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Screening and Identification of Potato *StSPS1*, a Potential Crucial Gene Regulating Seed Potato Vigor

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Abstract: Sucrose phosphate synthase (SPS), a crucial rate-limiting enzyme that catalyzes the synthesis of precursors of sucrose, plays an indispensable role in the regulation of seed potato vigor. Nonetheless, the genes that encode SPS in potato have not undergone complete analysis, and the primary genes responsible for the regulation of seed potato vigor have not been screened and identified. In this study, four StSPS family members were identified by comparing the potato genome database with homologous proteins. Using bioinformatics, the physicochemical properties, subcellular localization, sequence structure, conserved motifs, and phylogenetics of StSPS were analyzed. The expression levels of *StSPS* in different potato tissues, from dormancy to sprouting in the seed potato tubers, were measured via qRT-PCR. The expression data from the potato genome database and previous transcriptome and proteome studies of dormancy to sprouting were also compared. After combining the analysis of SPS enzyme activity in diverse tuber tissues and the correlation analysis between multiple varieties with different dormancy periods and the expression of StSPS1, the primary gene StSPS1 that might regulate seed potato vigor was identified. This study set a theoretical and experimental groundwork for further verification and clarification of the regulatory function of StSPS1 in alterations in seed potato vigor.

Keywords: seed potato vigor; sucrose phosphate synthase; dormancy; sprouting

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1. Introduction

Potato (*Solanum tuberosum* L., Solanaceae) stands as one of the four major food crops worldwide. Despite being a major producer of potatoes, China still faces obstacles in the form of low per-unit yields and a tardy growth rate [1]. In recent times, hybrid breeding has allowed China to develop early maturing, disease-resistant, and high-yield varieties. Additionally, regulating the seed potato vigor state to coincide with the optimal sowing date has been proven to be a highly effective method to boost production. Particularly in China's southwestern mixed cropping area, the center of potato production [2], regulating the seed potato vigor stands as a more critical factor over developing high-yield cultivated varieties into multi-seasonal planting modes. Seed potato vigor is generated from seed vigor—a term referring to the robustness of the seed potato. It includes sprouting and emergence rate, seedling growth potential, plant stress resistance, and production ability [3]. Despite its importance, there has been only a handful of studies conducted on the regulation of seed potato vigor. Identifying and screening its regulatory genes could hold significant value for rapidly detecting seed potato vigor and augmenting potato yields.

The physiological age of seed potato directly affects the yield post-sowing [4]. It is generally considered that the best seed potato age is when there are multiple robust lateral buds. It is commonly held that the optimal physiological maturity of seed potatoes is

attained when there are numerous robust laterals shoots. High in water content (approximating 80%), potato tubers can directly release dormancy without imbibition and water absorption for subsequent physiological processes [5]. Tuber dormancy is an irreversible process, and unless forcibly broken, only natural release of dormancy causes germination and growth in tubers [6]. Low-level physiological activities persist during dormancy. Considered a catalyst in the process of physiological metabolism, alterations in enzyme activities are indicative of the strength of physiological metabolic processes. The nutrients of potato pith are rapidly metabolized, furnishing energy for tuber sprouting, which is sourced from sucrose [7,8]. Seed vigor reaches its zenith during the period of physiological maturity [9], while seed potatoes must traverse through an irreversible dormancy period, and optimal seed vigor is achieved solely post-germination. The alterations in several enzymes implicated in sucrose metabolism are intimately intertwined with seed potato vigor and serve as a prerequisite for bud sprouting.

Sucrose is synthesized in the cytoplasm through the collaboration of two enzymes, SPS (EC 2.4.1.14) and sucrose phosphate phosphatase (SPP, EC 3.1.3.24). SPS uses UDP-glucose and fructose-6-phosphate as substrates to synthesize sucrose-6-phosphate, while SPP releases orthophosphate (Pi) from 6-phosphate-fructose to produce sucrose [10]. SPS and SPP can be activated and deactivated by protein phosphorylation in response to osmotic stress and changes in light conditions. The action sites may vary and could involve different serine residues. Meanwhile, SPS activity was stimulated by 6-phosphogluconic acid and inhibited by Pi [11]. Sucrose is mainly produced in mature leaves, but it can be resynthesized in sink tissue. Sucrose in sink tissue will be degraded into hexahedron or its derivatives by invertase (INV, EC 3.2.1.26) or sucrose synthase (EC 2.4.1.13) and then used in different ways. INV hydrolyzed sucrose into glucose and fructose, while sucrose is degraded by sucrose synthase in the presence of UDP and hydrolyzed into UDP-glucose and fructose [12].

Numerous studies have proven that SPS is a pivotal juncture in the sucrose synthesis pathway, and its activity directly reflects the ability for sucrose synthesis in plants [13,14]. As a polygenic family, SPS is divided into four subfamilies, namely, A, B, C and D, through phylogenetic analysis, of which subfamily D has only been found in gramineous monocotyledons thus far [15]. At present, SPS family members have been identified in Arabidopsis, rice, alfalfa, and other species [16]. As a member of the glycosyltransferase superfamily, SPS is a dimer or tetramer that consists of two or four subunits [17], with three conserved regions, including a sucrose synthase domain (sucrose synthase), a glycosyltransferase domain (glycos-trans-1), and a sucrose-6-phosphate phosphatase domain (S6PP) [18]. As a key enzyme in the metabolic process of sucrose, SPS predominantly regulates the accumulation of starch and sucrose in plants. In Arabidopsis leaves, SPS co-express with AtSWEET11/12, a plasma membrane monomer that exports sucrose from mesophyll cells to phloem plastids to participate in sucrose biosynthesis and transport [19]. Rice double gene mutant *sps1/sps11* exhibited a significant decrease in SPS enzyme activity compared to that of the wild type, with an obvious accumulation of starch content [20].

