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In silico Identification and Characterization of Starch Synthase (SS) Genes in Potato (*Solanum tuberosum*)

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ABSTRACT

Starch is the major source of storage carbohydrate, which is important product for plant and animal as well as for industrial use. Starch synthase (SS) enzyme encoded by several genes is the key factors in starch biosynthetic pathway. Genome wide exploration and characterization of these *starch synthase* (*SS*) genes are still limited in the literature. Therefore, intensive bioinformatic tools were approached to identify and *in silico* characterize the *SS* genes in potato genome. We identified several orthologues of *SS* in *Arabidopsis* by using the term starch synthase in *Arabidopsis* genome database (TAIR) and National Centre for Biotechnology Information (NCBI). These orthologues were blast in Sol Genomics network and ten *SS* genes were confirmed as member of the family following Hidden Markov Model (HMM). The genes were characterized *in silico* for chromosomal locations, phylogeny, exon-intron, domains and motif content, molecular weight and nature of proteins and predicted the functions using different online bioinformatic tools. Chromosomal map and phylogenetic tree were constructed for analyzing evolutionary pattern and relationship with the *SS* genes of other crop species. The *SS* genes are distributed on 4 chromosomes (chromosome number 2, 3, 7 and 8) out of 12 potato basic chromosomes and originated from *Arabidopsis*. The results of this study will be provided basic information in planning future research regarding the regulatory mechanism of search synthesis both under normal and stress condition.

Introduction

Potato is the most popular and diversified vegetable crop in the world, which is cultivated in tropical, subtropical and temperate regions. It is considered as a staple food of many countries due to presence of high starch content, different types of protein, vitamins, minerals and metabolites. Consumption of potato can be reduced the pressure on rice as source of higher amount of carbohydrate production per unit area compared to other cereal crops. Apart from being a staple food, it has wide range of uses including industrial raw materials and processed food. After rice, wheat and maize, it is the 4th most important crop in the world (FAOSTAT 2018). Bangladesh stands 4rd in Asia for potato production (Scott and suarez 2012). The production of this crop is continuously increased with reducing the amount of export since 2013 (BBS 2017). Breeder efforts reduced its exporting revenue about 77% from 2013 to 2017 (BBS 2018) but has chance to reduce export either by storing the left-over production in cold storage or by developing genotypes that can be stored in ambient temperature. In every year, farmer faces problem to get space in cold storage due to availability of storage and involvement of huge cost. Potato is suitable to produce different dehydrated processed food and storage product because of its high starch content. Starch content in dry matter of potato is varied upon cultivars and growing seasons and ranged from 66 to 80% (Liu et al. 2002). In addition, source and sink relationship also determined the remobilization and accumulation starch in storage organ, like potato tuber. High temperature can influence balance between source and sink (Gandin et al. 2011). Both genetic and environmental factors have influence on metabolism and physiochemical properties (composition and structure) of starch (Beta and Corke 2001, Liu et al. 2002).

Starch is the ubiquities storage form of glucose monosaccerides in plant and the main source of carbohydrate in human diet as well as an industrial polymer, which is isolated from the heterotopic storage organs of plant species. It is deposited as water insoluble

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granules of polysaccerides in cell cytoplasm, consisting of both branched (amylopectin) and linear glucose (amylose) chain. The biosynthetic pathways of starch metabolism go through a complex process involving multiple starch synthase and starch branching/ debranching isozymes (Harsselaar et al. 2017). In higher plants, starch is existed in two major metabolic types; transitory starch and reserve starch. The metabolism of transitory starch follows a day/night cycle, where starch is accumulated in the chloroplast at light phase and breakdown partially or completely at night for supplying necessary soluble sugars for metabolic activities in plant organelles. Reserve starch granules are produced over a long period of time, depending on the mobilization and accumulation of photosynthates from photoautotrophic cells to non-photosynthetic storage organs (Keeling and Myers 2010).

The control of starch metabolism in storage organs differs from that in leaves as it accumulates over a much longer period of time and degraded only once (Lloyd and Kossmann 2015). The synthesis of starch in storage organs is mediated by the several starch synthesis and degradation enzymes. A total of 77 loci are known to code for different isoforms of starch metabolic enzymes (Harsselaar et al. 2017). The key enzymes of starch synthesis are sucrose synthase, ADP glucose pyrophosphorylase, starch phosphorylase, starch synthase, starch branching and debranching enzymes (Bes and DBEs) as well as starch degradation enzymes (GWD, PWD, α-, β-, iso-amylase, SEX4) (Smith et al. 1997, Nakamura 2002, Subasinghe et al. 2014). Among them *SS*, a class of glucan-elongating enzymes, is considered to be one of the most important regulatory enzymes in starch metabolism. It catalyses and transfers the glucosyl moiety from ADP-glucose to the non-reducing end of a pre-existing glucan chain for extending the α-1,4 glucan chains (Szydlowski et al. 2009, 2011).

