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Merit Research Journal of Biochemistry and Bioinformatics Vol. 2(1) pp. 005-027, September, 2014
Available online http://www.meritresearchjournals.org/bb/index.htm

Original Research Article

Comparative bioinformatics study of 5′ regulatory and coding regions of sucrose synthesizing isozymes of selected monocot crops

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Abstract

Regulation of gene transcription is fundamental for function and development of all living organisms. The temporal and spatial expressions of genes are mostly conferred by the nature and frequencies of cis-acting element located within the regulatory region and by appropriate transcriptional factors. Putative cis-acting present within the 5' regulatory region of maize, rice and sorghum Sucrose Phosphate Synthase (SPS) and Sucrose Phosphate Phosphatase (SPP) were thus identified using bioinformatic tools such as PLACE, Plant Pan, plant CARE and Genomatrix Mat inspectors. Cell development, hormonal and environmental responsive elements were identified and these include: TATA, CAAT, ABRE, GARE, W-BOX, GT-1, CURE, MYC, MYB, DRE, RY-motif, CGGB-BOXAT, among others at different frequencies. Furthermore, the structural/evolutionary relationships between the genomic and proteomic sequences predicted using Clustal Omega: EMBL-EBI, Multiple Sequence Alignment and Constrainst Based Protein Multiple Alignment Tool (COBALT) programs respectively, showed very high conservation and homology between the isozymes. This work thus reveals the probable cis-acting regulatory elements that may work in combination with other transcription factors to confer the expression and regulation of these SPS and SPP isozymes. It also shows that these isozymes could have evolved as a result of independent gene duplication from one ancestral sucrose biosynthesis gene.

Keywords: (A) biotic stress, Cis-acting elements, gene homology, plant hormone, sucrose phosphate synthase (SPS), sucrose phosphate phosphatase (SPP), Transcription factors

INTRODUCTION

Sucrose is the main sugar transported product of photosynthesis in plants and it is utilized as the sole carbohydrate source for energy production in organs that are unable to photosynthesize. It is also used to synthesize other molecules such as starch or cellulose. The selection of sucrose has been related to its metabolically non-reducing characteristic, which makes it perfect for long distance transport or long-term storage compound, unlike glucose that is easily metabolized (Jang and Sheen, 1994; Smeekens, 2000).

Sucrose is synthesized as depicted in Figure 1 by transfer of the glycosyl moiety from UDP-glucose to fructose 6-phosphate to produce sucrose 6-phosphate [catalyzed by sucrose 6-phosphate synthase (SPS; EC

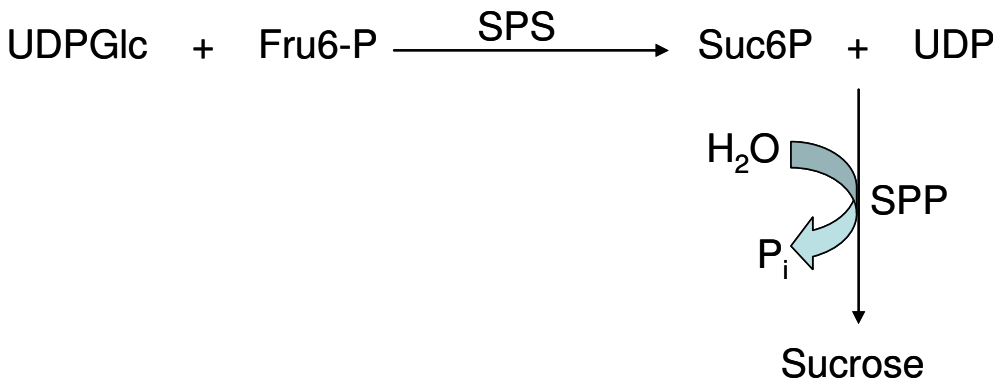


Figure 1. Sucrose synthesis Pathway

2.3.1.14)]; the product then dephosphorylates to yield sucrose as the final irreversible product (catalyzed by sucrose 6-phosphate phosphatase (SPP; EC 3.1.3.24)).

Sucrose production during photosynthesis is the most fundamental activity in plant life. Several sucrose signals are created in various plant parts (source/sink tissues) in response to photosynthesis and carbon metabolism. This therefore makes the process of sucrose synthesis, transport, utilization and storage to be tightly linked to the plant cell physiology, development, growth and environmental status (Smeekens, 2000).

The continuous exposure of plants to a variety of adverse environmental conditions such as extreme temperature, water shortage that leads to high salt concentration in soil, excessive exposure to UV light, pathogen, insect and human attacks, greatly affect their development and productivity. Plants response and adaptation to these diverse environmental stresses depend on physiological as well as biochemical alterations at the cellular and molecular levels, which could involve the expression of inducible proteins and production of anti-microbial compounds; for resistance, and accumulation of organic solutes such as sucrose that acts as an osmoregulator and also as a source of carbohydrate to meet the energy demands that usually arise during these environmental stress conditions (Shinozaki and Yamaguchi-Shinozaki, 2000).

Thus, the quantity of sucrose present in a particular plant tissue will be essentially related to, or largely controlled by the nutritional, developmental and/or environmental statuses that will allow specific transcriptional-translational expressions of SPS and SPP genes (Winter and Huber, 2000). Although several SPS and SPP isozymes have been isolated and/or cloned from various plants tissues (Lunn, 2003; Lunn and MacRae, 2003; Salerno and Curatti, 2003, Lutfiyya et al., 2007), however limited information is available about their expression, localization, function, regulation and mechanism of action during environmental stress. Studies have shown occurrence of evolutionary divergence between monocot and dicot SPS and SPP isozymes sequences, and was proposed that the several gene duplication that lead to creation of many isozymes could have developed at evolution before/after the monocot/dicot divergence, or after speciation in individual monocot or dicot plant (Lunn, 2003; Lutfiyya et al., 2007).

Cereals (such as maize, rice and sorghum) are of great agronomic importance, they represent one of the major carbohydrate foods for humans and as raw materials for industrial processes. Thus, understanding sucrose production that will consequently lead to carbohydrate accumulation in the grains of these plants will have great agricultural and economic benefits, most importantly during environment stress conditions, which they are regularly subjected to.

Transcription remains the most fundamental aspect for expression and regulation of genes for cellular functions and development in all living organisms (Jeffery, et al., 2007). Large portions of DNA sequence of genes are invested in the untranslated regions (about 1000 - 1500 base pairs upstream; called the promoter/regulatory regions), and are responsible for the coordinate expression, function, and regulation of the adjoining translated DNA regions (Qui, 2003; Wyeth and Albin, 2004; Rani, 2007). These untranslated regions are made up of short but specific DNA sequence (approximately 5 – 25 base pairs) called *cis*-acting regulatory element or the transcription factor binding site (TFBs) (Rani, 2007).

Often time's external cell stimulus activates a signal transduction cascade system that involves the recruitment of transcription factors (TFs), RNA polymerase and other factors binding together at specific *cis*-acting regulatory elements to activate or suppress gene transcription in response to development or change in environment status. *Cis*-acting regulatory elements are important components of the transcription network as they primarily determine the temporal and spatial expression of genes (Singh, et al., 2002; Qui, 2003; Wyeth and Albin, 2004; Rani, 2007). Therefore, knowledge of the *cis*-acting regulatory elements of a gene bound by TFs encoded in a genome can provide the information essential to build models for

Table 1. Shows accession numbers of SPS and SPP used for computational analyzes

Plant	Abbreviation	Accession No SPS	Accession No SPP
Maize (Zea mays)	Zm	**ZmSPS1** [NP_001105694.1, GRMZM5G875238; Gardiner et al., 2004; Worrell et al., 1991], **ZmSPS1*** [AFW61928.1, GRMZM2G055331; Schnable et al., 2009], **ZmSPS2** [AFW61929.1, GRMZM2G055331; Schnable et al., 2009], **ZmSPS3** [AFW61930.1, GRMZM2G055331; Schnable et al., 2009], **ZmSPS$^{\alpha}$** [ACG39958.1, GRMZM2G055489; Alexandrov et al., 2009], **ZmSPS$^{\beta}$** [ACG45751.1, GRMZM2G097641; Alexandrov et al., 2009]	**ZmSPP1$^{\alpha}$** [NP_001105006.1, GRMZM2G055489; Lunn 2003], **ZmSPP1** [AFW82209.1, GRMZM2G055489; Schnable et al., 2009], **ZmSPP2** [NP_001105652.1, GRMZM2G097641; Soderlund et al., 2009; Lunn, 2003]
Rice (Oryza sativa)	Os	**OsSPS1** [BAD87626.1, Loc_Os01g69030; Sasaki et al., 2002], **OsSPS3** [Q67WN8.1|SPSA3_ORYSJ, Loc_Os06g43630; IRGSP, 2005; Tanaka et al., 2008; Okamura et al., 2011], **OsSPS4** [Q6ZHZ1.1|SPSA4_ORYSJ, Loc_Os08g20660; Nagaki et al., 2004; IRGSP, 2005; Tanaka et al., 2008; Kikuchi et al., 2003; Lutfiyya et al., 2007; Okamura et al., 2011], **OsSPS5** [Q53JI9.1|SPSA5_ORYSJ, Loc_Os11g12810; IRGSP 2005; Rice Chromosome 11 & 12 Sequencing Consortia, 2005; Tanaka et al., 2008; Okamura et al., 2011]	**OsSPP1**-Japonica [AAT93996.1, Loc_Os05g05270; Chow, 2004], **OsSPP1** [Q94E75.1|SPP1_ORYSJ, Loc_Os01g27880; Sasaki et al., 2002; Lunn 2003; Kikuchi et al., 2003; Yu et al., 2005; Tanaka et al., 2008], **OsSPP2** [BAD13232.1, Q6YXW6.1|SPP2_ORYSJ, Loc_Os02g05030; Sasaki et al., 2001, IRGSP, 2005], **OsSPP3** [A3AZW5.1|SPP3_ORYSJ, Loc_Os05g05270; Lunn 2003; Cheng et al., 2005; IRGSP 2005; Yu et al., 2005; Tanaka et al., 2008]
Sorghum (sorghum bicolor)	Sb	**SbSPS$^{\alpha}$** [ACX94229.1, Sb04g005720; Liu et al., 2009], **SbSPS$^{\beta}$** [EES19952.1, Sb09g028570; Patterson et al., 2009], **SbSPS$^{\gamma}$** [EES08281.1, Sb05g007310; Patterson et al., 2009], **SbSPS$^{\delta}$** [EES04111, Sb03g043900; Patterson et al., 2009], **SbSPS$^{\varepsilon}$** [EER92301.1, Sb01g035890; Patterson et al., 2009], **SbSPS$^{\eta}$** [XP_002465161.1, Sb01g033060; Patterson et al., 2009], **SbSPS$^{\theta}$** [EER90105.1, Sb10g025240; Patterson et al., 2009], **SbSPS$^{\kappa}$** [EES06073.1, Sb04g038410; Patterson et al., 2009]	**SbSPP** [ACA28706.1, Sb04g020180; Dwivedi et al., 2008]

transcriptional regulatory networks.