Moreover, SPS functions as a mediator in various aspects of plant growth, development, and response to stress. The manifestation of this is apparent in the short phenotype of the plants, flowers, and pods of double and triple mutants in four AtSPS members, with the *atspsa1/atspsb/atspsc* triple mutant along with four *atsps* mutant inducing the seed phenotype of nongermination [21]. Similarly, the *OsSPS1* gene mutation in rice resulted in pollen sterility [22]. Interestingly, despite the inhibition of potato SPS gene expression, no changes in sucrose and starch contents occurred under water stress, indicating that SPS might be an essential component for water stress responsiveness [23]. By transferring SPS from maize to potato, SPS activity and postharvest yield were also improved [24]. The above studies collectively serve as evidence that SPS plays a pivotal role in not only regulating sucrose synthesis but also performing other vital biological functions.

The members of the SPS family in potato have not been systematically studied. Mining its main genes in the regulation of seed potato vigor is of great significance to comprehen-

sively analyze the specific functions of SPS and improve potato yield. In this study, the potato SPS family members were totally screened and analyzed through bioinformatics methods. By comparing the gene and protein expression levels of these family members and the SPS enzyme activity, the main gene potentially involved in the regulation of seed potato vigor was preliminarily screened. Eventually, the correlation analysis in multiple varieties demonstrated that *StSPS1* is a key gene regulating the seed potato vigor. This study provides experimental and data support for the in-depth study of the specific function of StSPS1 in seed potato vigor.

2. Materials and Methods

2.1. Plant Growth Conditions and Sampling Procedure

The virus-free tissue culture seedlings of the mid-early maturing cultivated variety 'Chuanyu 10' came from the Potato Research and Development Center, Agronomy College, Sichuan Agricultural University (30°42′20″ N, 103°51′37″ E), and all the experiments were conducted there. After propagation by potato tissue culture technology, around 400 seedlings with a physiological age of 30 days were planted in a nutrient matrix, and tubers were harvested after 90 days. Then, the harvested tubers were treated with room temperature and natural scattered light for 7 days, and tubers weighing approximately 8–9 g were selected for storage experiments. After wound healing, the tubers were transferred to 3 dark and relatively closed cartons at room temperature storage, each containing 150 tubers. According to previous studies, the sampling method of bud eye is slightly improved [25]. The sample does not contain the outer tuber pith tissue of the tuber, and only the tissue with a diameter of 3 mm and a height of approximately 1 mm (including periderm, cortex, and vascular bundle ring) is regarded as the bud eye. From the beginning of storage, samples were taken every 7 days a total of 8 times, and 10 tubers were randomly selected from each carton for sampling. That is to say, a total of 30 bud eyes were sampled and mixed for RNA extraction. Potato flower tissue was sampled at the full flowering stage (70 days after planting), and tuber sprout tissue was sampled after tuber sprouting (56 days storage). Other tissues including root, stem, leaf, and stolon were sampled during tuber harvest. After sampling, the samples were placed in liquid nitrogen for quick freezing and stored at −80 °C until further experiments.

2.2. Identification and Bioinformatics Analysis

Through the BLAST alignment of homologous proteins in the potato genome-wide database PGSC http://spuddb.uga.edu/index.shtml (accessed on 9 March 2022), information on all StSPS members was obtained and named from small to large according to the position order for each gene on the chromosome. The physical and chemical properties of the protein were analyzed online by Expasy-ProtParam, and the subcellular localization of the protein was predicted online by BaCelLo, YLoc, and WoLf-PSORT II. The gene structure was analyzed online by GSDS, and other functional sites of protein phosphorylation were analyzed online by NetPhos-3.1 and ScanProsite. The conserved motifs of proteins were analyzed online by MEME and annotated by InterPro and Pfam. A phylogenetic tree was constructed by using the neighbor-joining method (the parameter bootstrap was set to 2000 times) in MEGA v7.0 software [26].

2.3. Gene Expression Analysis and Enzyme Activity Determination

Total RNA was isolated by a SteadyPure Plant RNA Extraction Kit, and 1 μg of total RNA was reverse transcribed for cDNA synthesis by using an Evo M-MLV RT Kit. The concentration and quality of RNA and DNA were determined by microspectrophotometer and agarose gel electrophoresis to determine that they had not been degraded or contaminated. SYBR® Green Premix Pro Taq HS qRT-PCR reagent was used to determine gene expression. The above kits and reagents were purchased from Accurate Biotechnology (Hunan, China). The $2^{-\Delta\Delta Ct}$ method was selected to calculate the qRT-PCR results [27]. *EF1αL* (elongation factor 1 alpha like) was chosen as the internal reference gene [28], and the

expression of *StSPS1* in the early stage of tuber storage (7 days) was set to 1 as normalized data. The specific information about the primers is shown in Table 1. The expression data onto StSPS family members in the transcriptome and proteome came from our previous research [25,29]. The tissue expression data used for comparison and analysis came from Ensembl Plants http://plants.ensembl.org/index.html (accessed on 11 May 2022), and the bud eye tissue in this study corresponds to the tuber cortex tissue in the database. SPS enzyme activity was determined according to the instructions of the Plant Sucrose Phosphate Synthase ELISA Kit (Yubo Biotechnology, Shanghai, China). Three biological replicates and three technical replicates were performed respectively for the enzyme activity determination and qRT-PCR experiments.

Table 1. Primer information. EF1αL is an internal reference gene. F represents forward primer, and R represents reverse primer.