It was observed that elimination of this protein results five-fold decreased in the rate of starch synthesis and twelve-fold increased in the amount of ADP glucose in the endosperm (Prioul et al. 1994). In plants, there are five isoforms of *SS*, defined as *granule-bound starch synthase* (*GBSS*), *starch synthase* I (*SS*I), *starch synthase* II (*SS*II), *starch synthase* III (*SS*III), and *starch synthase* IV (*SS*IV), which have high degree of amino acid similarity (450 amino acid residues in the C-terminus) (Schwarte et al. 2013). Expression profaning of these isoforms has been studied in different model species, , such as potato (Edwards et al., 1999), rice (Crofts et al. 2015), maize (Huang et al. 2016), and *Arabidopsis* (Schwarte et al., 2013). Although, *SS* gene has been investigated in several model species, a comprehensive investigation study of this protein in potato is limited.

Therefore, in this study we performed comprehensive genome wide analysis of the *starch synthase* gene to computationally characterize the structure, chromosomal locations, function and evolutionary divergence and to classify them based on phylogenetic analysis.

Materials and Methods

Identification of starch synthase gene

For genome wide identification of potato *starch synthase* (*SS*) genes, the key word starch synthase was used as a query to find out orthologue in *Arabidopsis* using *Arabidopsis* genome database (TAIR) and National Centre for Biotechnology Information (NCBI). The redundant sequences were discarded from blast search in Sol Genomic network (http://solgenomics.net/) and NCBI (https://www.ncbi.nlm.nih.gov/) and finally 10 genes were identified and confirmed through Hidden Markov Model (HMM) ENSEMBLE PLANT (http://plants.ensembl.org/hmmer/index.html).

Arabidopsis starch synthase domain sequences were used to compare with predicted potato starch synthase domain sequences for the confirmation using the web tool from EMBL (http://smart.embl-heidelberg.de/). CDS (coding sequence) and protein sequences of the potato *SS* genes were obtained from SGN (https://solgenomics.net/). To verify further the identified sequences were checked in NCBI (https://www.ncbi.nlm.nih.gov/) and iTAK; Plant Transcription factor & Protein Kinase Identifier and Classifier (http://bioinfo.bti.cornell.edu/cgi-bin/itak/index.cgi).

Sequence analysis of potato starch synthase genes

ProtParam (http://web.expasy.org/protparam/) was used to analyze the primary structure, comprising the molecular weight (MW), the length and isoelectric point (pI) of the assumed potato starch synthase proteins. The exon/intron structures of the potato *SS* genes were analyzed by GSDS (http://gsds.cbi.pku.edu.cn/) web tool using the potato *SS* genes CDS and their corresponding genomic sequences. To analyze the potato starch synthase protein motifs, software MEME (http://memesuite.org/) was used. The following parameters were used to identify distinctive motifs; (1) maximum number of motifs 10, (2) width of the optimum motif ≥ 6. Clustal omega web tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyze the similarity among the 10 potato starch synthase proteins. ProtComp 9.0 from SoftBerry (http://linux1.softberry.com/berry.phtml) was used to predict the sub-cellular locations of the SS proteins of potato.

Phylogenetic analysis of potato starch synthase (SS) proteins

The deduced amino acid sequences of *Arabidopsis*, tomato, tobacco and rice SS proteins were obtained from TAIR, SGN, NCBI, respectively and aligned with the help of DNAMAN web server. These aligned sequences were used to construct a phylogenetic tree using UPAGMA method with 1000 bootstrap replicates in MEGA-X software. A second phylogenetic tree of the full-length amino acid sequences of 10 potato SS proteins was constructed using the maximum parsimony method following complete deletion of amino acids with 1000 bootstrap replicates.

Chromosomal locations, gene duplication analysis of potato SS genes

The start and end positions of each potato *SS* gene, including sub-genome information, were obtained from SGN and the position of each genes were analyzed using the MapGene2Chromosome2 (http://mg2c.iask.in/) web tool. An NCBI BLAST search was conducted based on the query coverage percentage of the potato *SS* genes against each other to identify duplicated genes and to identity of each gene. When the query coverage percentage and identity of the candidate genes was ≥80%, they were considered to be segmentally duplicated genes. Paralogous genes were considered to be tandemly duplicated when two genes were separated by five or fewer genes in a 100-kb region on a chromosome.