Presently, study on the transcription factors/*cis*-acting regulatory elements that are involved in the expression/regulation of SPS and SPP isozymes during abiotic or biotic stress is very scarce. It's been shown that some regulatory sites are lacking in some SPS and SPP isozymes, which could be the rationale for having differential expression, functionality and/or response to environmental signals (Lunn, 2003; Lutfiyya et al., 2007).

Thus, our aim was to use existing bioinformatics tools to comparatively analyze and reveal the nature and frequencies of essential *cis*-acting regulatory elements that are present within the 5' untranslated (5'UTR) DNA sequence (as a source of promoter region) of SPS and SPP isozymes of maize (*Zea mays*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*) in a bid to potentially understand and predict their transcriptional expressions and regulatory interactions during normal plant development and/or under environmental stress. We also use their genomic and amino acids sequences to predict their structural/evolutionary relationships.

We believe this study will aid in identifying the combinatorial *cis*-acting regulatory elements and the probable transcription factors that may be involved in the spatial and temporal expressions and/or regulation of these sucrose synthesizing genes and their structural/ evolutionary relationships. We propose this will provide additional insight into their structure-function relationship, the developmental/environmental factor that could turn on/off the gene expression, which could assist in deducing other specific function(s) in addition to sucrose synthesis. In future this information may help in genetic processes in plant breeding programmes for the development of hybrid varieties that have higher yields with tolerance to environmental stress.

RESULTS

Table 1, shows the accension numbers of SPS and SPP isozymes as obtained from Entrez Protein Sequence database on NCBI website. They were used in obtaining the amino acid, genomic and 1.5kb upstream regulatory sequences from Phytozome v 9.1, and these sequences

Table 2. Cis-acting regulatory elements identified within the 5' regulatory region of SPS and SPP isozymes of maize, rice and sorghum. PLACE, Plant CARE, Plant Pan and Genomatix Matinspector professional databases were used to predict and analyze the cis-acting regulatory elements present within 1.5 kb upstream of 5' regulatory region.

Cis-acting elements	Sequence	Main function involved in	Specific function involved in	References
CAAT	CAAT	Core promoter elements	Essential for the recruitment of RNA polymerase II correctly to Transcription start site	Smale and Baltimore, 1989; Dean and Schmidt, 1995
CCAAT	CCAAT			
InR	YTCNTYY			
TATA	TATA; TATAAAT; TATTAAT; TATATAA; TTATTT; TATTTAA			
-10PEHVPSBD	TATTCT	Cellular development	Involved in the expression of the plastid gene expression; circadian rhythms; light regulation	Thum, et.al, (2001)
CGCGBOXAT	VCGCGB		A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signaling pathways in plants	Yang et al., (2002)
CIACADIANLELHC	CAANNNNATC		Region necessary for circadian expression of gene	Piechulla et al., (1998)
DOFCOREZM	AAAG		Binds Dof1 and Dof2 transcription factors, which are associated with expression of multiple genes involved in carbon metabolism	Yanagisawa et al., (2000)
GTGANTG10	GTGA		Found in the promoter of genes required for late pollination	
Pyrimidine box	TTTTTTCC; CCTTTT		Partially involved in sugar repression. It requires Gibberellin for its induction	Washio (2003)
RY-motif	CATGCA; CATGCAT; CATGCAY; CATGCATG		Cis-acting regulatory element involved in seed-specific regulation	Ezcurra et al. (1999); Ezcurra et al. (2000)
SREATMSD	TTATCC		Sugar Repressive Element (SRE) found in genes involved in sugar repression	Tatematsu et al., (2005)
ARF (AuRE)	GGTCCAT; TGTCTC	Hormonal regulation	Cis-acting regulatory element involved in early auxin responsiveness	Ulmasov et al. (1999); Hagen and Guilfoyle (2002); Goda et al., (2004)
ABRE	(C/A)ACG(T/C)G(T/C/G)		Abscisic acid responsive element; a cis-acting regulatory element involved in abscisic acid responsiveness	Kaplan et al. (2006)
ABRELATERD1	ACGTG		ABRE-like sequence required for etiolation-induced expression of erd1 (early response to dehydration)	Nakashima, et al., (2006)
DPBFCOREDCDC3	ACACNNG		Required in genes induced by or responsive to Abscisic acid	Kim et al., (1997)
ERE	A(A/T)TTCAAA		Ethylene responsive element, involved in senescence	Itzhaki et al. (1994); Lin et al. (2007)
GARE	TAACAA(G/A); TAACGTA; CAACTC		Gibberellin Responsive Element; a cis-acting regulatory element involved in Gibberellin responsiveness and also partially involved in sugar repression	Gubler and Jacobsen (1992); Ogawa et al. (2003); Sutoh et al., (2003)
NTBBF1ARROLB	ACTTTA		Required for tissue-specific expression and auxin induction.	Baumann et al., (1999)
T/GBOXATPIN2	AACGTG; CGTCA; TGACG		Involved in jasmonate (JA) responsiveness	Kim et al. (1993); Rouster et al. (1997); Boter et al., (2004)

Table 2. Continue

DRE; CBFHV	A/GCCGAC; RYCGAC	Abiotic stress	Salt/dehydration responsive element	Dubouzet et al. (2003); Diaz-Martin et al. (2005); Svensson et al., (2003)
Erd1	ACGTG; ACGT		Cis-acting regulatory element required for early response to dehydration (etiolation-induced expression)	Simpson et al. (2003)
GATA; I-BOX	GATA; GATAA; GATAAGR; GATAAG		Cis-acting regulatory element conserved in sequence upstream of high level light-regulated and tissue specific expression	Lam and Chua (1989); Hiratsuka and Chua (1997); López-Ochoa et al. (2007)
LTRE	ACCGACA; CCGAAA; GTCGAC		Low Temperature Response Element; a cis-acting regulatory element required in cold inducible gene expression	Nordin et al. (1993); Baker et al., 1994; Brown et al. (2001); Dunn et al. (1998)
MYB	WAACCA; TAACTG; CNGTTR; YAACKG; GGATA; CAACTG; CCWACC		Cis-acting regulatory element involved in regulation of drought inducible gene expression	Shinozaki and Yamaguchi-Shinozaki (2000); Abe et al. (2003)
MYC	CATGTG; CACATG; CANNTG		Cis-acting regulatory element involved in early response to drought and abscisic acid induction	Shinozaki and Yamaguchi-Shinozaki (2000); Abe et al. (2003)
SORLIP	GCCAC ; GGGCC		Cis-acting regulatory element over- represented in promoter of light-induced gene	Hudson and Quail, (2003)
BIHD1OS	TGTCA	Biotic stress	Cis-acting regulatory element required in disease resistance responses	Luo et al., (2005)
ELRE	TTCGACC		Elicitor Responsive Element; a cis-acting regulatory element essential for elicitors responsiveness	Rushton et al. (1996); Fukuda (1997)
GCC-Box	GCCGCC		Cis-acting regulatory element found in many pathogen-responsive genes such as PDF1.2, Thi2.1 and PR4. Also found to function as ethylene-responsive element.	Sessa et al. (1995); Brown et al. (2003)
GT-1	GAAAAA; GGTTAA; GRWAAW		Cis-acting regulatory element required for rapid response to pathogen attack, salinity and salicylic acid inducible gene expression	Park et al. (2004)
W-box	TTGAC; TGACT; TGACY; TGAC TTTGACY CTGACY		Cis-acting regulatory element involved in direct fungal elicitor stimulated transcription of defense genes and activation of genes involved in response to wounding	Maleck et al. (2000); Eulgem et al. (2000); Chen et al. (2002); Yamamoto et al. (2004); Zheng et al. (2006); Ross et al. (2007)
CuRE	GTAC		Copper responsive element and also involved in oxygen response	Quinn et al. (2000)
SuRE	AATACTAAT; GAGAC		Sulphur responsive element	Maruyama-Nakashita et al. (2005)

were saved in Fasta format (Supplementary; Data 1) and were subsequently used for the computational analyzes.

Cis-acting regulatory elements analyzes

Using the Genomatix Matinspector professional, PLACE, Plant Pan and Plant CARE many essential cis-acting regulatory elements that are associated with core promoter activity, plant

growth and development, hormonal regulation and environmental stress responses were identified. The names and function of the elements generally found in all of the SPS and SPP isozymes are as contained in Table 2. The spatial localization and frequencies of occurrence of these elements within the 1.5kb upstream region are presented in Supplementary; Data 2 and Figure 2, respectively for SPS isozymes, and in Supplementary; Data 3 and Figure 3, respectively for SPP isozymes. In Figures 2 and 3, the elements are grouped and represented in frequencies according to their general function and /or response. Overall assessment of these groups shows that the cumulative frequency of hormonal regulation associated cis-acting regulatory elements were generally found to be very low in all the isozymes of SPS and SPP, while the abiotic elements were found at a very high cumulative frequencies in all the isozymes of SPS and SPP (Figure 4).

Although the frequencies of TATA were found to be high in some of the maize, rice and sorghum SPS isozymes, overall the CAAT element was present at higher frequencies in all the SPS isozymes (Figure 2A). InR and CCAAT frequencies were however found to be very low compared to CAAT and TATA elements. In SPP isozymes, CAAT was the only core element found to be at high frequencies, with the exception of ZmSPP2 where the TATA element was found at comparable frequency with CAAT (Figure 2A). Similar to SPS, InR and CCAAT elements were found at very low frequencies in SPP.