Name	Primer	Purpose
StSPS1	qStSPS1-F: CAAGCTCAGTGGATGGCAAA qStSPS1-R: TGACTGACCCATGCTTCCAT	qRT-PCR
StSPS2	qStSPS2-F: AAGGGGGTTTTCATCAATCC qStSPS2-R: GCTTCAAAAGTGCATCAGCA	
StSPS3	qStSPS3-F: AGGAGTCAATTGCCATGGTC qStSPS3-R: GAAAACGCATGACCTCAGAC	
StSPS4	qStSPS4-F: ATCTGATGCTTTTGGCTGCT qStSPS4-R: TAAGGCCACACTGCTTTTCC	
EF1αL	qEF1αL-F: CTTGTACACCACGCTAAGGAG qEF1αL-R: GTCAATGCAAACCATTCCTTG	

2.4. Correlation Analysis

The tubers of 7 tetraploid cultivated potato varieties, each with different dormancy periods, were produced via tissue culture and seedling substrate cultivation. The dormancy period for 'Weiyu 3' and 'Favorita' is around 50 days, while for 'Chuanyu 10' and 'MK wu' it is approximately 60 days. For 'Bashu 10' and 'Chuanyu 117', the dormancy period is approximately 80 days, whereas for 'Burbank', it is around 110 days. Then, the storage experiments were conducted in accordance with the guidelines provided in Section 2.1. Next, samples were taken from the bud eyes at the initial stage of storage, the stage about to sprout (when the buds showing white), and the end stage of dormancy (about 10% of 150 tubers sprouting). Sprouting rates were observed over ten-day intervals and recorded. Thereafter, qRT-PCR tests were utilized to identify the relative expression levels of *StSPS1* during these three stages. Finally, Pearson's method was used to perform a correlation analysis between *StSPS1* and the dormancy period.

2.5. Statistical Analysis and Mapping

In this study, EvolView, Microsoft PowerPoint 2019 software, and SVG edit online were used for creating and embellishing visual figures. DNAMAN v6.0 and SnapGene v5.3.1 software programs were used for gene and protein sequence alignment. The heatmap was drawn by HemI v1.0 software. Microsoft Excel 2019 and IBM SPSS Statistics 26 were used for tabulation and statistical analysis, and $p \leq 0.01$ and $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Identification and Characteristics of the StSPS Family

From conducting a blast alignment of four tomato SPS family protein sequences, four members of StSPS were derived from the PGSC database, which were located on chromosomes 7, 8, 9, and 11. Except for StSPS4, the lengths of the coding regions of the other members were more than 3000 bp and 1000 aa. Because the numbers and sequences

of genes and proteins in different databases were different, the numbers in the EBI database are also listed (Table 2). The physical and chemical properties analysis showed that the molecular weight of StSPS proteins was approximately 120 kD, except for StSPS4. The theoretical isoelectric points (PIs) of StSPS proteins were between 6.02 and 6.45, the average hydrophilic coefficient was negative, the instability index was greater than 40, and the number of positive amino acid residues was less than that of negative amino acids. Due to the different online software predictions based on the different models and databases, three online websites were selected for prediction. More than two websites indicated that StSPS4 was located in the chloroplast and that the other members were located in the nucleus. In conclusion, it was determined that StSPS family members are hydrophilic unstable proteins, and compared with other members, StSPS4 had the shortest sequence length and different subcellular localization (Table 3).

Table 2. Information of StSPS family members. PGSC is the potato genome database, while EBI is the European bioinformatics institute database. Coding sequence refers to the length of gene and protein sequences.

Gene	PGSC Number	Chromosome Locus	Coding Sequence	EBI Number
StSPS1	Soltu.DM.07G003160.1	7: 3688426–3697173	3165/1054	M1CPB7/Q43845
StSPS2	Soltu.DM.08G010240.1	8: 30404288–30393496	3120/1039	M1CXH8
StSPS3	Soltu.DM.09G029230.1	9: 65340125–65331377	3195/1064	M1CI66
StSPS4	Soltu.DM.11G017190.1	11: 33423533–33413792	2598/865	M1BN62/M1BN65

Table 3. Physicochemical properties and subcellular localization prediction of StSPS family proteins. MW: molecular weight. PIs: theoretical isoelectric points. Gravy represents the average hydrophilic coefficient. "+/−" represents basic/acidic.

Gene	MW (kD)	PIs	Gravy	Instability Index	+/− Residues	Localization
StSPS1	118.43	6.02	−0.428	46.83	133/147	Nucleus
StSPS2	116.68	6.19	−0.374	45.72	132/144	Nucleus
StSPS3	119.57	6.15	−0.496	47.38	140/152	Nucleus
StSPS4	96.48	6.45	−0.239	45.67	101/107	Chloroplast

3.2. Gene and Protein Structure Analysis

The analysis of the gene structure to StSPS family showed that *StSPS1/2* contained 13 exons, and it has been discerned that these exons manifest an exalted degree of similarity. *StSPS3* contains 12 exons, and exon 2 was differentiated into exons 1 and 2 in *StSPS1/2*. Because exons with high homology between these family genes were concentrated at the C-terminus of the coding region, the gene structure diagram showed a negative chain. Taking *StSPS1* as an example, exons 13-1 are labeled from left to right (File S1). It was also found that the sequence length and overall structure of *StSPS3* were similar to those of *StSPS1*. *StSPS4* contained only 11 exons and lacked exons 1–3 compared with other members, and exons 4–6 had low similarity compared with other members (Figure 1A). Through protein comparison and structural analysis, it was found that all the StSPS proteins contained sucrose synthesis, glycosyl transfer, and sucrose-6-phosphate phosphatase domains and possessed multiple sites, including phosphorylation, amidation, glycosylation, and myristoylation sites. StSPS2 did not contain an amidation site, and StSPS3 also possessed a nuclear localization signal site (Figure 1B). The identification of conserved protein motifs showed that of the 25 motifs obtained, 10 motifs had been annotated by the database. These 10 motifs were involved in sucrose phosphatase synthesis, of which only StSPS4 did not contain motif 9. Most of the other unannotated motifs were highly conserved in the StSPS family, and a few motifs, such as motifs 16 and 25, existed only in two or three members (Figure 1C).

