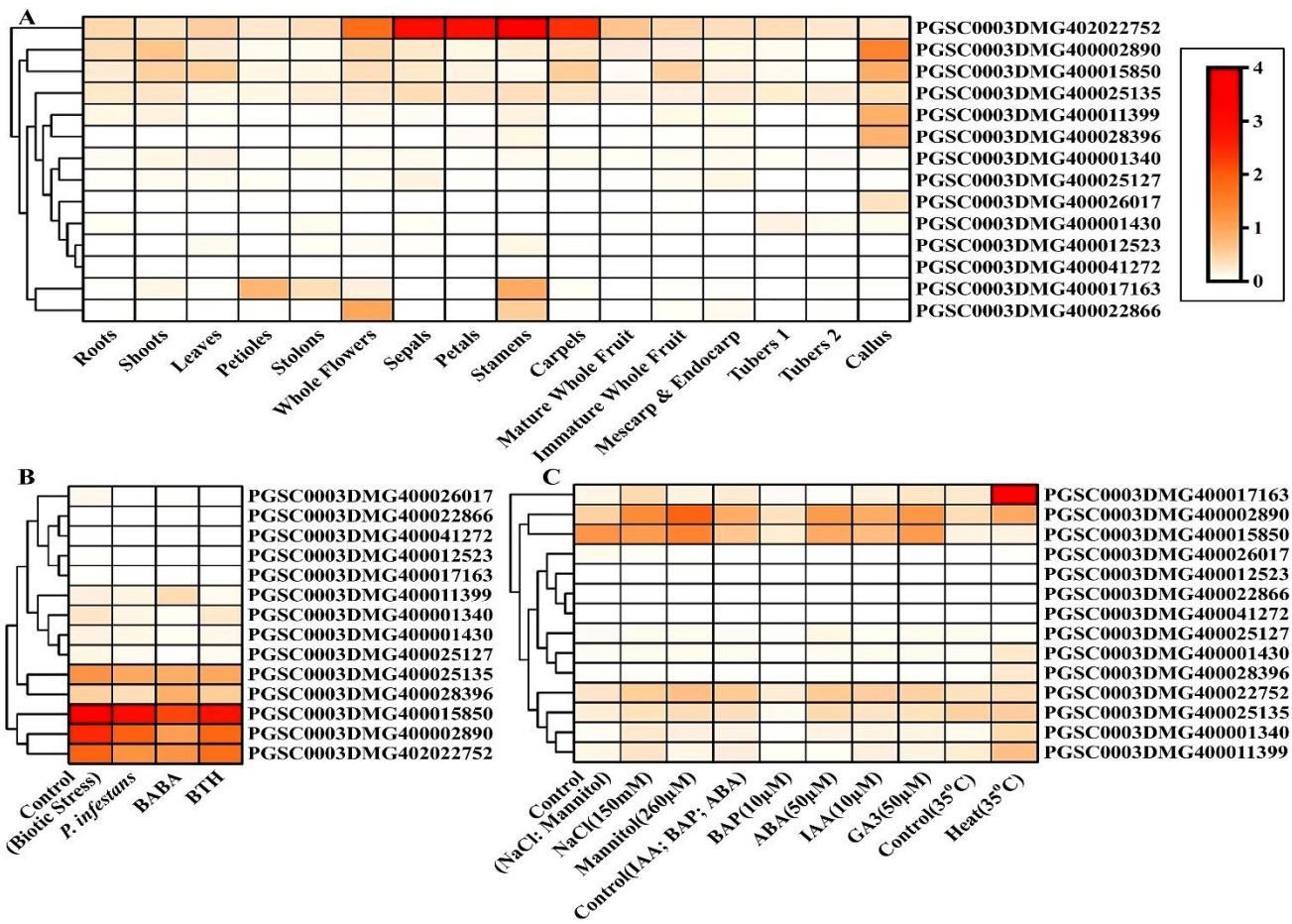

Fig. 7. Expression profiles of *Solanum tuberosum* SPX family genes in different tissues, biotic stress, and abiotic stress. (a) Expression profiles of *Solanum tuberosum* SPX family genes in different plant parts (roots, shoots, leaves, petioles, stolon, whole flowers, sepals, petals, stamens, carpels, mature fruits, immature fruits, mesocarp & endocarp, tubers (tubers 1 and tubers 2) and callus). (b) Expression profiles of *Solanum tuberosum* SPX family genes in biotic stress with *Phytophthora infestans* and two chemical inducers (BTH and BABA) treatments. (c) Expression profiles of *Solanum tuberosum* SPX family genes in abiotic stress with salt stress (150mM NaCl), drought stress (260μM mannitol), BAP (10uM) treatment, ABA (50μM) treatment, IAA (10μM) treatment, GA3 (50μM) treatment, heat stress (35°C). FPKM values for different tissues, biotic stress, and abiotic stress were used to construct the heatmaps. The color scale represents the relative signal values as shown in the right upper corner of each heatmap.