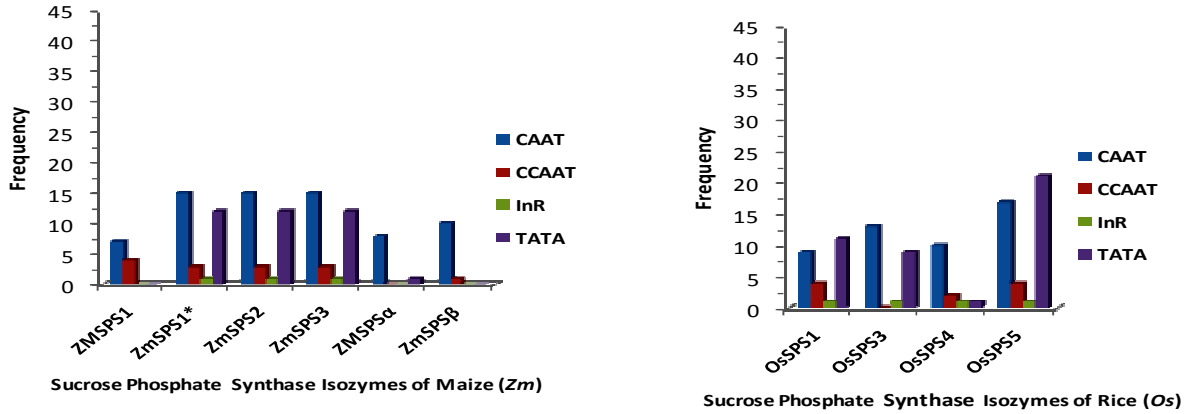
CGCG BOXAT was found to be at high frequencies in ZmSPS1, OsSPS4 and SbSPSδ for SPS isozyme and ZmSPP1α and ZmSPP1 for SPP isozyme, when compared to their counterpart isozymes (Figures 2B and 3B). Of worth noting is the Dof COREZM element that was present at very high amount in all of maize, rice and sorghum SPS isozymes, however it was more evident in SPP isozymes (Figures 2B and 3B). RY-motif was found to be present at substantial amount only in ZmSPS1, OsSPS1, SbSPSα, SbSPSβ and SbSPSθ, its presence in SPP was found to be at very low frequency. GTGANTG10 was found to be at low frequencies in maize and rice SPS isozymes with the exception of ZmSPSα and OsSPS3, where it is present at considerable amount. However it was found at appreciable amount in virtually all of sorghum SPS isozymes, with the exception of SbSPSη and SbSPSκ (Figure 2B). However in SPP isozymes, it was found to be at appreciable amount, only in OsSPP1-Japonica and OsSPP3 was it found at low amount (Figure 3B). Pyrimidine Box was present at a very low frequency in all of maize, rice and sorghum SPS and SPP isozymes, with the exception of SbSPP that has considerable amount (Figures 2B and 3B). -10PEHVPSBD, CIACADIANLEHC and SREATMSD elements were all found at very low frequencies in all of maize, rice and sorghum SPS and SPP isozymes (Figures 2B and 3B).

In general, the frequencies of the hormonal responsive elements were very low, however some SPS and SPP isozymes do have considerable amount (Figures 2C and 3C). ABRE was found to be in a considerable frequency only in ZmSPS1, OsSPS1, OsSPS4, SbSPSδ, ZmSPP1α, ZmSPP1 and SbSPP. ABRELATERD1 was present in appreciable amount only in ZmSPS1, ZmSPSβ, OsSPS1, OsSPS4, SbSPSθ, ZmSPP1α, ZmSPP1, ZmSPP2, OsSPP3 and SbSPP. DPBFCOREDCDC3 was found in appreciable only in ZmSPSα, OsSPS1, OsSPS3, SbSPSα, ZmSPP1α and ZmSPP1. GARE was noticeable however at low frequencies in ZmSPS1, ZmSPSβ, OsSPS1, SbSPSγ, SbSPSε, ZmSPP2 and OsSPP1. In all the SPS and SPP isozymes, ARF, T/GBOXATPIN2, NTBBF1ARROLB and ERE were found at very low frequencies. However, NTBBF1ARROLB was found only at considerable amount in ZmSPP2, OsSPP1-Japonica and OsSPP3, while ERE was found only at considerable amount in ZmSPP2.

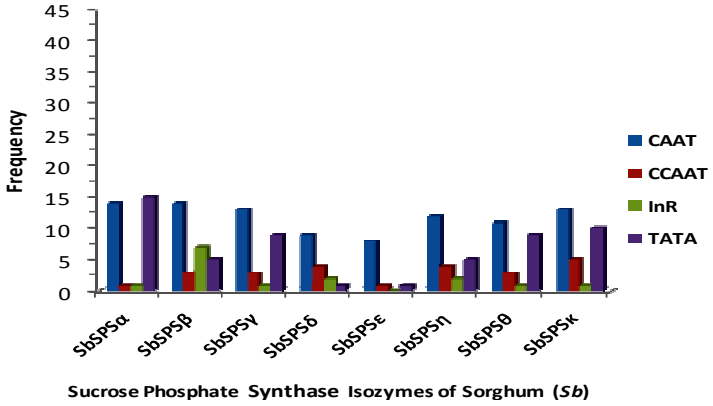
In all the SPS and SPP isozymes, abiotic stress responsive elements were found at high frequencies. Of most prominent are the GATA; I-BOX, MYB and MYC were found at high frequencies (Figures 2D and 3D). Erd1 was found to be at high frequencies only in ZmSPS1, OsSPS1, OsSPS4, SbSPSγ and SbSPSδ, while it was present at low frequencies in the rest isozymes (Figures 2D and 3D). LTRE was present at noticeable frequencies only in ZmSPSα, OsSPS4, SbSPSα, SbSPSγ, SbSPSδ, SbSPSε, ZmSPP1α and ZmSPP1. SORLIP was found at high frequencies only in OsSPS1, OsSPS4, SbSPSδ, SbSPSη and SbSPP, it was however present at low frequencies in the rest SPS and SPP isozymes (Figures 2D and 3D). DRE; CBFHV was present at very low frequencies in SPS and SPP isozymes, however considerable amount was only present in ZmSPSβ, OsSPS1, OsSPS4, SbSPSγ, SbSPSκ, ZmSPP1α and ZmSPP1.

Of the biotic responsive elements, GT1 and W-BOX were found to be at high frequencies in all the SPS and SPP isozymes, with exception of ZmSPS1 that has very low frequency of W-BOX. CuRE element was found at appreciable frequencies in all the SPS and SPP isozymes (some high, while some are low), with exception of ZmSPP1α and ZmSPP1 which have very low frequencies (Figures 2E and 3E). GCC-Box was found at high frequencies only in OsSPS4, SbSPSδ, SbSPSκ, OsSPP1-Japonic and OsSPP3, while it was either at low frequencies or completely absent in the rest SPS and SPP isozymes. OsSPS1, SbSPSα, SbSPSγ, SbSPSε, SbSPSη, SbSPSθ, OsSPP1-Japonica, OsSPP1 and OsSPP2 all have considerable frequencies of BIHD1OS (Figures 2E and 3E). EIRE and SuRE elements are either present in low frequencies or completely absent in the SPS and SPP isozymes (Figures 2E and 3E).

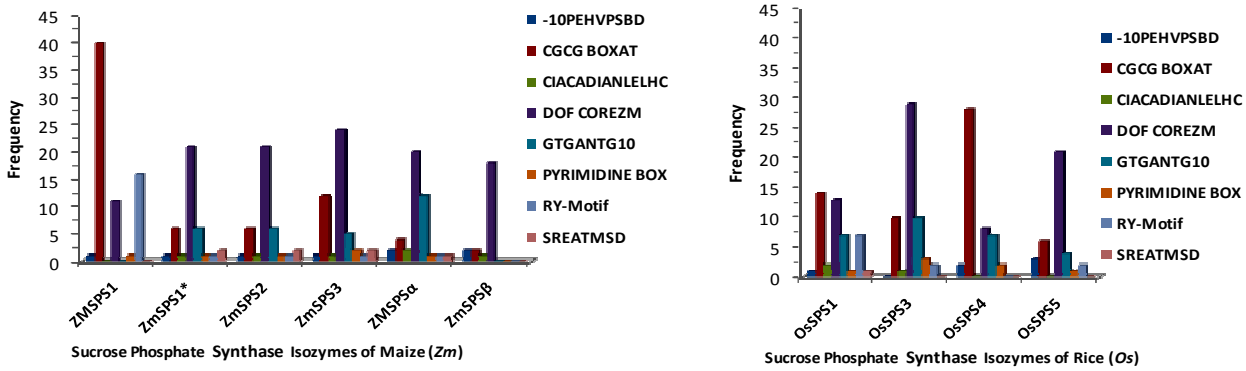
Core Promoter Elements



(A)