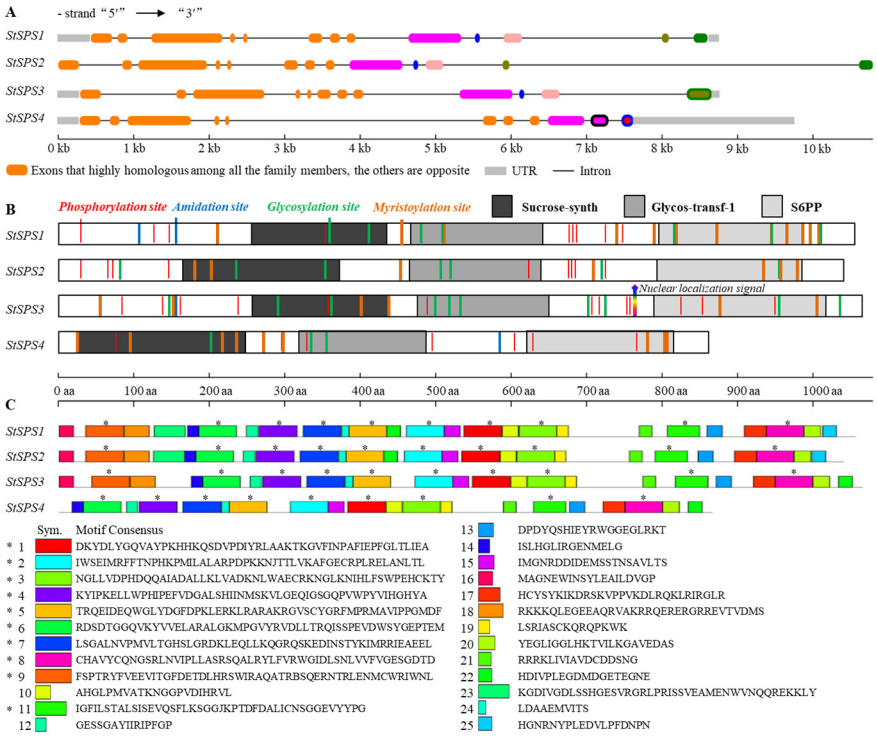

Figure 1. Analysis of the gene and protein structures of StSPS family proteins. (**A**) Gene structure of the *StSPS* family genes. (**B**) Typical domains and functional sites of StSPS family proteins. (**C**) Prediction of conserved motifs of StSPS family proteins. '*' represents a conservative motif that has been annotated in the database.

3.3. Phylogenetic Analysis of StSPS Family Members

Phylogenetic analysis of SPS family members in *Solanum tuberosum*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Nicotiana tabacum*, *Oryza sativa*, and *Triticum aestivum* (Table 4) showed that these family members could be divided into four subfamilies, namely, A, B, C and D, of which StSPS1/2 belonged to subfamily A, StSPS3 belonged to subfamily B, StSPS4 belonged to subfamily C, and subfamily D had no StSPS member. Subfamily D contained only monocotyledon members, including OsSPS2/6/8 and TaSPS2/3/4 (Figure 2). In addition, StSPS family members were closely related to tomato SPS. StSPS1/2/3/4 were also separately highly homologous to SlSPS1/2/3/4, and they were all distributed on chromosomes 7, 8, 9, and 11.

Table 4. Information and literature sources of SPS family members in various plants.

Species	Gene Name	Number in Original Literature	Literature
Arabidopsis thaliana	*AtSPS1/4/5.1/5.2*	AAF40445/CAB39764 AAK64015/CAC03459	[30]
Solanum lycopersicum	*SlSPS1/2/3/4*	Solyc07g007790/Solyc08g042000 Solyc09g092130/Solyc09g092130	[31]
Nicotiana tabacum	*NtSPS1/2/3*	AAF06792/ABA64521/ABA64520	[16]
Oryza sativa	*OsSPS1/2/6/8/11*	Rice SPS1/Rice SPS2/Rice SPS6 Rice SPS8/Rice SPS11	[15]
Triticum aestivum	*TaSPS1/2/3/4/5*	Wheat SPS1a/Wheat SPS2a/Wheat SPS3a Wheat SPS4a/Wheat SPS5a	