Results and Discussion

Identification and sequence analysis of StSS genes and their putative proteins

We found 10 potato genes which encode starch synthase proteins. These 10 potato *SS* genes were designated as *StSS001* to *StSS010* (Table 1). The *SS* genes in potato are distributed on 4 chromosomes of 12 basic chromosomes. Among the 10 *SS* genes, 4, 2, 2 and 2 genes are located on chromosome 2, chromosome 3, chromosome 7 and chromosome 8, respectively. Ten SS genes have exon content ranged from 1 to 16 indicates that some genes of this family under-went extensive alternate splicing in the process of evolution and produced several introns shuffling from exon which increased the gene diversity (Souza et al. 1996). A noticeable range of variation found in length of the coding DNA sequence (CDS) of *StSS* from 330 to 4230 bp for *StSS006* and *StSS005*, respectively (Table 1). The identified potato SS protein length ranged from 54 (*StSS010*) to 767 (*StSS009*) amino acids (AA), the molecular weight (MW) of SS proteins ranged from 6596.48 (*StSS010*) to 113750.89 (*StSS003*) kDa, and the iso-electric point (pI) ranged from 5.49 to 9.43, indicates that genes are acidic to basic in nature. Alignment structure of the potato SS protein (Fig. 1) showed that many amino acids are conserved in the genes throughout.

Evolutionary analysis of potato SS proteins through phylogenetic classification

In chloroplast-containing organisms, *SS* are grouped into five classes by DNA sequence, termed as, soluble SS (*SS* I, *SS* II, *SS* III, *SS* IV) and Granule Bound SS (GBSS) (Leterrier et al. 2008, Deschamps et al. 2008) with considerable number of aminoacidic similarity in the C-terminus that comprises the catalytic and starch-binding domains (Schwarte et al. 2013). For establishing the evolutionary and functional relationships within the *SS* gene family, a phylogenetic tree was constructed using protein sequences of 10 potato *SS* genes, 18 putative tomato *SS* genes, 6 *Arabidopsis SS* genes, 6 rice *SS* genes and 5 tobacco *SS* genes (Fig. 2). The phylogenetic tree classified the 45 SS proteins into eight subgroups (I-VIII) based on clade and evolution of species in the topology of the trees. With exception of subgroup II and III, potato *SS* genes were distributed in all 6 subgroups. Subgroup VI and VIII both contained three *StSS* genes. Monocot and dicot *SS* genes were mixed up in all the subgroup. Subgroups II and III contained the highest amount of tomato *SS* genes i.e. 8 genes out of 18. Tobacco *SS* genes are absent in subgroup VI. (Fig. 2). Most of the potato *SS* genes are distributed with the *Arabidopsis SS* genes indicating that potato *SS* genes are originated from the ancestral plant *Arabidopsis*. Until now, it was reported that under normal growth conditions, SS I, SS II and SS III catalyse the same reaction of glucan chain elongation, SSIV is involved in starch granule initiation and GBSS performs synthesis of amylose (Brust et al. 2014). Nevertheless, the functional relevance of SS V and SS VI in storage starch metabolism is still uncharacterized (Helle et al. 2018). However, in phylogenetic tree, the similar functional proteins are distributed in different subgroups. For example, genes for SS I (*StSS6, StSS8*), SS II (*StSS9*) and SS III (*StSS10*) are located in subgroups VI, IV, I, VII; genes for GBSS (*StSS1 and StSS2*) are found in subgroups VI and V; and genes for SS VI (*StSS5, StSS7*) are found in subgroup VI and VIII, respectively (Tab. 1, Fig. 2).

Gene structure analysis of potato SS gene family

To further investigate the diversity of the potato *SS* genes, we analyzed StSS protein motifs using the MEME online server. Ten conserved motifs were identified, motif 1 to 10 (Fig. 3). Motif 6 and 7, the most common motifs, comprise the starch synthase dimer domain. These two motifs mostly found in the genes of largest subgroup VIII (Fig. 2 and Fig. 3). Among the 10 gene products, 8 genes contained motif. The remain two (*StSS1* and *StSS5*) did not show motif site (Fig. 3) which

are located in the same subgroup (IV) (Fig. 2). Among 8 motif containing *SS* genes, motif 6 and 7 are absent in *StSS6* and *StSS10.* Both of them had only one motif, motif 1 and motif 10, respectively. The genes, *StSS3 and StSS8* both had 2 motifs absent: motif 1, 4 and motif 3, 5, respectively. To gain further insights into the structural diversity of *SS* genes in potato, a phylogenetic tree was constructed with 10 *StSS* genes using their full-length DNA sequences (Fig. 4a). The *StSS* genes were classified into 3 subfamilies in this phylogenetic tree (Fig. 4a). Analyzing the genetic structural diversity among the proteins of a multigene family is a useful way to perform evolutionary analysis. We therefore, deduced the exon-intron organization of individual *StSS* genes to examine their structural diversity (Fig. 4b). Among 10 *StSS* genes only two genes (*StSS006* and *StSS010*) had no intron and included in the same subfamily. Most of the *StSS* genes bear larger intron region compared to exon region. Only two *StSS* genes showed upstream/downstream region (*StSS005* with upstream region and *StSS008* with both upstream and downstream regions). Most closely related members in the same subfamily shared almost identical exon-intron organization (Fig. 4a and b). However, the exon-intron organization was not always conserved for most sister gene pairs. For example, *StSS005-006* and *StSS007-008* have different numbers of exons and introns (Fig. 4a and b). Exon-intron structure, distribution and abundance reflect the evolutionary lineage of a certain gene. Exon has been recognised to carry the mRNA for regulating transcriptional process to code proteins since 40 years back (Gilbert 1987). From the last decade a large breakthrough in exploring the function of introns of a gene there was a breakthrough in understanding the function of introns (Robart and Zimmerly 2005, Rogozin et al. 2005) and concluded as functional to harbour different elements regarding transcription control, such as untranslated RNAs (Mattick and Gagen 2001) and splicing elements (Majewski and Ott 2002).