Cellular Development Responsive Elements



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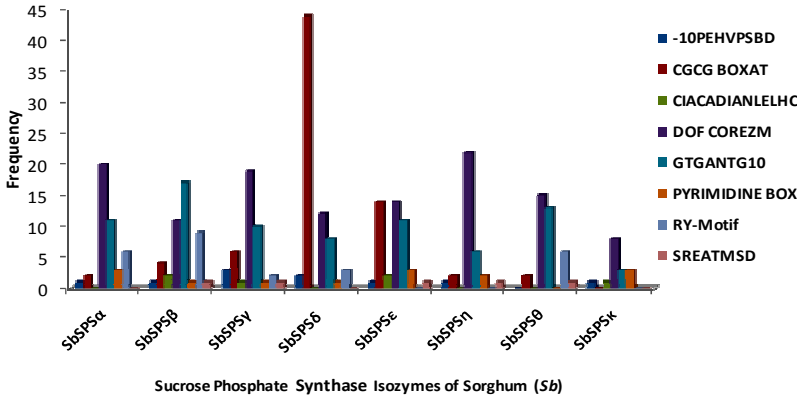
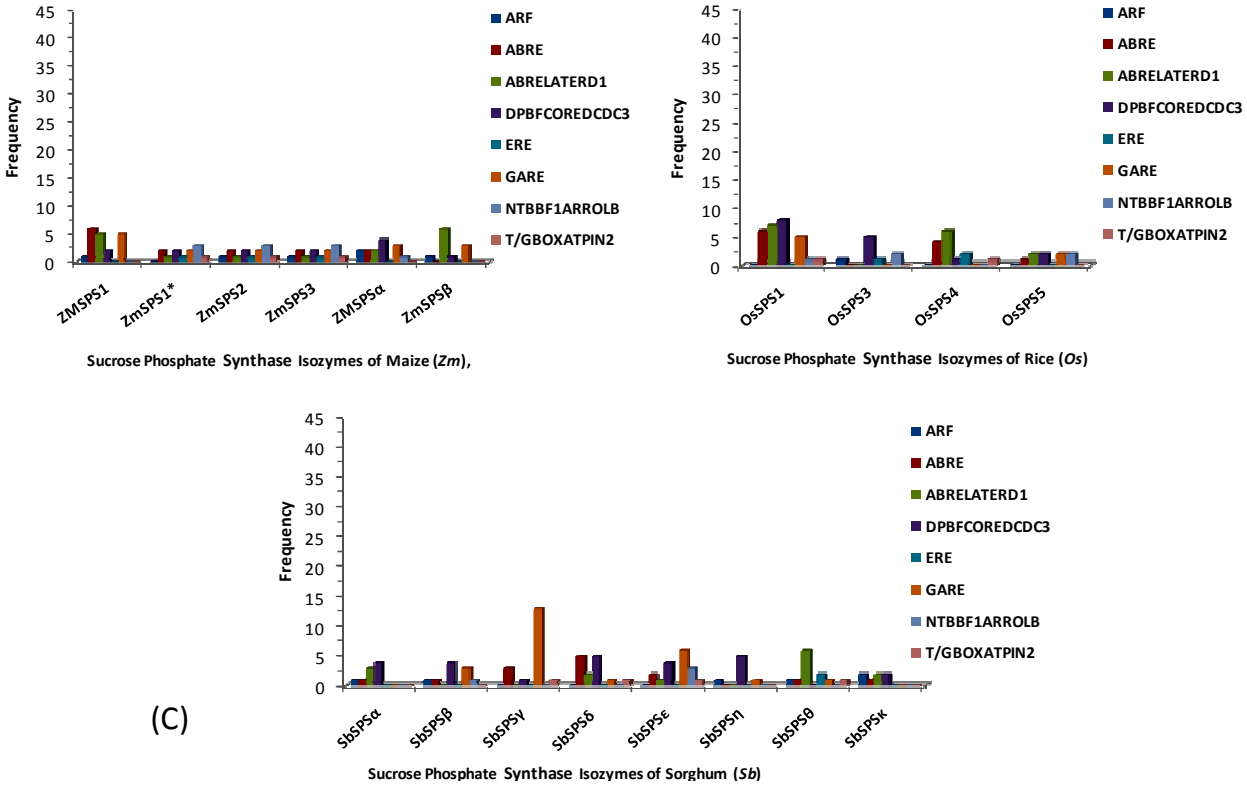
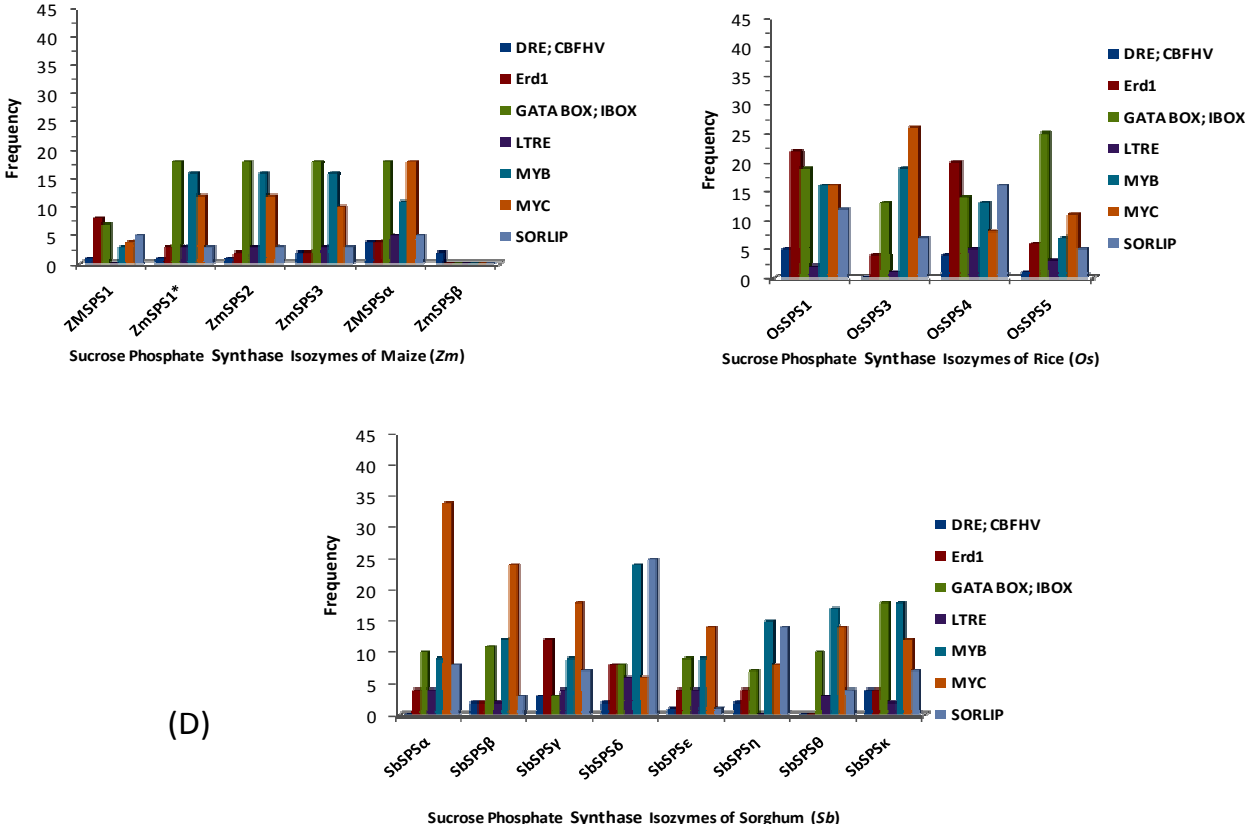


Hormonal Responsive Element



(C)

Abiotic Stress Responsive Elements



(D)

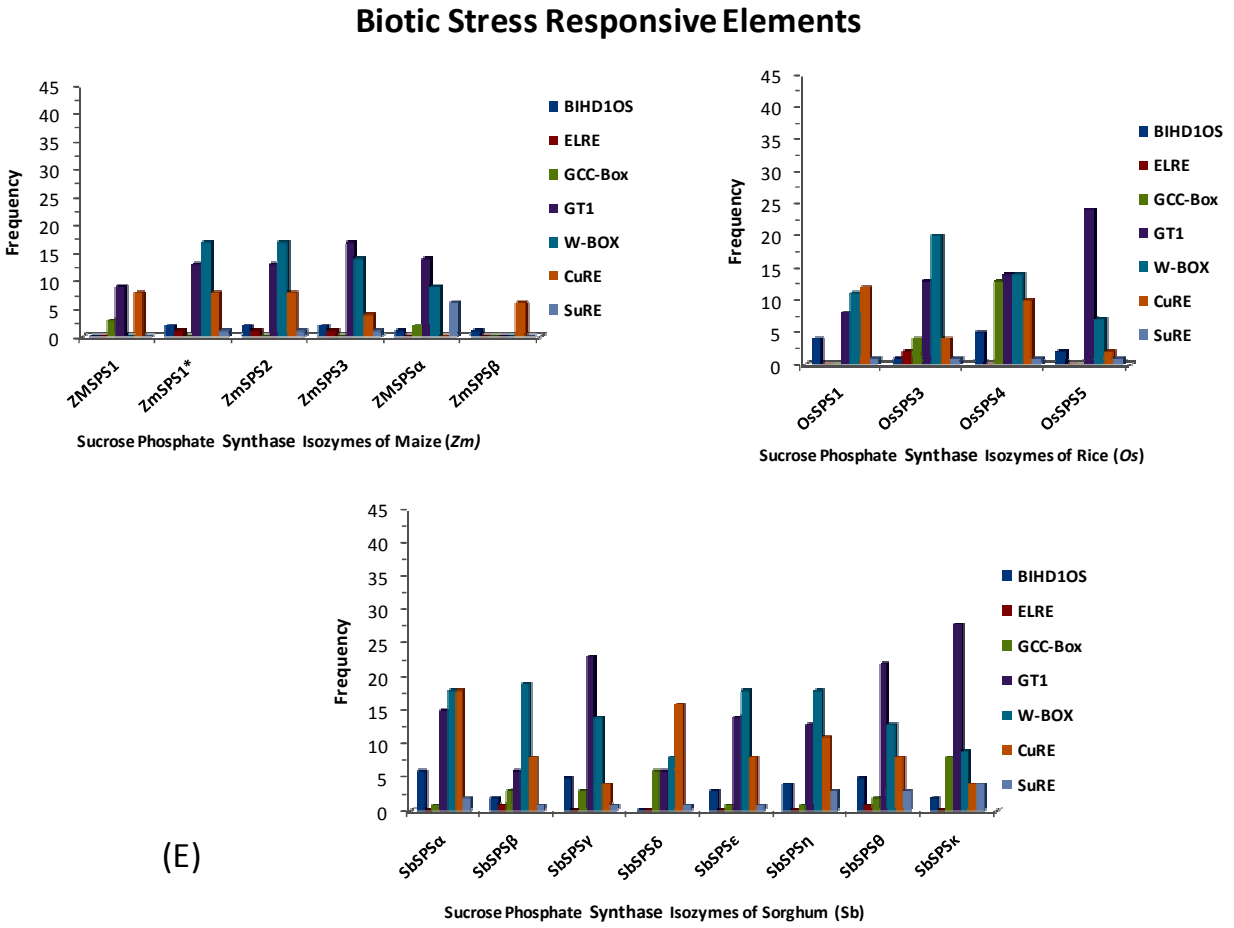
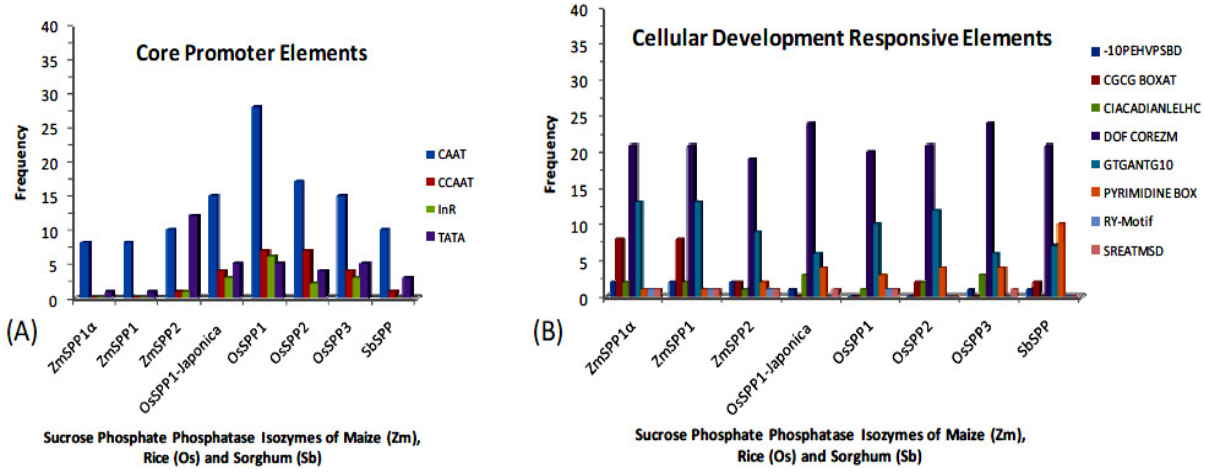