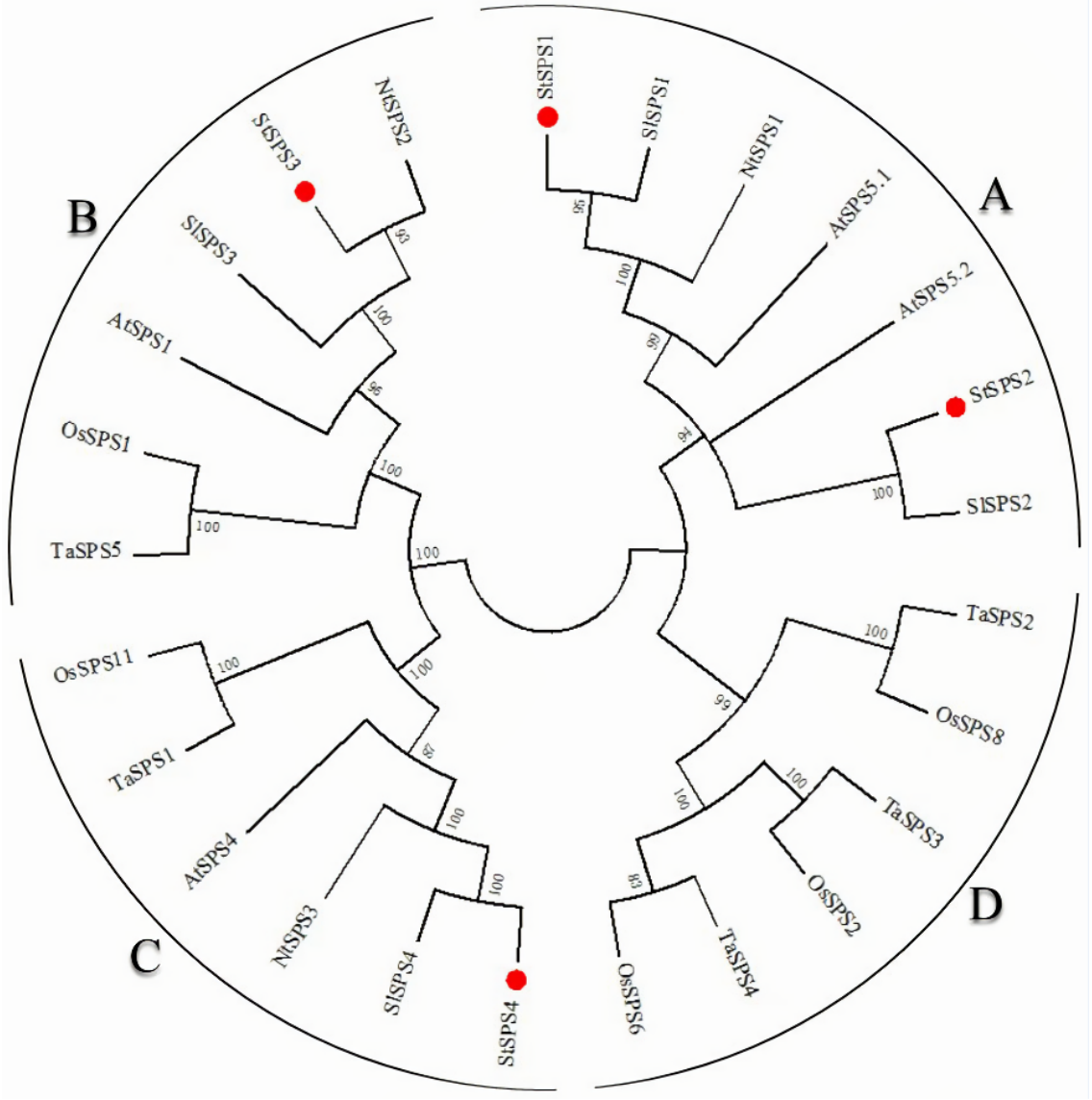

Figure 2. Phylogenetic analysis of SPS family members in *Solanum tuberosum*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Nicotiana tabacum*, *Oryza sativa*, and *Triticum aestivum*. A/B/C/D represents the four subfamilies. Specific sequence information is shown in File S2.

3.4. SPS Enzyme Analysis in Different Tuber Tissues

To elucidate the SPS activity levels during the dormancy and sprouting phases of potato tubers, the enzyme activities of the tuber cortex, pith, and sprout were detected subsequent to their storage over a duration of 7 and 56 days. The results showed that the levels of SPS activity in tuber tissues during sprouting were significantly higher than those during dormancy, and there was no significant difference in SPS activity between the tuber pith and tuber cortex during tuber dormancy. In addition, the SPS activity in the tuber sprouts was the lowest when the tubers sprouted, which was significantly lower than the activity levels in the tuber cortex and tuber pith. Although the enzyme activity difference between the tuber cortex and tuber pith reached a significant level, the average difference value was not high (Figure 3). It is indicative that SPS enzyme activity intensifies during the process of tuber sprouting, with a marked accumulation phenomenon observed in the cortex.

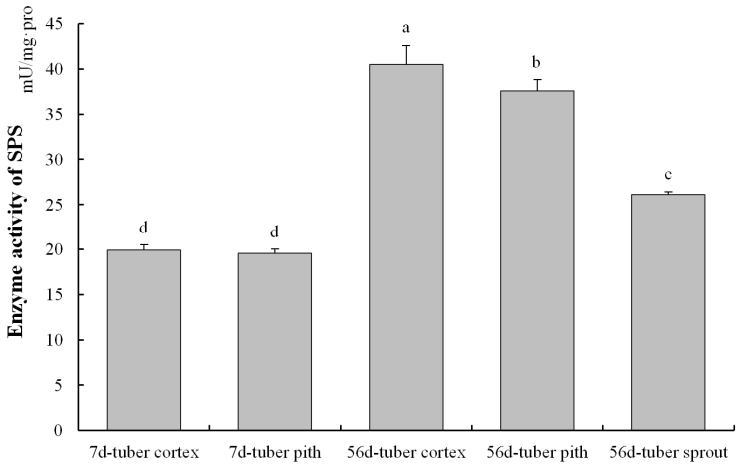

Figure 3. SPS enzyme activity in different tuber tissues at the dormant and sprouting stages. The error bar represents the standard error, calculated through three biological replicates. The uniformity of letters denotes a lack of disparity in SPS enzyme activity between two distinct tissues. Contrarily, the presence of different letters illustrates significant difference at a statistical threshold of $p < 0.05$. For example, the marked discrepancy between the SPS enzyme activity of the '56d-tuber cortex' and '56d-tuber pith' groups is delineated by the use of letters 'a' and 'b'.