Table 1. Details information about *StSS* genes and corresponding proteins in potato (*Solanum tuberosum*). In *StSS*, *St* stands for *Solanum tuberosum* and *SS* stands for starch synthase. ORF: open reading frame, bp: base pair, AA: amino acid, CN: chromosome number, MW: Molecular Weight, pI: isoelectric point. Information about these genes, including the chromosome locations and exons, is provided in the table. Most of starch synthase genes i.e. six genes out of ten located in the membrane bound chloroplast.

Gene name	Locus name	Name of the enzyme	ORF (bp)	Location	CN	Length (AA)	MW (kDa)	pI	Exons	Subcellular location
StSS001	PGSC0003DMG400012112	granule-bound starch synthase/ waxy	2490	PG5C_DIvf-v3_219_608:41398639..41401252	8	416	44987.35	7.59	2	Plasma membrane
StSS002	PGSC0003DMG400012111	granule bound starch synthase 1/ gbss1	2544	PG5C_DMv3_219_608:41394811..41398415	8	607	66575.78	6.92	12	Membrane bound chloroplast
StSS003	PGSC0003DMG400008322	Starch synthase IV	3006	PG5C_DMv3_219_602:51580615..51590189(-)	2	1001	113750.89	5.59	9	Membrane bound chloroplast
StSS004	PGSC0003DMG400030619	Starch synthase V	2197	PGSC_DIvf-v3_219_602:63356318..63362993(+)	2	390	43562.14	6.46	4	Membrane bound chloroplast
StSS005	PGSC0003DMG401013540	Starch synthase VI	4230	PGSC_DMv3_219_ch.07:38230264..38238416(+)	7	392	45447.42	9.18	1	Extracellular
StSS006	PGSC0003DMG401018552	Soluble starch synthase 1, chloroplastic/amyloplastic	330	PG5C_DMv3_219_603:17079543..17079872(-)	3	109	11684.41	9.43	1	Extracellular
StSS007	PGSC0003DMG402013540	Starch synthase VI	1892	PGSC_DIvf-v3_219_607:38220166..38227412(+)	7	412	46327.42	5.96	14	Membrane bound chloroplast
StSS008	PGSC0003DMG402018552	soluble starch synthase 1/SS1	1626	PGSC_DIvf-v3_219_ch03:17071123..17080138	3	641	70608.89	5.49	16	Membrane bound chloroplast
StSS009	PGSC0003DMG400001328	soluble starch synthase 2/SS2	3395	PG5C_DM-6_219_602:67445011..67452158	2	767	85221.52	5.90	8	Membrane bound chloroplast
StSS010	PGSC0003DMG400016481	soluble starch synthase 3/ SSIII	2159	PGSC_DIvf-v3_219_ch02:57632525..57634683	2	54	6596.48	5.61	8	Extracellular