Figure 2. Illustration of the frequencies of the cis-acting regulatory elements identified within the regulatory regions of Sucrose Phosphate Synthase (SPS) isozymes of maize (*Zea mays*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*). Where (A), (B), (C), (D) and (E) in the Figure represent, core promoter elements, cellular development responsive elements, hormonal responsive elements, abiotic stress responsive elements and biotic stress responsive elements, respectively.



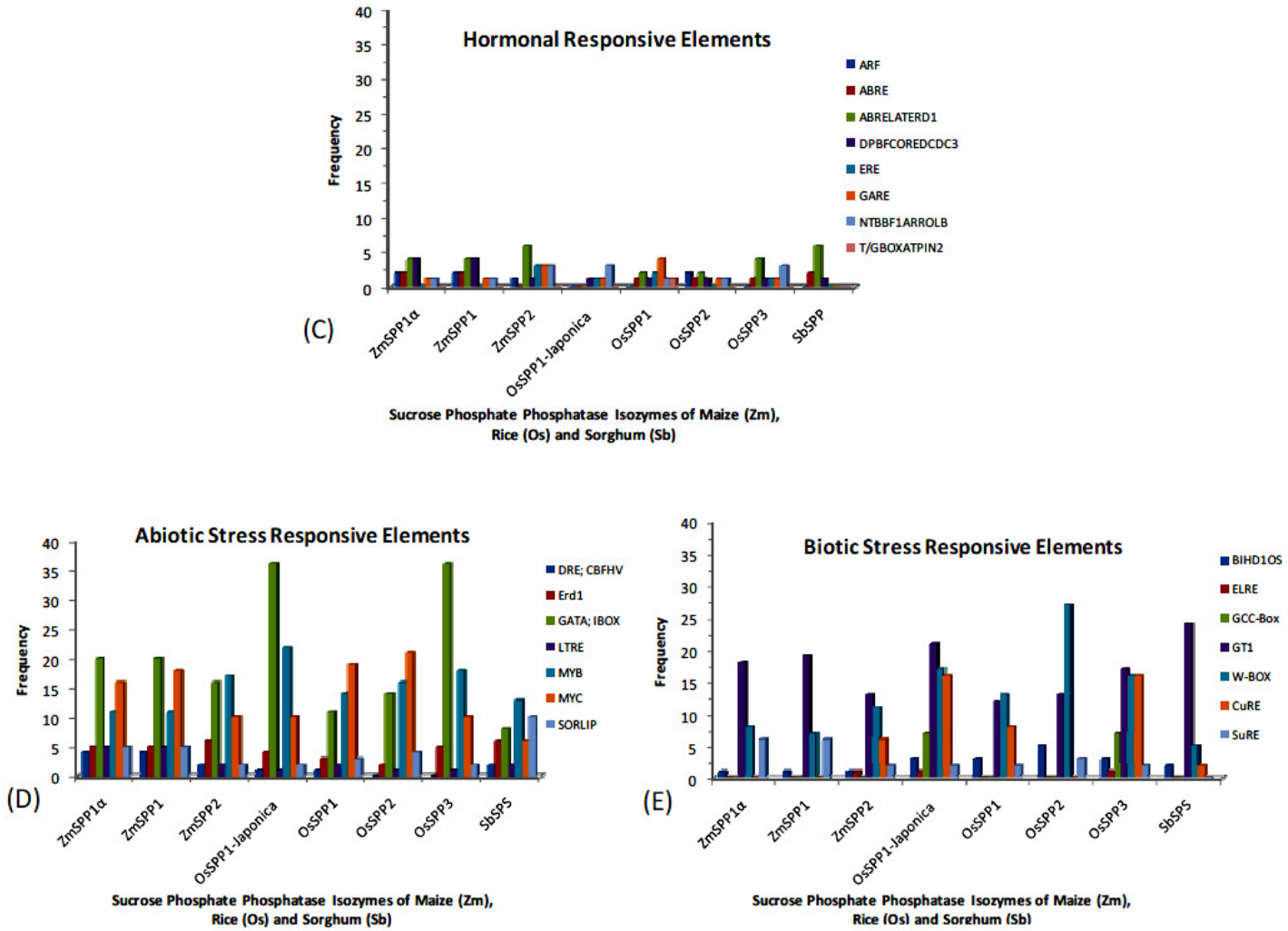


Figure 3. Illustration of the frequencies of the cis-acting regulatory elements identified within the regulatory regions of Sucrose Phosphate Phosphatase (SPP) isozymes of maize (*Zea mays*), rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*). Where (A), (B), (C), (D) and (E) in the Figure represent, core promoter elements, cellular development responsive elements, hormonal responsive elements, abiotic stress responsive elements and biotic stress responsive elements, respectively.

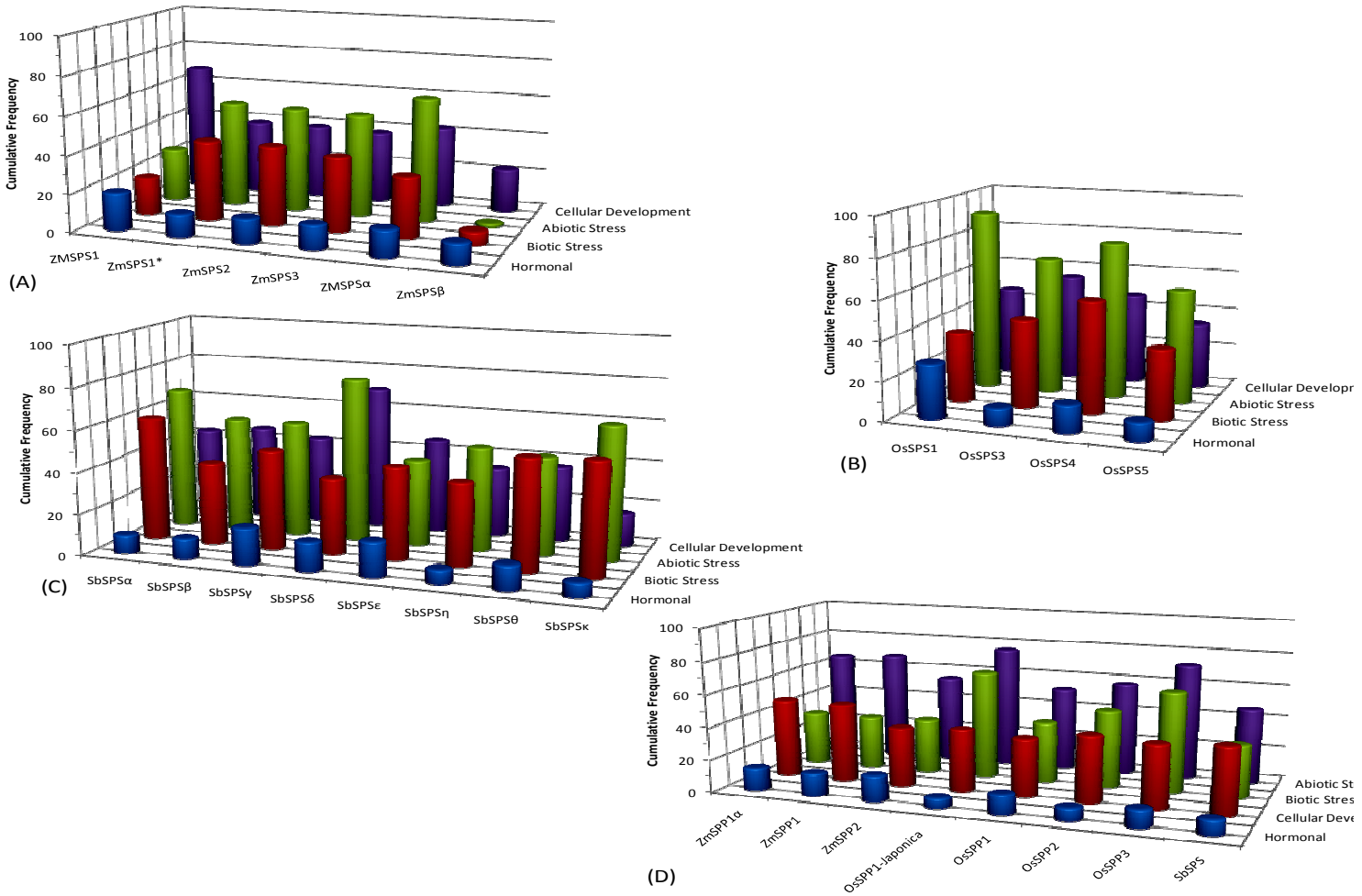


Figure 4. Cumulative frequencies of the cis acting regulatory elements identified within the regulatory regions of Sucrose Phosphate Synthase (SPS) and Sucrose Phosphate Phosphatase (SPP) isozymes of maize (*Zm*), rice (*Os*) and sorghum (*Sb*). Where (A), (B) and (C) are cumulative frequencies for maize (*Zm*), rice (*Os*) and sorghum (*Sb*) Sucrose Phosphate Synthase (SPS) isozymes, respectively. (D) shows cumulative frequencies for maize (*Zm*), rice (*Os*) and sorghum (*Sb*) Sucrose Phosphate Phosphatase (SPP) isozymes.