3.5. Expression Analysis of StSPS Family

In the analysis of the transcriptome pertaining to the dormancy and sprouting of tubers, it has been observed that four StSPS genes exhibit changes in expression on the transcriptional level. Among them, the expression of *StSPS1* was relatively high, and its RPKM value was more than 100 in both the dormant and sprouting stages, while the RPKM values of the other members were less than 15. Among them, the expression of *StSPS2/4* was particularly low, and the expression of *StSPS2* was not detected during dormancy. In the proteomic study at the early stage of tuber sprouting, only StSPS1/3 was found to be expressed, and the expression of StSPS1 protein reached approximately 9139, while the expression of StSPS3 was only 1305, and that of StSPS2/4 was not detected (Table 5). The above studies show that the transcription and protein expression levels of StSPS1 were significantly higher than those of other members, indicating that StSPS1 is a major functional member in the StSPS family.

Table 5. Expression of StSPS in transcriptome and proteome research on tuber dormancy and sprouting. Number stands for the ID in PGSC and EBI databases. RPKM represents reads per kilobase million bases in transcriptome research.

Gene	Number	RPKM Value in Transcriptome		Protein Expression in Proteome
		Dormancy	Sprouting	Sprouting
StSPS1	PGSC0003DMT400071807 M1CPB7	124.72	111.79	9139.23
StSPS2	PGSC0003DMT400076855 M1CXH8	NA	0.12	N/A
StSPS3	PGSC0003DMT400067951 M1CI66	14.15	5.12	1305.77
StSPS4	PGSC0003DMT400049042 M1BN62	2.86	1.60	N/A
	PGSC0003DMT400049047 M1BN65	2.65	2.27	N/A

To further verify whether StSPS1 functions as the principal regulator of seed potato vigor, the expression changes from tuber dormancy to sprouting were evaluated. The results showed that the expression of *StSPS1* was significantly higher than those of other family genes at all stages, the expression of *StSPS3* was less than 0.18 from dormancy to sprouting, the expression of *StSPS2/4* was less than 0.04, and the expression of *StSPS2* was not even detected in the first three stages. In addition, the expression of *StSPS1* gradually decreased in early storage and gradually increased in late storage. Among them, the expression of *StSPS1* changed the most. When tuber dormancy was broken at 56 days of storage, the expression of *StSPS1* was more than three times that at 7 days (Figure 4). At the same time, the tissue expression level was explored (Figure 5). On the basis of the TPM (transcripts per million) values (File S3) of four *StSPS* genes in the Ensembl Plants database, it was found that the expression of *StSPS1* was the highest in stem and tuber tissues and the lowest in the stolon. Compared with other tissues, *StSPS3* is highly expressed in flowers, tuber pith, and tuber cortex. The expression levels of *StSPS2/4* in various tissues were very low or below the minimum threshold. Through qRT-PCR detection, it was found that the expression of *StSPS1* was the highest in stem and tuber pith (outer pith), slightly lower in leaf and stolon, and the lowest in other tissues. In addition, except for *StSPS2*, which was not detected in tuber sprouts, four *StSPS* were expressed in all tissues, although the expression of *StSPS2* in all tissues was very low.

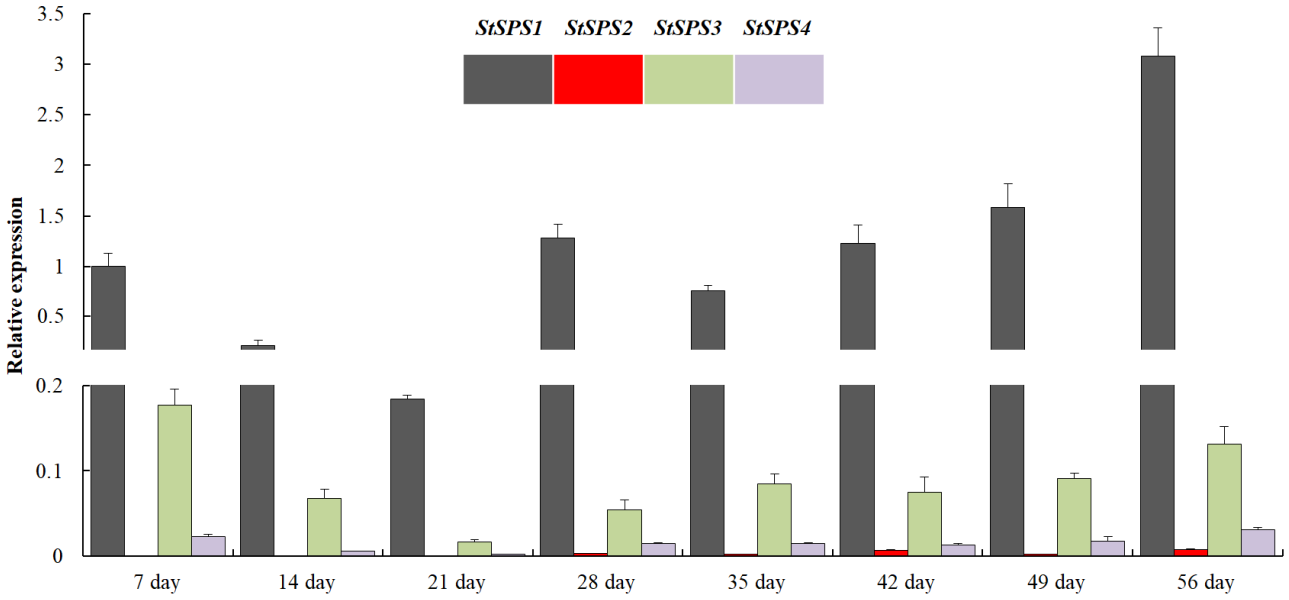

Figure 4. Expression of *StSPS* family genes in bud eyes tissue during tuber storage (qRT-PCR).