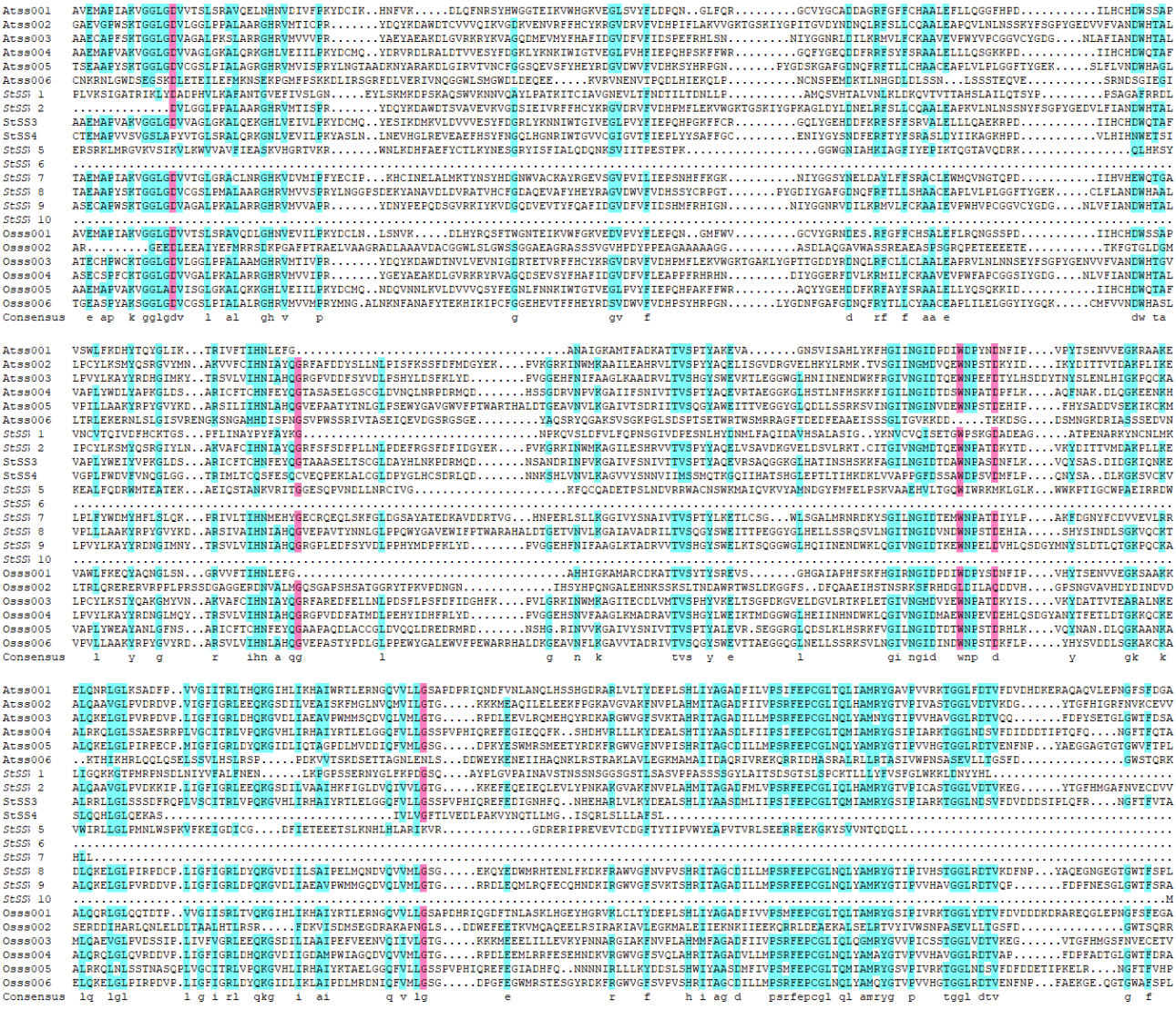

Figure 1. Sequence alignment of StSS (Solanum tuberosum starch synthase) proteins and starch synthase proteins from *Arabidopsis* and rice (Oryza sativa). Identical amino acids are indicated by pink and the amino acids with >50% similarity is indicated by aqua color background