Analyzes of Structural/Evolutionary Relationship

Analyzes of the 5' untranslated (5'UTR) DNA sequences of maize, rice and sorghum SPS and SPP isozymes, using Clustal Omega: EMBL-EBI, Multiple Sequence Alignment program is presented in Supplementary; Data 4. It was revealed that these regions were well conserved in some isozymes while it was diverse in some. Table 3 illustrates the range of homology within these regions as expressed in percent between the different groups of maize, rice or sorghum SPS and SPP isozymes.

Table 3. Range of 5' upstream regulatory region sequence homology expressed in percent between the groups of maize, rice or sorghum SPS and SPP isozymes.

Isozyme [A]	Isozyme [B]	Range of Homology (%)	Isozyme [A]	Isozyme [B]	Range of Homology (%)
ZmSPS	ZmSPS	28 – 100	SbSPS	SbSPS	29 - 50
	OsSPS	28 – 46		ZmSPP	28 – 46
	SbSPS	29 – 64		OsSPP	27 – 46
	ZmSPP	28 – 100		SbSPP	28 – 33
	OsSPP	28 – 47	ZmSPP	ZmSPP	31 – 100
	SbSPP	28 – 34		OsSPP	31 – 35
OsSPS	OsSPS	25 – 34		SbSPP	26 – 28
	SbSPS	26 – 41	OsSPP	OsSPP	33 - 100
	ZmSPP	29 – 37		SbSPP	27 – 32
	OsSPP	25 – 48			
	SbSPP	27 - 32			

Table 4. Range of genomic sequence homology expressed in percent between the groups of maize, rice or sorghum SPS and SPP isozymes.

Isozyme [A]	Isozyme [B]	Range of Homology (%)	Isozyme [A]	Isozyme [B]	Range of Homology (%)
ZmSPS	ZmSPS	40 – 100	SbSPS	SbSPS	34 - 59
	OsSPS	33 – 66		ZmSPP	31 – 39
	SbSPS	31 – 81		OsSPP	29 – 100
	ZmSPP	33 – 100		SbSPP	31 – 37
	OsSPP	33 – 56	ZmSPP	ZmSPP	50 – 100
	SbSPP	34 – 73		OsSPP	48 – 56
OsSPS	OsSPS	44 – 55		SbSPP	56 – 73
	SbSPS	34 – 66	OsSPP	OsSPP	36 - 59
	ZmSPP	34 – 39		SbSPP	51 – 60
	OsSPP	34 – 37			
	SbSPP	31 - 39			

Table 5. Range of amino acid sequence homology expressed in percent between the groups of maize, rice or sorghum SPS and SPP isozymes.

Isozyme [A]	Isozyme [B]	Range of Homology (%)	Isozyme [A]	Isozyme [B]	Range of Homology (%)
ZmSPS	ZmSPS	19 – 63	SbSPS	SbSPS	14 - 84
	OsSPS	17 – 86		ZmSPP	0 – 25
	SbSPS	0 – 94		OsSPP	0 – 23
	ZmSPP	19 – 99		SbSPP	0 – 23
	OsSPP	18 – 85	ZmSPP	ZmSPP	63 – 99
	SbSPP	19 – 96		OsSPP	71 – 85
OsSPS	OsSPS	49 – 65		SbSPP	65 – 96
	SbSPS	22 – 89	OsSPP	OsSPP	71 - 93
	ZmSPP	17 – 24		SbSPP	68 – 88
	OsSPP	16 – 22			
	SbSPP	16 - 21			

Furthermore, using same Clustal Omega: EMBL-EBI, Multiple Sequence Alignment program, the result of alignment of genomic and protein sequences of maize, rice and sorghum SPS and SPP isozymes, is as presented in Supplementary Data; 5. Although there appear to be very high conservation in the genomic and protein sequences of some isozyme to each other, some are however also very diverse. Table 4 and 5 show the range of genomic and amino acid sequences homology expressed in percent between the groups of maize, rice

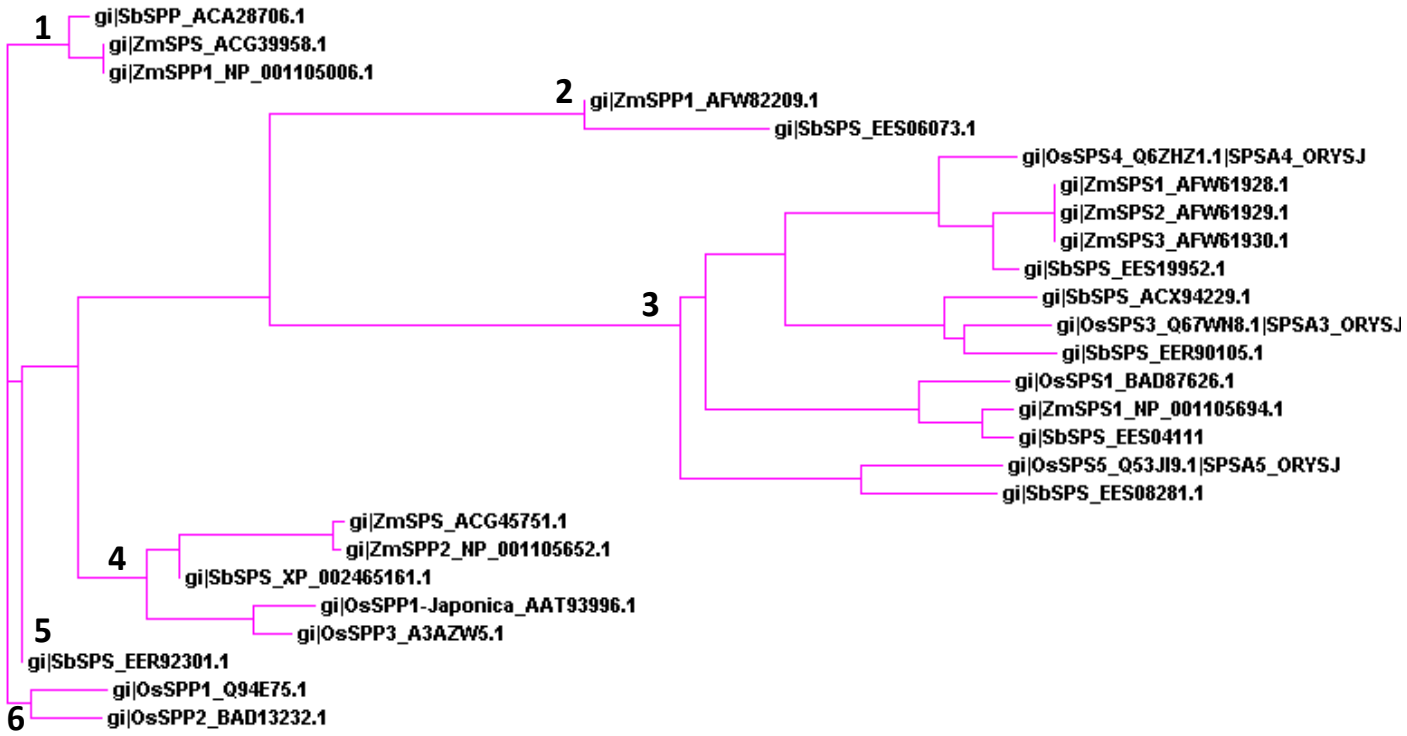


Figure 5. The phylogenetic tree for 18 SPS and 8 SPP isozymes of maize, rice or sorghum obtained from Constraints Based Protein Multiple Alignment Tool (COBALT) using their protein sequences. The SPS and SPP isozymes accession numbers are as described in Table 1.

or sorghum SPS and SPP isozymes.

The coding genomic region of the SPP isozymes appears to be a bit more conserved within the maize, rice and sorghum with a range of 36 – 100 % than the SPS, which has a range of 31 – 100 %. From Table 4, it may be deduced that the SPP isozymes of maize, rice and sorghum are more closely related than the SPS isozymes in terms of their structural and evolutionary origin. High ranges of percent homology of 63 – 99 exist within the SPP isozymes group, while 0 – 94 % homology exists within SPS isozymes group. ZmSPS isozymes showed high homology of 19 – 99% with ZmSPP, OsSPP and SbSPP, as compare to OsSPS and SbSPS isozymes that showed low homology of 16 – 24% and 0 – 25%, respectively.

Figure 5 shows the phylogenetic tree for 18 SPS and 8 SPP isozymes of maize, rice or sorghum obtained from Constrainst Based Protein Multiple Alignment Tool (COBALT) using their protein sequences. The inter-evolutionary relationship between the SPS and SPP isozymes is as revealed by the distance/length of the bars. In the phylogenetic tree, the 18 SPS and 8 SPP isozymes fall into 6 distinct independent groups. From the Figure it may be deduced that there are tight inter-structural and evolutionary relationship between the SPS and SPP isozymes with the exception of group 3 and 6 that are predominantly made of SPS and SPP isozymes, respectively.