To conclude, the SPS enzyme activity undergoes significant alteration in tandem with variations in the seed potato vigor. While StSPS1 shows marginally lesser expression in flowers compared to StSPS3, it consistently demonstrates greater expression than other StSPS genes. More importantly, the divergence in expression of *StSPS1* was the most conspicuous from tuber dormancy to sprouting, indicating that *StSPS1* is a vital gene regulating seed potato vigor.

3.6. Correlation Analysis between StSPS1 and Seed Potato Vigor

We conducted an exploration of the expression patterns and correlation analysis in seven potato varieties to delve deeper into the relationship between *StSPS1* and seed potato vigor. It could be discovered that 'Weiyu 3' and 'Favorita' both had brief dormancy periods, whilst 'Burbank' had a notably longer dormancy span. The rest of the varieties had relatively moderate dormancy periods. During all three periods studied in all varieties, the expression of *StSPS1* increased towards the end of dormancy. Furthermore, it seemed apparent that the shorter the dormancy period, the higher the initial-stage expression of

StSPS1. Conversely, as the length of the dormancy period increased towards the end of dormancy, the expression of StSPS1 decreased. Through correlation analysis, we ascertained that *StSPS1* had a significant negative correlation with the length of dormancy period across all seven varieties (Figure 6). This further illustrated that *StSPS1* was a potential crucial gene regulating seed potato vigor.

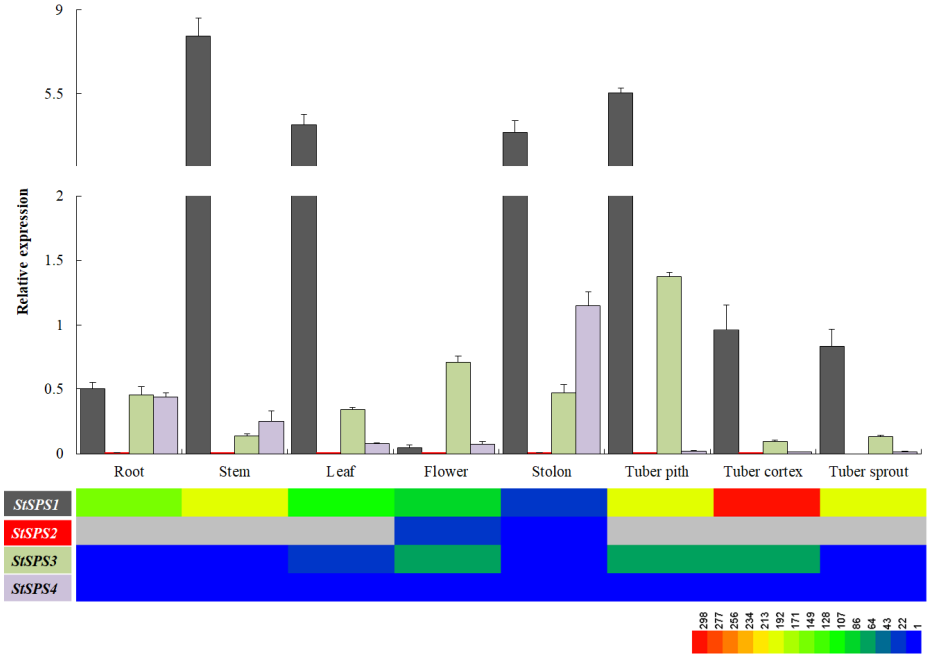

Figure 5. Expression analysis of *StSPS* family genes in different tissues (qRT-PCR). Flowers were sampled at the full flowering stage (70 d after planting), tuber sprout tissue was sampled after tuber sprouting (56 days storage), and other tissues were sampled at harvest time. The heat map below shows the tissue expression in the Ensembl Plants database.