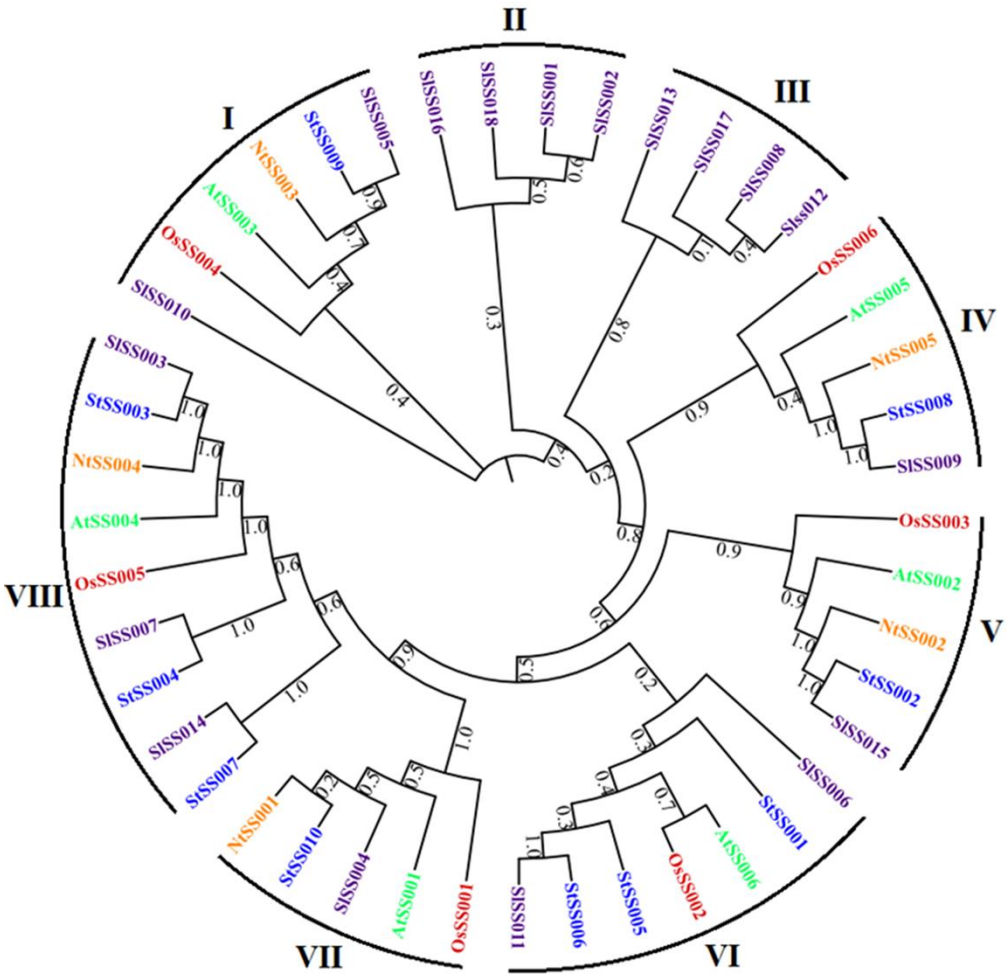

Figure 2. Phylogenetic relationship of Arabidopsis (*AtSS*), rice (*OsSS*), potato (*StSS*), tobacco (*NtSS*), and tomato (*SlSS*) *SS* genes. The conserved starch synthase dimer domain sequences of *Arabidopsis,* rice, potato, tobacco, and tomato genes were aligned using muscle, and the tree were constructed by the UPAGMA method with MEGA 7.0. The numbers on the branches indicate bootstrap support values from 1000 replications. The tree was divided into eight subfamilies according to bootstrap support values and evolutionary distances.

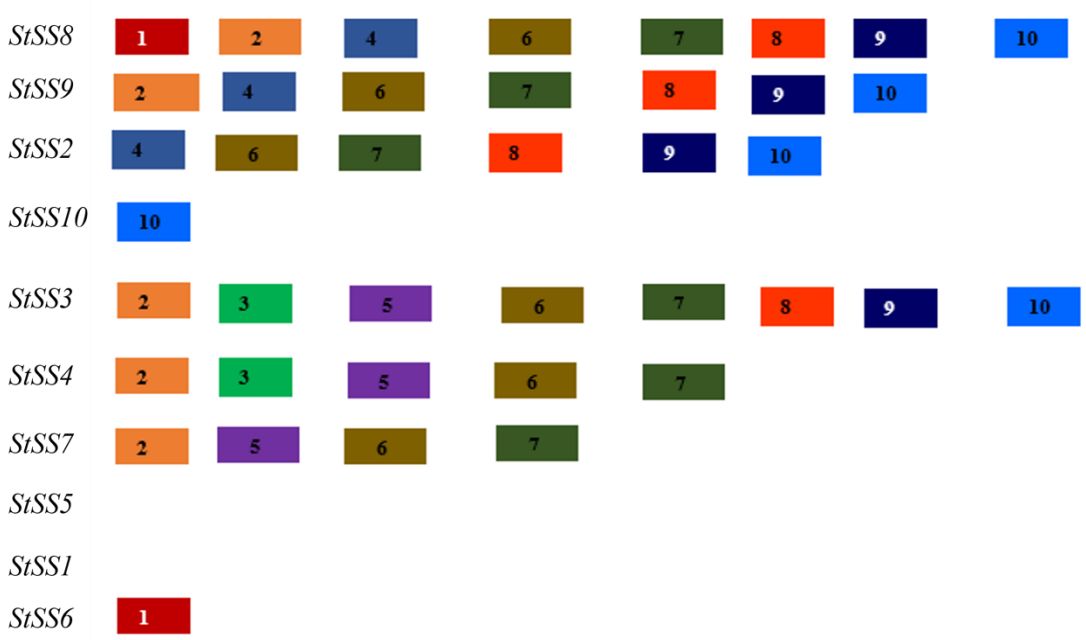

Figure 3. Schematic representation of the 10 conserved motifs in StSS proteins. StSS protein motifs were identified using the online MEME program. Different colored boxes represent different motifs, where the number in center of each boxes indicates their name (Motif 1 to 10). The colored boxes were drawn and ordered manually according to the results of MEME analysis. Boxes don't indicate the exact location and size of motif but represent sequence of motif. Genes are ordered according to phylogenetic tree.