DISCUSSION

Sucrose plays an essential role in plant metabolism, among which are; it's the main assimilate transported from source organs to sites of utilization, acts as storage reserve for carbon and energy and as a signal compound (Smeekens, 2000). Sucrose phosphate synthase (SPS) and Sucrose phosphate phosphatase (SPP) are key enzymes in the sucrose biosynthesis-related proteins (SBRPs) and they have been linked to quantitative trait loci that control plant growth and yield (Causse et al., 1995a; Causse et al., 1995b; Prioul et al., 1999; Winter and Huber, 2000; Ishimaru et al., 2003). SPS was predicted to have evolved from gene fusion of glucosyl-transferase domain (GTD)-like and a phosphohydrolase domain (PHD)-like primordial domains which are present in all organisms of all kingdoms, while SPP was thought to evolve from the duplications of PHD and GTD during endosymbiotic chloroplast organism diversification at the time of the cyanobacterial phylogenetic radiation to plant (Giovannoni, et al., 1988; Cumino et al., 2002). This assumption is well supported by the fact that plant SBRPs share similar homology in protein phylogeny with the corresponding cyanobacterial protein phylogeny (Cumino et al., 2002, Salerno and Curatti, 2003). The expansion or presence of multiple isozymes of SPS and SPP was thought to have arisen from in-dependent gene duplications of the SBRP family in plants

(Cumino et al., 2002, Salerno and Curatti, 2003). Quiet a number of SPS and SPP isozymes are reported in the literatures, however what we reported here are the available maize, rice and sorghum SPS and SPP isozymes with complete genomic and proteomic sequences as hosted in Phytozome v 9.1.

Although 3 groups of SPS and SPP isozymes were previously reported to exist (Langenkämper et al., 2002), 5 different groups were actually reported to be present in monocotyledons (Castleden et al., 2004); where 3 of them were associated as subfamilies in dicotyledons (Langenkämper et al., 2002; Lunn and MacRae, 2003; Castleden et al., 2004). From our result 6 distinct groupings were identified and going by the work of Langenkämper et al. (2002) these groups exist within the B-b2 group of monocotyledon SPS. Our results thus clearly reflect that there will exist many subgroups within those that are already established, as more isozymes are isolated and characterized more subgroups will be emerging, which will share more light into the already existing groupings.

Analyzes of the non-coding regulatory regions of the SPS and SPP isozymes identified numerous putative cis-acting elements that may be involved in the cellular transcriptional expression and regulation of these isozymes. As the transcription of any gene is a well coordinated and regulated network that involves the cooperative action of many effectors / regulators that binds to specific cis-acting elements forming machineries that activates RNA Polymerase II to initiate transcription (Zhu, 1996), analyzing the nature and frequencies of these putative cis-acting elements will assist in understanding the expression and response of such gene to normal cellular activity and/or environmental stimuli. The predicted expression or regulatory network may or not necessary be functional, however *in-silico* analysis is a forerunner that assist in confirmatory *in vitro* experiments to expressional and functional analyzes.

The putative cis-acting elements identified within the translational start site upstream to 1.5kb revealed lots of divergence both in nature and frequencies, within the SPS and SPP isozymes regulatory regions. This well corroborated similar studies that show that isozymes usually lose common cis-acting elements and that the upstream regulatory regions may be well diverse as a result of evolution or gene duplication that may arises from their ancestral parent gene (Qui, 2003; Ibraheem et al., 2010). This likewise gives support to the degenerative complementation model that offers that gene usually keeps a small fragment of the cis-acting elements of their ancestral parent. Thus SPS and SPP isozyme genes having close sequence homology and frequencies of cis-acting elements (Supplementary; Data 4) may have evolved from same parent gene and may therefore have similar transcription factors and effectors that controls them, thus, they may have similar cellular expression and (or) regulatory patterns influenced by environmental factors. Having common cis-acting regulatory elements is one of the main characteristics of co-regulated genes and often times they interact with complex protein machineries, though single cis-element may also interact with these protein machineries, consequently subjecting the gene to multiple regulatory controls (Wang, 2004).

TATA, CAAT, CCAAT and InR motifs the core promoter elements required for basal promoter activity were found at differential amount within the 1.5kb upstream regulatory region; however TATA and CAAT are more prominent in the isozymes than the rest. The core promoter elements of plant genes are known to be very heterogeneous in composition and they influence the activity and respond of the genes to various activators, enhancers and repressors by mediating between them and their target genes. The presence of high frequencies of TATA and CAAT well suggest that SPS and SPP isozymes may well be inducible genes, since TATA motif is very significant in most final stages of environmental signal transduction, unlike the housekeeping genes that have lower diversity in their cis-acting regulatory motifs and may not have an identifiable TATA motif (Dean and Schmidt, 1995).

Going by the high cumulative frequency of cellular development related cis-acting regulatory motifs present at the regulatory regions of the maize, rice and sorghum SPS and SPP isozymes (Figure 4), infers that their expression and involvement in cellular activities may be tightly regulated by the cellular developmental state and function of the cells/tissues where they are localized. Plant hormone related cis-acting regulatory motifs were very low across the SPS and SPP isozymes, however relatively higher amount found in ZmSPS1 of maize, OsSPS1 of rice, SbSPSγ and SbSPSε of sorghum, may well show that these isozymes are most likely to be involved in cascades of metabolic actions that may involved plant hormone participation and (or) regulation. With the exception of only ZmSPSβ that has very low cumulative frequency of abiotic stress related cis-acting regulatory motifs, all the maize, rice and sorghum SPS and SPP isozymes have high cumulative frequencies (Figure 4). This trend was found similarly in the biotic stress related cis-acting regulatory motifs where relatively high cumulative frequencies were found with the exception of ZmSPSβ (Figure 4). Thus it may be assumed that the expression and regulation of the cereals; maize, rice and sorghum SPS and SPP isozymes will be tightly linked to environmental factors, with the exception of ZmSPSβ which may only be involved in cellular related functions.

Hormone Responsive Cis-elements

Plant hormones are group of unrelated chemical substances that affect plant morphogenesis; they include auxins, cytokinins, gibberellins, ethylene and abscisic

acid. In multicellular organisms, cell division is normally coordinated with growth and cellular differentiation (Meyerowitz, 1997). Sucrose is a good candidate for modulating cell division rates by its availability to proliferating cells located within the meristems, which reflects on the overall photosynthetic capacity (Koch, 1996). The direct evidence for the role of sucrose in stimulating cell division comes from the response of plant cells in intact or excised plant tissues to sucrose and cell division within a tissue was synchronized by withholding and resupplying sugars (Webster and Henry, 1987).

Diverse cis-acting elements identified within the 5' regulatory region of the SPS and SPP isozymes of the studied monocot crops include:

ABA responsive elements (ABRE, ABRELATERD1 and DPBFCOREDCDC3)

Abscisic acid (ABA) has been known to assist plants in desiccation tolerance in seeds, and its also known to impose growth inhibition in developing seeds and acts in the response of vegetative tissues to water stress (Finkelstein and Gibson, 2002). The accumulation of ABA is an immediate response to water deficit and the processes stimulated by ABA include physiological changes such as: seed development, germination and seedling growth (Finkelstein and Gibson, 2002; Garciarrubio et al., 1997).

ABA responsive elements although found relatively low in all the SPS and SPP isozymes, appreciable amount were found in some isozymes such as ZmSPS1, ZmSPP1, ZmSPP1α, ZmSPP2 in maize; OsSPS1, OsSPS4, and OsSPP3 in rice; SbSPSδ, SbSPSθ and SbSPP in sorghum. It may be infer that these isozymes will play essential roles in cellular activities or cascade mechanisms that may involve ABA and sugar (in form of sucrose), perhaps during dehydration or osmolytic stress that may involve urgent accumulation of solute in a bid to overcome the effect. The ABA responsive elements have been shown to work cooperatively to confer ABA response in plant (Busk and Pages, 1998), thus the differential amount of these elements might add further to this assertion. ABA was shown in Arabidopsis plants to deprive germinating seeds and young seedlings nutrients essential for growth (Garciarrubio et al., 1997), thus the expression of SPS and SPP isozymes may well tightly be regulated by ABA, which may deprive the young tissues carbohydrate (in form of sucrose) required for their development.

Jasmonic acid responsive element (T/GBOXATPIN2)

Jasmonic acid (JA) is a known key regulator of plant responses to pathogens and insects and also plays in the defence system during mechanical wounding and pathogen elicitors (Creelman et al., 1992; Gundlach et al., 1992). JA accumulates within the first hour in response to these external stimuli, thus JA has been used as a marker to response to stress. Furthermore, they have been found to inhibit seed germination, increase leaf senescence and induce accumulation of vegetative storage proteins (Wasternack and Parthier, 1997; Sembdner and Parthier, 1993). Although the frequency of JA responsive element was relatively low in all the SPS and SPP isozymes of maize, rice and sorghum, its presence alone suggests that SPS and SPP isozymes may be involved in the plants responses towards these environmental factors.

Auxin responsive element (ARF, AuRE andNTBBF1ARROLB)

Auxin has been implicated in numerous cellular developmental activities such as cell elongation and differentiation, root formation, apical dominance and tropism by inducing alterations in gene expression of proteins involved in these cellular activities (Hagen et al., 1984; Hagen and Guilfoyle, 2002). Although auxin have been reported to increase sucrose loading in plant decapitated stems and whole shoot (Patrick, 1979) it was found to antagonise active sucrose uptake in detached leaves (Harms et al., 1995). Thus the role of auxin might be either to activate or inhibit gene expression depending on the cellular localization and function of the gene. The relative low frequencies of the auxin responsive elements may point to the fact that SPS and SPP isozymes activity may not necessary be influenced by auxin or the transcription factors that confers auxin responsiveness. However, ZmSPS1*, ZmSPS2, ZMSPS3 and ZmSPP2 in maize; OsSPS3, OsSPP1-Japonica and OsSPP3 in rice; SbSPSε in sorghum do have relatively more than the rest isozymes, they may therefore be the isozymes that may respond to auxin.

Gibberellin responsive element (GARE)

Gibberellin is involved in many plant cellular developmental activities such as shoot elongation, seed germination, fruit and flower maturation (Bewley et al., 1997; Derkx et al., 1994). GARE has been implicated to confer response to GA in genes, thus the relatively high frequencies of GARE in ZmSPS1, ZmSPSβ, ZmSPP2 in maize; OsSPS1, OsSPP1 in rice; SbSPSγ, SbSPSε in sorghum, may implied that these isozymes are involved in the plants cellular activity that will require sucrose to be synthesized and exported to cells that are in need of carbohydrate or energy supply. It has been shown that increased OsSPS1 activity in transgenic rice plant increases the plant height and leaf expansion rate when compared to the control (Seneweera et al., 1995;

Ishimaru et al., 2003).