SPX-RING subfamily had two members (PGSC0003DMG400011399, PGSC0003DMG400022866). One gene showed high levels of expression in shoots, stamens, and callus (Fig. 7a). The other gene (PGSC0003 DMG400022866) showed high levels of expression in stolon, whole flowers, sepals, stamens, carpels, mature whole fruit, immature whole fruit, mesocarp & endocarp, tubers (tubers 1 and tubers 2), and callus. We speculated that the two members of the SPX-RING subfamily may play pivotal roles in modulating phosphate (Pi) responses in the aforementioned tissues of *Solanum tuberosum*.

The new subfamily named SPX-EXS-DNL subfamily which contained one member gene (PGSC0003DMG 400025127), showed high levels of expression in shoots,leaves, petioles, whole flowers, sepals, petals, immature whole fruit, mesocarp, endocarp, and callus (Fig. 7a). The SPX-EXS-DNL subfamily has not been reported in other plants. Therefore, it is very necessary to conduct in-depth research on its potential functions in terms of phosphorus utilization.

Expression analysis of *Solanum tuberosum* SPXs under biotic stress conditions: For biotic stress, *Phytophthora infestans* (Pi isolate US8: Pi02-007) and two chemical inducers, BTH and BABA were used to induce biotic stress. Analysis of SPX family gene expression in *Solanum tuberosum* leaves infected with *Phytophthora infestans* revealed a striking downregulation of nearly all members of this gene family, in comparison to the uninfected control group, except for one gene (PGSC0003DMG400041272), which was too low to be detected in leaf (Fig. 7b). 11 *SPX* family genes were down-regulated in *Solanum tuberosum* leaf treated with BABA, two genes (PGSC0003DMG 400028396 and PGSC0003DMG400011399) were up-regulated, and one (PGSC0003DMG400041272) was too low to be detected (Fig. 7b). For the two up-regulated genes, PGSC0003DMG400028396, a member of the SPX-EXS subfamily, and PGSC0003DMG400011399, belonging to the SPX-RING subfamily. 12 *SPX* family genes were down-regulated in *Solanum tuberosum* leaf treated with BTH, one gene (PGSC0003DMG400028396) belonged to the SPX-EXS subfamily was up-regulated, and one (PGSC0003 DMG400041272) was too low to be detected (Fig. 7b). In summary, the expression level of *SPX*s generally exhibited a downward trend under biotic stress.