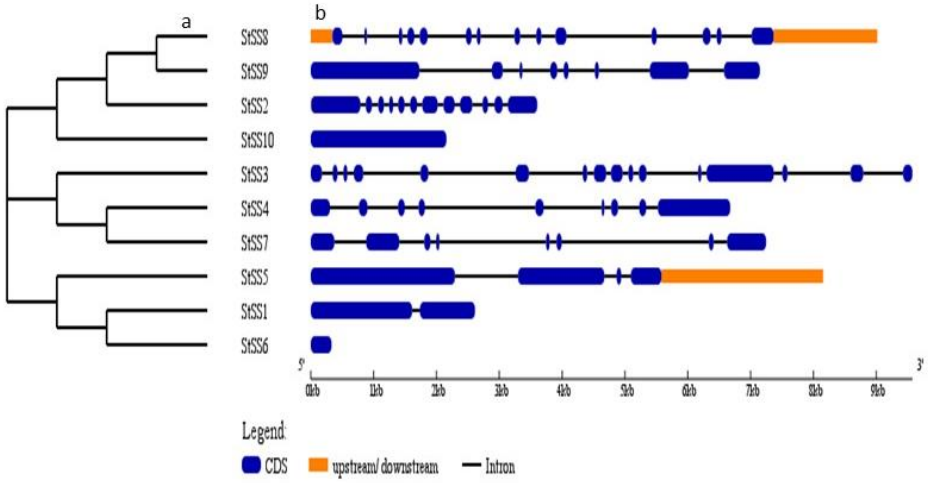

Figure 4. Phylogenetic relationships and gene structures of *StSS* genes. a. Phylogenetic tree constructed among the 10 *StSS* genes using full length DNA sequences with clustal omega online tools following the UPGMA method with 1000 bootstrap replicates. b. Exon-intron organization of *StSS* genes constructed by using GSDS online tools. Exon and introns are represented by blue boxes and ash lines, respectively. Untranslated regions (upstream/ downstream) are indicated by yellow boxes.

Conclusion

Starch synthase (SS) gene family has been characterized in several plants. In this study, we identified 10 *SS* genes in *Solanum tuberosum* and categorize them with other crops. These results showed the relationship among them. The results of this study provide foundation for further investigation of biological function of this gene family in potato as well as in other crops under both normal and stress conditions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

BBS, 2018. 45 years agriculture statistics of major crops (aus, amon, boro, jute, potato and wheat). Bangladesh Bureau of Statistics (BBS), Statistics and Informatics Division (SID), Ministry of Planning.

BBS, 2017. Bangladesh Statistics 2017. Bangladesh Bureau of Statistics (BBS) Statistics, Informatics Division (SID), Ministry of Planning.

Beta, T., Corke, H. 2001. Genetic and environmental variation in sorghum starch properties. Journal of Cereal Science, 34: 261-268. https://doi.org/10.1006/jcrs.2000.0379

Brust, H., Lehmann, T., D'Hulst, C., Fettke, J. 2014. Analysis of the functional interaction of *Arabidopsis* starch synthase and branching enzyme isoforms reveals that the cooperative action of SSI and BEs results in glucans with polymodal chain length distribution similar to amylopectin. Plos One, 9: 1-14. https://doi.org/10.1371/journal.pone.0102364

Crofts, N., Abe, N., Oitome, N.F., Matsushima, R., Hayashi, M., Tetlow, I.J., Emes, M.J., Nakamura, Y., Fujita, N. 2015. Amylopectin biosynthetic enzymes from developing rice seed form enzymatically active protein complexes. Journal of Experimental Botany, 66 (15): 4469-4482. https://doi.org/10.1093/jxb/erv212

Deschamps, P., Moreau, H., Worden, A.Z., Dauvillée, D., Ball, S.G. 2008. Early gene duplication within chloroplastid and its correspondence with relocation of starch metabolism to chloroplasts. Genetics Society of America, 178: 2373-2387. https://doi.org/10.1534/genetics.108.087205

FAOSTAT. 2018. Statistics of the Food and Agriculture Organization of the United Nations.

Gandin, A., Gutjahr, S., Dizengremel, P., Lapointe, L. 2011. Source-sink imbalance increases with growth temperature in the spring geophyte *Erythronium americanum*. Journal of Experimental Botany, 62 (10): 3467-3479. https://doi.org/10.1093/jxb/err020

Gilbert, W. 1987. Why genes in pieces. News and views, 271: 501. https://doi.org/10.1038/271501a0

Harsselaar. V.J.K., Lorenz, J., Senning, M., Sonnewald, U., Sonnewald, S. 2017. Genome-wide analysis of starch metabolism genes in potato (*Solanum tuberosum* L.). BMC Genomics, 18: 1-18. https://doi.org/10.1186/s12864-016-3381-z

Helle, S., Bray, F., Verbeke, J., Devassine, S., Courseaux, A., Facon, M., Tokarski, C., Rolando, C., Szydlowski, N. 2018. Proteome analysis of potato starch reveals the presence of new starch metabolic proteins as well as multiple protease inhibitors. Frontiers in Plant Science, 9: 1-14. https://doi.org/10.3389/fpls.2018.00746