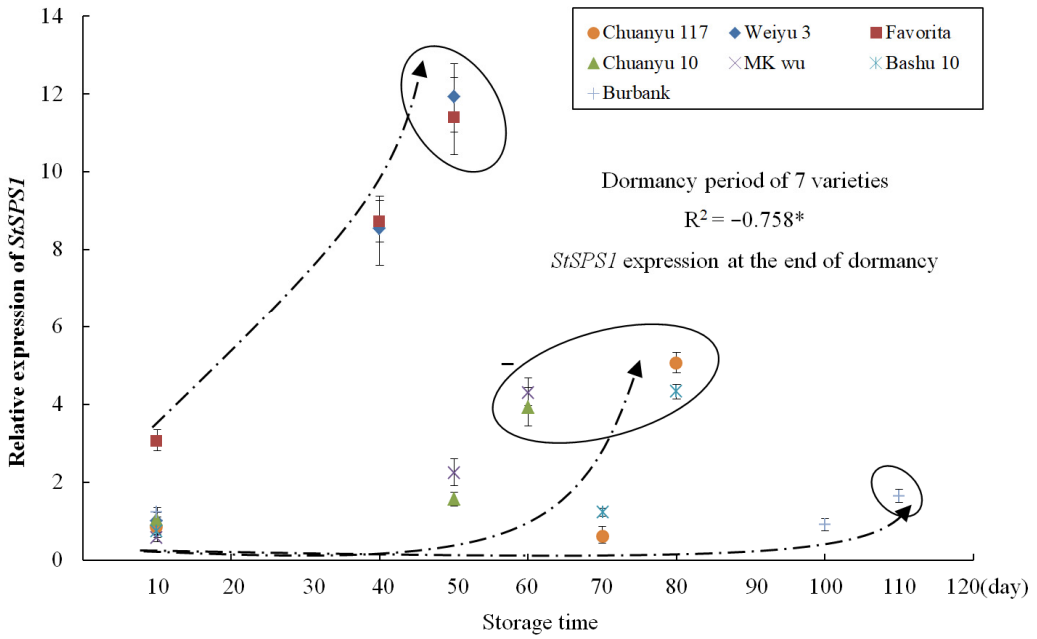

Figure 6. Correlation analysis between *StSPS1* expression and tuber dormancy period of different varieties. Names of 7 potato varieties were listed in the rectangle 'R2' represents a correlation coefficient. '*' represents a significant difference level at $p < 0.05$, and the circle marks the end of dormancy. The dotted arrows from top to bottom indicate the expression trend line of *StSPS1*.

4. Discussion

Seed potato vigor is an important index for measuring the quality of seed potatoes. It is the general indicator of a seed potato's internal physiological and metabolic activity and its ability to adapt to the environment. Affected by internal and external factors such as genotype, development degree, storage, and sowing season, it includes comprehensive indices such as different dormancy periods, sprouting periods, emergence rates, seedling growth, numbers of main stems, and stress resistance. Under a specific sowing date and environmental conditions, a high-vigor seed potato has accurate sprouting time, fast, neat, and strong emergence; strong resistance to adverse environments; and full growth and development of plants to obtain high and stable yield. Therefore, seed potato vigor is a prerequisite for giving full play to the excellent characteristics of varieties. No matter how good a variety is, if it fails to sprout on time or has aged, crop reduction or even harvest failure will occur. How to accurately grasp seed potato vigor and make timely adjustments is of great significance to improve potato yield.

High levels of sucrose possess the ability to promote tuber sprouting [32]. SPS is the key factor controlling sucrose synthesis and plays important roles in plant growth and yield improvement [33,34]. In this study, SPS activity increased significantly during tuber sprouting, which indicated that the changes in sucrose-synthesis-related enzymes and genes were also prerequisite factors affecting the increase in seed potato vigor. At present, research on SPS family proteins mostly focuses on the isolation and identification of coding proteins. The first SPS protein was isolated and purified from corn and spinach [35,36].

However, as a multigene family, the major SPS genes regulating sucrose metabolism in different plants and the various biological functions involved in these genes are not the same. With the whole-genome sequencing of multiple plants, SPS family members have been gradually identified. The number varies from four in Arabidopsis and potato to eight in sugarcane [37]. A previous study found that there were three or possibly four SPS members with different protein molecular weights in potato [38]. In this paper, four SPS members of potato were identified through comparative genomics and sequence analysis. The gene and protein structures of these four StSPS members are relatively conservative, and they all contain typical SPS domains.

There are differences in the expression patterns of different SPS members of the same plant. Four *SPSs* in Arabidopsis are expressed in all tissues, *AtSPS5.1/5.2* are mainly expressed in cotyledons and mature leaves, and *AtSPS1* can also be expressed in embryos. In addition, it was also found that the four *SPSs* in Arabidopsis have functional redundancy in organs and tissues. Inactivation of one gene does not affect the growth of Arabidopsis [39]. Rice *OsSPS1* and *OsSPS11* were specifically expressed in source and dark tissues, respectively, while *OsSPS2/6/8* were expressed in both source and sink tissues, and the expression of *OsSPS1/6* was negatively correlated with sucrose content [40]. The above studies suggest that *SPSs* with different expression levels have different specific functions. In this study, the expression level of *StSPS1* was always higher than those of other *StSPS* in different tissue and developmental stages. The overall expression of the *StSPS2/4* gene in potato is very low, indicating that they may not be key genes encoding SPS. This phenomenon of high expression of *SPS1*, low expression of *SPS3*, and extremely low expression of *SPS2/4* also exists in the four *SPSs* of tomato [31]. The phylogenetic relationship between potato and tomato is extremely close, and the gene structures of homologous gene pairs are similar. Compared with *SPS3/4*, *SPS1/2* lost some introns and were distributed on the same chromosome in potato and tomato. This result illustrates that the homologous genes in potato and tomato may have similar functions and that *SPS1* may be a vital gene in the regulation of sucrose metabolism, growth, and development in potato and tomato.

5. Conclusions

Four genes encoding sucrose phosphate synthase were discovered in the potato genome in this article. By applying bioinformatics techniques, the gene and protein

structure, alongside systematic evolution analysis, were carefully scrutinized. In light of qRT-PCR and enzyme activity analysis, it was discovered that *StSPS1* exhibited a greater level of expression in nearly all tissues and periods compared to other members. An investigation was conducted across seven distinct varieties featuring potatoes of different seed potato vigor, indicating the existence of a negative correlation between *StSPS1* and dormancy period length. These findings imply StSPS1 has the potential ability to enhance seed potato vigor. This study constituted a foundational examination of SPS gene function and seed potato vigor regulation, carrying important implications for future research.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae9040511/s1, Supplementary File S1 (File S1): Each exon sequences of four StSPS family genes. Supplementary File S2 (File S2): The protein sequences used for phylogenetic analysis in this paper. Supplementary File S3 (File S3): Expression values of the four StSPS genes in the Ensembl Plants database.

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