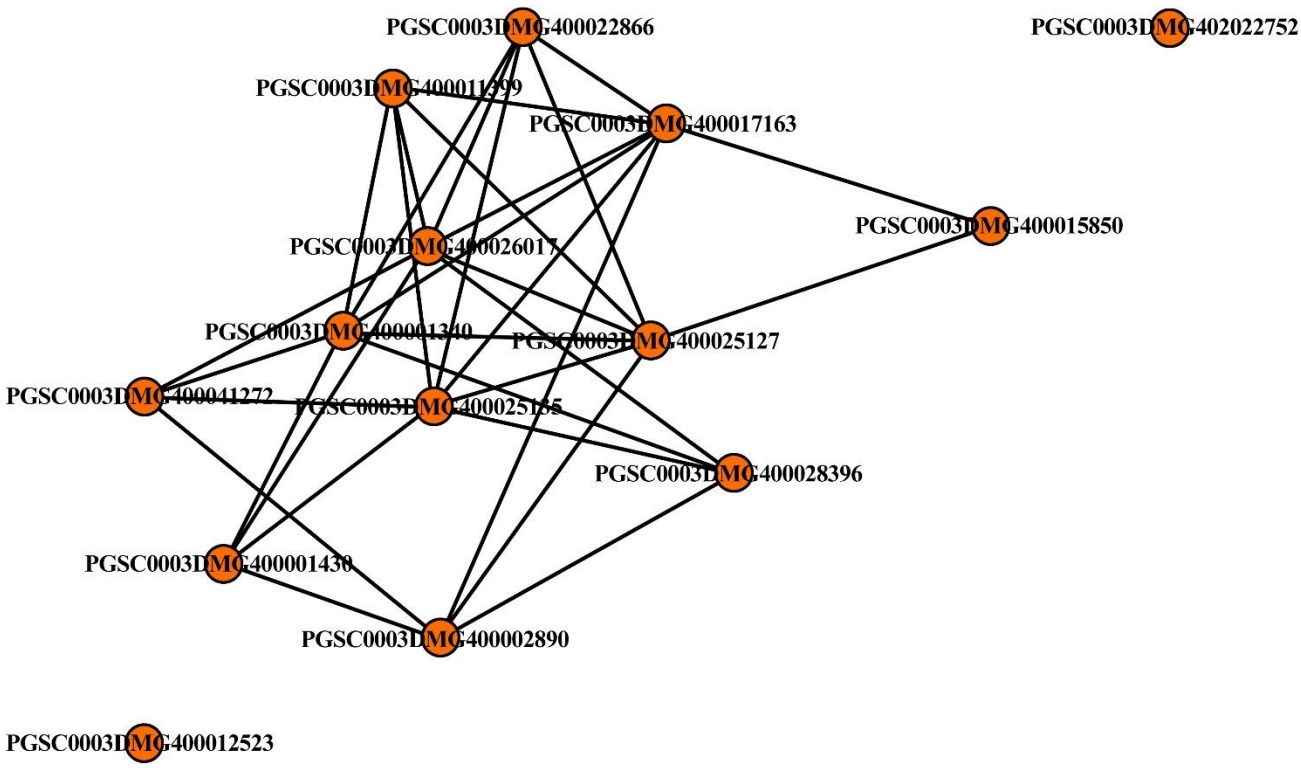

Fig. 8. Interaction network of *Solanum tuberosum* SPX family genes.

Expression analysis of *Solanum tuberosum* SPX family genes upon abiotic stress conditions: Expression analysis of *Solanum tuberosum* SPX family genes upon abiotic stress was indicated in Figure 7c. For salt stress (150 mM NaCl), 11 SPX family genes of *Solanum tuberosum* were up-regulated compared with the control group, two genes (PGSC0003DMG400015850 and PGSC0003DMG4000 26017) were down-regulated, and one (PGSC0003DMG 400041272) was too low to be detected. For drought stress (260 μM mannitol), 11 SPX family genes of *Solanum tuberosum* were up-regulated, two genes (PGSC0003DMG 400026017 and PGSC0003DMG400022866) were down-regulated, and one (PGSC0003DMG400041272) was too low to be detected. For the two down -regulated genes, one (PGSC0003DMG400026017) belonged to the SPX subfamily and the other (PGSC0003DMG400022866) to the SPX-RING subfamily. In all, the expression level of SPX family genes mainly showed increasing trend by salt (150 mM NaCl) and drought stress (260 μM mannitol) (Fig. 7c). Further, we performed expression analysis of *Solanum tuberosum* SPX family genes in hormone-induced stress. 12 SPX family genes of *Solanum tuberosum* were down-regulated under BAP (10 uM) treatment compared with the control group, and two genes (PGSC0003DMG400041272 and PGSC0003 DMG400022866) were too low to be detected. Five SPX family genes of *Solanum tuberosum* were down-regulated under ABA (50 μM) treatment, eight genes were up-regulated, and one (PGSC0003DMG400041272) was too low to be detected. Eight SPX family genes of *Solanum tuberosum* were down-regulated under IAA (10 μM) treatment, five genes up-regulated, and one (PGSC0003DMG400041272) was too low to be detected. Five SPX family genes of *Solanum tuberosum* were down-regulated under GA3 (50 μM) treatment, eight genes were up-regulated, and one (PGSC0003DMG 400041272) was too low to be detected. The number of the down-regulated and up-regulated genes by ABA (50 μM) was the same as by GA3 (50 μM), but they contained different genes. The SPX family genes exhibited different expression patterns in response to hormone-induced stress in *Solanum tuberosum*. In addition, the expression levels of the *Solanum tuberosum* SPX family genes were analyzed under heat (35°C). Eleven SPX family genes of *Solanum tuberosum* were up-regulated under heat stress (35°C), two genes (PGSC0003DMG400015850 and PGSC0003DMG4000 25127) were down-regulated, one (PGSC0003DMG4000 41272) was too low to be detected. We found that one member (PGSC0003DMG 400017163) of the SPX-EXS subfamily was specifically highly expressed under heat treatment (35°C) (Fig. 7c), implying it may play a vital role for heat stress resistance in *Solanum tuberosum*.