Huang, B., Keeling, P.L., Hennen-Bierwagen T.A., Myers, A.M. 2016. Comparative in vitro analyses of recombinant maize starch synthases SSI, SSIIa, and SSIII reveal direct regulatory interactions and thermosensitivity. Archives of Biochemistry and Biophysics, 596: 63-72. https://doi.org/10.1016/j.abb.2016.02.032

Keeling, P.L., Myers, A.M. 2010. Biochemistry and genetics of starch synthesis. Annual Review of Food Science and Technology, 1: 271-303. https://doi.org/10.1146/annurev.food.102308.124214

Leterrier, M., Holappa, L.D., Broglie, K.E., Beckles, D.M. 2008. Cloning, characterisation and comparative analysis of a starch synthase IV gene in wheat: Functional and evolutionary implications. BMC Plant Biology, 8: 1-21. https://doi.org/10.1186/1471-2229-8-98

Liu, Q., Eber, E.W., Fan, M.Z., Yada, R. 2002. Physicochemical properties of potato dry matter isolated from various cultivars at different times during growth. ZYWNOSC, 4(33): 181-190.

Lloyd, J.R., Kossmann, J. 2015. Transitory and storage starch metabolism: two sides of the same coin? Current Opinion in Biotechnology, 32: 143-148. https://doi.org/10.1016/j.copbio.2014.11.026

Majewski, J., Ott, J. 2002. Distribution and characterization of regulatory elements in the human genome. Genome Research, 12: 1827-1836. https://doi.org/10.1101/gr.606402

Mattick, J.S., Gagen, M.J. 2001. The evolution of controlled multitasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. Molecular Biology and Evolution, 18(9): 1611-1630. https://doi.org/10.1093/oxfordjournals.molbev.a003951

Nakamura, Y. 2002. Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: Rice endosperm as a model tissue. Plant and Cell Physiology, 43(7): 718-725. https://doi.org/10.1093/pcp/pcf091

Prioul, J.L., Jeannette, E., Reyss, A., Gregory, N., Giroux, M., Hannah, L.C., Causse, M. 1994. Expression of ADP-Glucose pyrophosphorylase in maize (*Zea mays* L.) grain and source leaf during grain filling. Plant Physiology, 104: 179-187. https://doi.org/10.1104/pp.104.1.179

Robart, A.R., Zimmerly, S. 2005. Group II intron retroelements: function and diversity. Cytogenetic and Genome Research, 110: 589-597. https://doi.org/10.1159/000084992

Rogozin, I.B., Sverdlov, A.V., Babenko, V.N., Koonin, E.V. 2005. Analysis of evolution of exon-intron structure of eukaryotic genes. Briefings in Bioinformatics, 6: 118-134. https://doi.org/10.1093/bib/6.2.118

Schwarte, S., Brust, H., Steup, M., Tiedemann, R. 2013. Intraspecific sequence variation and differential expression in starch synthase genes of *Arabidopsis thaliana*. BMC Research Notes, 6(48):1-14. https://doi.org/10.1186/1756-0500-6-84

Scott, G., Suarez, V. 2012. The rise of Asia as the centre of global potato production and some implications for industry. Potato Journal, 39(1): 1-22.

Smith, A.M., Denyer, K. and Martin, C., 1997. The synthesis of the starch granule. Annual Review of Plant Physiology and Plant Molecular Biology, 48: 67-87. https://doi.org/10.1146/annurev.arplant.48.1.67

Subasinghe, R.M., Liu, F., Polack, U.C., Lee, E.A., Emes, M.J., Tetlow, I.J. 2014. Multimeric states of starch phosphorylase determine protein-protein interactions with starch biosynthetic enzymes in amyloplasts. Plant Physiology and Biochemistry, 83: 168-179. https://doi.org/10.1016/j.plaphy.2014.07.016

Szydlowski, N., Ragel, P., Hennen-Bierwagen, T.A., Planchot, V., Myers, A.M., Mérida, A., D'Hulst, C., Wattebled, F. 2011. Integrated functions among multiple starch synthases determine both amylopectin chain length and branch linkage location in *Arabidopsis* leaf starch. Journal of Experimental Botany, 62: 4547-4559. https://doi.org/10.1093/jxb/err172

Szydlowski, N., Ragel, P., Raynaud, S., Mercedes, Lucas. M., Roldán, I., Montero, M., Muñoz, F.J., Ovecka, M., Bahaji, A., Planchot, V., et al. 2009. Starch granule initiation in *Arabidopsis* requires the presence of either class IV or class III starch synthases. The Plant Cell, 21: 2443-2457. https://doi.org/10.1105/tpc.109.066522