***Solanum tuberosum* SPX family genes interaction network analysis:** The predicted protein-protein network interaction of 14 *Solanum tuberosum* SPX family genes revealed that most genes formed an association network, except two numbers (PGSC0003DMG402022752 and PGSC0003DMG400012523) (Fig. 8). Three members (PGSC0003DMG400001340, PGSC0003DMG400025135, and PGSC0003DMG400026017) of the SPX subfamily all interacted with each member of the SPX-EXS subfamily, SPX-RING subfamily, and SPX-EXS-DNL subfamily, which seemed to be the central proteins to interact with others subfamily. A member of the SPX subfamily (PGSC0003DMG400002890) interacted with each constituent of the SPX-EXS subfamily as well as the member of the SPX-EXS-DNL subfamily. Another gene (PGSC0003DMG400015850) interacted with one member

(PGSC0003DMG400017163) of the SPX-EXS subfamily and with the member of the SPX-EXS-DNL subfamily. The remaining two genes (PGSC0003DMG400012523 and PGSC0003DMG402022752) of the SPX subfamily did not interact with other SPX family genes. Above results showed that the member (PGSC0003DMG400017163) of the SPX-EXS subfamily interacted with the five members (PGSC0003DMG400001340, PGSC0003DMG400002890, PGSC0003DMG400015850, PGSC0003DMG400025135, and PGSC0003DMG400026017) of the SPX subfamily. Besides the five members of the SPX subfamily, we found the member (PGSC0003DMG400017163) of the SPX-EXS subfamily interacted with two members (PGSC0003DMG 400011399 and PGSC0003DMG400022866) of the SPX-RING subfamily. Further, based on the results mentioned above, the member (PGSC0003DMG400025127) of the SPX-EXS-DNL subfamily also interacted with the five members (PGSC0003DMG400001340, PGSC0003DMG 400002890, PGSC0003DMG400015850, PGSC0003DMG 400025135, and PGSC0003DMG400026017) of the SPX subfamily, and also interacted with two members (PGSC0003DMG400011399 and PGSC0003DMG4000 22866) of the SPX-RING subfamily. Additionally, our predicted protein-protein interaction network analysis failed to uncover any discernible interaction relationships among the members within the SPX subfamily, the SPX-EXS subfamily, or the SPX-RING subfamily.

Discussion

The SPX proteins are categorized into four subfamilies in plants, including SPX subfamily, SPX-MFS subfamily, SPX-EXS subfamily, and SPX-RING subfamily (Secco *et al*., 2012). Not all plant's genomes cover all of the four subfamilies. To date, the plants whose genomes contain all four SPX subfamily genes including *Arabidopsis thaliana*, rice, *Brassica napus*, wheat, maize, *Solanum lycopersicum* (Secco *et al*., 2012; Du *et al*., 2017; Kumar *et al*., 2019; Xiao *et al*., 2021; Li *et al*., 2021). *Phyllostachys edulis* genome only contains the SPX subfamily genes, SPX-EXS subfamily genes, and SPX-MFS subfamily genes, and lacks SPX-RING subfamily genes (Luo *et al*., 2023). The *Solanum tuberosum* genome also only covered three subfamily genes including the SPX subfamily genes, the SPX-EXS subfamily genes, and SPX-RING subfamily genes, but *Solanum tuberosum* genome contains a new subfamily gene named SPX-EXS-DNL subfamily gene.

The SPX subfamily of proteins serves as pivotal regulators in the intricate process of Pi (phosphate) uptake and mobilization within plant cells (Duan *et al*., 2008; Wang *et al*., 2009; Liu *et al*., 2010; Zhang *et al*., 2016). The *Arabidopsis* SPX subfamily comprises four distinct members, as reported by Duan *et al*., (2008), while the *Solanum lycopersicum* genome harbors seven members of this subfamily, as evidenced by the findings of Li *et al*., (2021). In *Solanum tuberosum* genome, we also found 7 SPX subfamily (Fig. 2). The 4 members of *Arabidopsis thaliana* SPX subfamily genes have three exons (Duan *et al*., 2008). Five members of *Solanum lycopersicum* SPX subfamily genes had a similar gene structure to *Arabidopsis thaliana* SPX subfamily genes, and the rest two possessed two exons or six exons (Li *et al*., 2021). We found that five *Solanum tuberosum* SPX subfamily genes (PGSC0003DMG400001340, PGSC0003DMG40000 2890, PGSC0003DMG400015850, PGSC0003DMG4000 25135 and PGSC0003DMG400026017) also had a similar gene structure to *Arabidopsis thaliana* SPX subfamily genes, but the rest two (PGSC0003DMG400012523 and PGSC0003DMG402022752) possessed 10 exons. Further analysis of the *Solanum tuberosum* SPX subfamily shows that the genes with a similar structure contains the same motifs (Fig. 3b, c). The *Arabidopsis thaliana* SPX subfamily genes were distributed to 2 chromosomes (Duan *et al*., 2008), *Solanum lycopersicum* SPX subfamily genes distributed to 4 chromosomes (Li *et al*., 2021), and *Solanum tuberosum* SPX subfamily genes also distributed to 4 chromosomes (Fig. 4). The result further indicates that *Solanum tuberosum* SPX subfamily genes have a similar chromosomal distribution with *Solanum lycopersicum* as distributed to chr1, chr2, chr8, and chr12. In *Arabidopsis thaliana*, the SPX subfamily proteins have been precisely localized to the cytoplasm, plasma membrane, and nucleus, as evidenced by the studies conducted by Duan *et al*., (2008) and Wang *et al*., (2009). *Solanum tuberosum* SPX subfamily proteins also had a broad range of subcellular localization. One member (PGSC0003DMG400001340) of *Solanum tuberosum* SPX subfamily proteins was localized to cytoplasm, four members (PGSC0003DMG 400002890, PGSC0003DMG400012523, PGSC0003 DMG400015850, and PGSC0003DMG400026017) localized to plasma membrane, and two members (PGSC 0003DMG402022752 and PGSC0003DMG400025135) localized to nucleus (Table 1). Distinctive expression profiles of the SPX subfamily genes have been discerned in both *Arabidopsis thaliana* and *Solanum lycopersicum* (Wang *et al*., 2009; Li *et al*., 2021). In *Solanum tuberosum*, the SPX family genes also exhibited a diverse array of expression patterns, but showed certain expression characteristics between certain tissues. Compared with leaves, almost all SPX subfamily genes were decreased in levels of expression in petioles and tubers including tubers1 and tubers 2, on the contrary, the expression increased in whole flowers and sepals. Additionally, comparative analysis revealed that the expression levels of all members of the SPX subfamily were notably upregulated in stolons as opposed to petioles, whereas a downregulation was evident in petals in comparison to sepals (Fig. 7a). Above results implied that the *Solanum tuberosum* SPX subfamily genes may act as important regulators in different tissues to phosphate homeostasis. Interestingly, one gene of the SPX subfamily genes showed the highest levels of expression across floral organs, encompassing sepals, petals, stamens, and carpels (Fig. 7a), suggesting its possible involvement in the regulation of phosphate homeostasis, but the specific function requires further investigation. It has been reported that one SPX subfamily gene has been demonstrated to be involved in the response to cold stress (Zhao *et al*., 2009). We also analyzed the expression of *Solanum tuberosum* SPX family genes under biotic and abiotic stress conditions, the results showed *Solanum tuberosum* SPX family genes respond to different stresses (Fig. 7b, c).

The SPX-EXS subfamily plays a wide range of roles, such as loading Pi into the xylem, involving in phosphate efflux out of the cell, and mediating in the long-distance Pi transfer *et al* (Hamburger *et al*., 2002; Wang *et al*., 2004; Wang *et al*., 2008; Secco *et al*., 2010; Stefanovic *et al*., 2011; Zhao *et al*., 2019). In *Arabidopsis thaliana*, the SPX-EXS subfamily is composed of 11 members (Wang *et al*.,

2004), and in the *Solanum lycopersicum* genome, their consists of 6 members (Zhao *et al*., 2019; Li *et al*., 2021). In *Solanum tuberosum* genome, we identified 4 SPX-EXS subfamily genes (Fig. 2). Members of the SPX-EXS subfamily displayed various gene structure variations in *Arabidopsis thaliana* (Wang *et al*., 2004) and *Solanum lycopersicum* (Li *et al*., 2021). The *Solanum tuberosum* SPX-EXS subfamily not only demonstrated a certain level of complexity in gene structure but also exhibited diversity in motif composition (Fig. 3b, c). The *Arabidopsis thaliana* SPX-EXS subfamily genes were distributed to 4 chromosomes including chr1, chr2, chr3, and chr4 (Wang *et al*., 2004). *Solanum tuberosum* SPX-EXS subfamily genes were distributed to chromosome 2, 5, 8, and 9, which exhibited the same chromosome distribution with *Solanum lycopersicum* (Li *et al*., 2021). One member of The *Arabidopsis thaliana* SPX-EXS subfamily was localized to Golgi structures (Wege *et al*., 2016). The predictive analysis of subcellular localization suggests that the genes belonging to the *Solanum tuberosum* SPX-EXS subfamily are likely to reside in the plasma membrane, nucleus, and cytoplasm (Table 1). The *Solanum tuberosum* SPX-EXS subfamily exhibited diverse tissue expression patterns similar to *Arabidopsis thaliana* (Wang *et al*., 2004) and *Solanum lycopersicum* (Li *et al*., 2021). A notable member within the *Arabidopsis thaliana* SPX-EXS subfamily has been implicated in the intricate response mechanisms to both biotic and abiotic stress conditions, as reported by Ribot *et al*., (2008). In contrast to the marked upregulation of this member of the *Arabidopsis thaliana* SPX-EXS subfamily upon infection with *Pseudomonas syringae* (Ribot *et al*., 2008), all members of the *Solanum tuberosum* SPX-EXS subfamily exhibit a coordinated downregulation in response to *Phytophthora infestans* infection (Fig. 7b). For salt (NaCl) and drought stress (mannitol), this member of the *Arabidopsis thaliana* SPX-EXS subfamily was induced up-regulation (Ribot *et al*., 2008), and all members of the *Solanum tuberosum* SPX-EXS subfamily except for one (PGSC0003DMG400041272) being too low to be detected was induced up-regulation also (Fig. 7c). For ABA treatment, this member of the *Arabidopsis thaliana* SPX-EXS subfamily was induced strong expression (Ribot *et al*., 2008), two members (PGSC0003DMG400001430 and PGSC0003DMG400028396) of the *Solanum tuberosum* SPX-EXS subfamily was up-regulated, one member (PGSC0003DMG400017163) was down-regulated, and one (PGSC0003DMG400041272) was too low to be detected (Fig. 7c). For heat treatment, no induction of this member of the *Arabidopsis thaliana* SPX-EXS subfamily expression was detected following a heat shock at 37°C for 1 h, and all members of the *Solanum tuberosum* SPX-EXS subfamily except for one (PGSC0003DMG400041272) being too low to be detected was induced up-regulation (Fig. 7c). Surprisingly, one member (PGSC0003DMG400017163) of the SPX-EXS subfamily was specifically highly expressed under heat treatment (35°C) (Fig. 7c), we speculated it may play a vital role under heat stress.

The SPX-RING subfamily has been implicated in the intricate regulation of phosphate homeostasis (Peng *et al*., 2007; Kant *et al*., 2011). In *Arabidopsis thaliana*, the sole representative of the SPX-RING subfamily has been identified (Peng *et al*., 2007; Kant *et al*., 2011), and the *Solanum lycopersicum* SPX-RING subfamily consisted of two members (Li *et al*., 2021). The *Solanum tuberosum* genome contained two members of the SPX-RING subfamily (Fig. 2). The *Arabidopsis thaliana* SPX-RING subfamily gene possessed 6 exons (Kant *et al*., 2011), the *Solanum lycopersicum* SPX-RING subfamily genes possessed 6 exons (Li *et al*., 2021), and the *Solanum tuberosum* genes possessed 6 exons too (Fig. 3b). The above results implied that the SPX-RING subfamily gene maybe possessed the same number of exons. In addition, we found that the two members of *Solanum tuberosum* SPX-RING subfamily were composed of the same motifs including motifs 1, 14 and 16 (Fig. 3c). The *Arabidopsis thaliana* SPX-RING subfamily gene located in chromosome 3 (Kant *et al*., 2011), the two genes of *Solanum tuberosum* SPX-RING subfamily were located in chromosomes 9 and 11 (Fig. 4) like as *Solanum lycopersicum* SPX-RING subfamily genes (Li *et al*., 2021). The *Arabidopsis thaliana* SPX-RING subfamily gene was localized to the nucleus (Peng *et al*., 2007), the two genes of *Solanum tuberosum* SPX-RING subfamily were localized to the nucleus and plasma membrane, respectively (Table 1). The *Arabidopsis thaliana* SPX-RING subfamily gene was expressed throughout the plant (Peng *et al*., 2007). The two genes *Solanum lycopersicum* SPX-RING subfamily displayed different expression pattern, one was expressed in varies tissues, and the other was expressed higher in bud and flower (Li *et al*., 2021). The two genes of *Solanum tuberosum* SPX-RING subfamily also displayed different expression pattern, one gene is highly expressed in the majority of tissues, the other showed high levels of expression in shoots, stamens, and callus (Fig. 7a). There is no research has been reported about expression analysis of SPX-RING subfamily genes in biotic and abiotic stress. Our results showed that the one gene of the *Solanum tuberosum* SPX-RING subfamily was up-regulated for salt (150 mM NaCl), drought (260 μM mannitol), and heat (35°C) stress, the other was up-regulated for salt (150 mM NaCl), ABA (50 uM), IAA (10 uM), GA3(50 uM), heat (35°C) treatments (Fig. 7c). Additionally, our results showed that all members of the *Solanum tuberosum* SPX-RING subfamily was induced down-regulation after infection with *Phytophthora infestans* (Fig. 7b).

The new SPX-EXS-DNL subfamily gene possessed thirteen exons (Fig. 3b), encompassed 5 motifs, which included motifs 1, 2, 5, 7, and 10 (Fig. 3c), located in chromosome 5 (Fig. 4), localized to the plasma membrane (Table 1), and was induced up-regulation expression for salt (NaCl 150 mM), drought (mannitol 260 uM), ABA (50 uM) and GA3 (50 uM) treatments (Fig. 7c).

Gene duplication events, comprising WGD/SD and TD, represent the preeminent mechanism underlying the expansion of gene families and the emergence of novel genes (Cannon *et al*., 2004; Cui *et al*., 2015; Hajibarat *et al*., 2022). The expansion for some members of *Arabidopsis thaliana* SPX family genes like as SPX-EXS subfamily mainly was followed by SD (Wang *et al*., 2004). For *Solanum tuberosum* SPX family gene duplication events, 5 pairs of WGD/ SD were found but no pairs of TD (Fig. 5), which suggested that *SPX* genes in *Solanum tuberosum* may be mainly produced by WGD/ SD. In addition, synteny analysis of *SPX* genes in *Solanum tuberosum*, *Solanum lycopersicum*, and *Arabidopsis thaliana* (Fig. 6) indicated that the syntenic relationship of the *SPX* family gene in *Solanum tuberosum* was closer to the *Solanum lycopersicum* than those in *Arabidopsis thaliana*. And chromosomal distributions analysis showed *Solanum tuberosum SPX* family genes were distributed to chromosomes 1, 2, 5, 8, 9, 11, and 12 like *Solanum lycopersicum*.

According to a recent study, some SPX domains could interact with each other in in yeast (Pipercevic *et al*., 2023). The predicted protein-protein network interaction of 14 *Solanum tuberosum* SPX family genes revealed that most genes could interact finally formed an association network except two numbers (PGSC0003DMG402022752 and PGSC0003DMG400012523) (Fig. 8), but the real interaction situation needs further research.

Conclusions

In this study, a comprehensive analysis of the *Solanum tuberosum* genome identified 14 genes belonging to SPX family. These genes can be classified into four subfamilies: SPX, SPX-EXS, SPX-RING, and a new SPX-EXS-DNL subfamily gene that has not been reported before. We comprehensively analyzed the gene structures, phylogenetic relationships, motif composition, chromosomal distributions, duplication events and synteny prediction, expression patterns, and interaction network of *Solanum tuberosum* SPX family genes. The *Solanum tuberosum SPX* family genes exhibited diverse expression patterns across different tissues and demonstrated varying degrees of response to both biotic and abiotic stresses. And all most *Solanum tuberosum* SPX family genes could interact finally formed an association network. The present findings lay a solid foundation for the subsequent exploration of the multifaceted functions of *Solanum tuberosum* SPX family genes, with a particular emphasis on unraveling their pivotal roles in phosphorus utilization, thereby advancing our understanding of the intricate mechanisms underlying plant nutrient acquisition and utilization.

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