Ethylene responsive element (ERE)

Ethylene plays vital roles in numerous plant cellular activities and biotic and abiotic responses. Ethylene has been shown to be involved in fruit ripening and senescence, in drought and osmotic imbalance, and in wounding and pathogen attacks (Alexander and Grierson, 2002; Manavella et al., 2006; Woltering and van Doorn, 1988; Xu et al., 1994). The presence of relatively higher frequencies of ethylene responsive element in ZmSPSα, ZmSPP2, OsSPS 4, SbSPSθ may well indicate that these isozymes are tightly regulated by cellular activity that is responsive to ethylene. Ethylene has been shown to be involved in enhancing sucrose formation and transport in plant tissues (Tupy, 1985; Ishizawa and Esashi, 1988; Chervin et al., 2006; Dusotoit-Coucaud et al., 2009), thus these isozymes may partake directly in synthesis of sucrose from simple sugars in these tissues.

Cellular Development Responsive Cis-elements

The overall sink Sugars are essential metabolic products required by plants for diverse cellular metabolic processes. The expressions of many genes have been shown to be connected to sugar biosynthesis or degradation (Smeekens, 2000). Cellular processes such as photosynthesis, growth, development and environmental stress resistance, have been shown to be well regulated by sugars (Smeekens, 2000). Sucrose is essential important because of its non-reducing properties making it appropriate to be transported from site of synthesis to site of utilization in plant. Sucrose is used by plants as an energy molecule that moves from cell to cell until it gets to the energy demanding cell where it is been hydrolysed to glucose and fructose which then generates ATP for the cell functions or for synthesis of other macromolecules required by the cell. Thus the role of sucrose in plant growth, development and adaption or resistance to external stimuli cannot be overemphasized. In view of this, the main enzymes that are essentially required by the cell for sucrose synthesis (SPS and SPP) are therefore core enzymes in the fundamental plant metabolic and (or) physiological state. They will be well linked to the source-sink transition of energy.

General carbon metabolism and signaling responsive elements (-10PEHVPSBD, CGCGBOXAT, CIACADIAN-LELHC, DOFCOREZM)

These elements have been reported to involve in the expression and regulation of the downstream coding regions of their genes. DOFCOREZM and CGCGBOXAT have both been shown to be involved in several signalling pathways of genes that are involved in carbon metabolism (Yanagisawa et al., 2000; Yang et al., 2002). These elements were present at very high frequencies in all the SPS and SPP isozymes. This is not so unexpected based on the function of SPS and SPP which is to produce carbon in the form of sucrose, thus the expression of the isozymes will be highly regulated by these elements in combination with other transcriptional factors. On the other hand, -10PEHVPSBD and CIACADIANLELHC elements were found to be very low in the SPS and SPP isozymes. These elements were shown to be involved in expression of plastid genes and genes that functions in plant circadian rhythms (Piechulla et al., 1998; Thum, et.al, 2001). It may be well to say that the low frequencies points to the fact that SPS and SPP genes may not necessarily partake in pathways or reactions that may involved these processes. However this doesn't necessary infer that the expression of SPS and SPP will not be regulated by these cis-acting elements, they might only have a minimally influence.

Seed and pollination responsive elements (RY-motif and GTGANTG10)

These elements are found at the regulatory regions of genes that are specifically involved in seed development and during late pollination, and are necessary for the expression and regulation of the genes (Ezcurra et al., 2000; Rodgers et al., 2001). These elements were found to be differentially present in the SPS and SPP isozymes, only at exception cases are they present at high frequencies. Such are ZmSPS1 in maize, OsSPS1 in rice and SbSPSα, SbSPSβ and SbSPSθ in sorghum; for RY-motif. For the late pollination motif (GTGANTG10), it is only ZMSPSα in maize, OsSPS1, OsSPS3 and OsSPS4 in rice, and very high in SbSPSβ in sorghum. Both motifs are found to be relatively low in SPP isozymes. It could then be said that these isozymes with higher frequencies are more involved in promoting sucrose formation in sink organs.

Sugar repressive elements (Pyrimidine-Box and SREATMSD)

Metabolic processes such as sugar synthesis from photosynthesis, sugar breakdown (glycolysis), nitrogen and amino acid metabolism, and sugar and carbohydrate metabolism sugar are often repressed by sugar (Smeekens, 2000). The frequencies of these elements were found to be very low in both SPS and SPP isozymes. This perhaps is an indication that Pyrimidine-Box and SREATMSD motifs might not necessarily have

influence on the expression and regulation of SPS and SPP isozymes. Since SPS and SPP are enzymes in sucrose biosynthesis in tissues, and they may either be mopping up excess hexoses to sucrose for export, or for storage (depending on the kind of tissue), it may then be put forward that inhibiting these enzymes may only be necessary when there is a peculiar need to keep the concentration of hexose high, such as in injured or starved tissue that will require carbon and energy to be made available for cellular activities rather than stored.

Abiotic Stress Responsive Cis-elements

The overall sink-source dynamics determines how much, where and when carbohydrates will be allocated. Abiotic stress causes the alteration of sink-source dynamics depending on the severity of the stress and plant growth period (Thomashow et al., 1990; Clifford et al., 1998). Cereals such as rice, maize, and sorghum have the ability to buffer sink-source interaction by storing sucrose in the stem tissues when the production of sucrose from the whole plant is more than the quantity the whole plant needs. These reserves improve yield capability by providing an alternative source when photosynthetic ability is reduced during the later phase of grain filling or during period of abiotic stress. Sucrose plays a major role during plant growth and development under abiotic stresses by regulating carbohydrate metabolism (Smeekens, 2000; Lutfiyya et al., 2007). Stress responsive genes have been reported to be induced by sucrose, which plays a role in the response to environmental stress.

Light responsive element (GATA, I-Box and SORLIP)

Numerous plant cellular processes such as flowering, seed development, chloroplast development, circadian rhythms, phototropism, etc. have been shown to be related to light (Natr and Lawlor, 2005). A number of light responsive cis-acting elements which include among others are the GATA, I-Box and SORLIP. They have been shown to be highly involved in light regulated or responsive genes, such that their deletion or alteration negates the ability of the genes to respond to light and ultraviolet radiation (Gilmartin et al., 1990; Lam and Chua, 1989). Very high frequencies of these cis-elements were found in all SPS and SPP isozymes maize, rice and sorghum. This is not surprising as the substrates (glucose and fructose) utilized by SPS and SPP are products of photosynthesis. It thus reflects that SPS and SPP will be highly regulated by light via photosynthesis, or through signals that may involve some specific light receptors. A light responsive element cannot confer light responsiveness alone; it is combination of the elements that work together with relevant transcription factors to form machinery relevant for the light responsiveness (López-Ochoa et al., 2007). Therefore the high frequencies of both GATA and I-Box in the entire isozymes further buttress that SPS and SPP are light responsive and regulated genes, although SORLIP is relatively lower in maize SPS isozymes.

Drought/Salinity stress responsive elements (DRE, CBFHV, Erd1, MYB and MYC)

Drought and salinity are known abiotic stress that adversely affects the plant development and productivity by their action which causes excessive water loss via the stomata pore and through the roof zone as a result of hyperionic or hyperosmotic effect that ensure during high soil salinity. One of the ways plants cope with this phenomenon is increase in solute concentration which will help in maintaining cellular hydration and relieving the cell/tissue from the stress. Sucrose is one of the plant major osmoprotectant, and sucrose accumulation has been shown not only to protect the cell against the dehydration, but also to provide the urgent energy needed by a stressed tissue (Everard et al., 1994; Binzel and Reuveni, 1994; Clifford et al., 1998; Manavella et al., 2006). It was reported in rice and Arabidopsis that drought/salinity stress responsive elements bind to NAC-domain transcription factors forming a complex that facilitate the expression of the downstream genes (Tran et al., 2004; Gao et al., 2010). Very high frequencies of MYC and MYB motifs were found in all the SPS and SPP isozymes, however DRE, CBFHV and Erd1 were found to be high in some isozymes. Of noteworthy are ZmSPS1 and ZmSPSβ in maize; OsSPS1 and OsSPS3 in rice; SbSPSα, SbSPSβ and SbSPP in sorghum. It therefore may be inferred that during drought or salinity stress, these isozymes may well partake in the increase of sucrose level in a bid to alleviate the stress.

Low temperature responsive element (LTRE)

Sucrose was reported to be one of the major osmolytes that protects cell membrane from cold temperature (Thomashow, 1990). Sucrose act to counter the effect of cold temperature such as membrane expansion that disrupt cells physical integrity, denaturation and eventual precipitation of macromolecules like proteins, and finally leading to cell dehydration ,then cell death (Thomashow, 1990). Sucrose act as an anti-freeze against these deleterious effects by stabilizing the cell membrane, restore membrane fluidity and viscosity of the adjoining environment and prevent change in protein conformation (Anchordoguy et al., 1987; Steponkus and Webb, 1992). Although low frequencies of LTRE were present in the isozymes, appreciable amount were found in ZmSPSα, ZmSPP1α, ZmSPP1 in maize; OsSPS4 in rice; SbSPSδ

in sorghum. The expression of these isozymes may therefore be highly regulated by low temperature condition, and they may be the isozymes that are essential employed by these plants to synthesis sucrose required for the maintenance of the cell integrity during cold temperature.

Biotic Stress Responsive Cis-elements

Plants suffer enormously from biotic environmental factors owe to their sedentary nature. They are often prone to numerous diseases caused by pathogens such as bacteria, fungi and virus, mechanical injury and/or herbivore attacks. Plants on their own have developed innate defence against these deleterious effects of these agents. Defence strategies such as production of waxy epidermal cuticle, thick bark, production of toxins, pathogen degrading enzymes and/or purposeful suicide (Freeman and Beattie, 2008). Availability of carbohydrate that will supply the essential energy required for the cascades of reaction mechanisms that will ensue is very vital for the survival of the plant. Sucrose has been reported to act as an endogenous inducer that causes the up-regulation of pathogen-related genes in rice plant (Gómez-Ariza et al., 2007). Thus sucrose availability and metabolism is tightly regulated by physiological state of plant tissue, and more in particular in an injured tissue that suddenly become a sink; energy consuming tissue.

Disease, elicitor and pathogen responsive elements (BIHD10S, EIRE, GCC-Box and GT1)

These elements have been found in regulatory regions of many pathogenesis–related genes and reported to work cooperatively with each other and transcription factors that facilitate their transcriptional induction and expression of their downstream coding regions (Rushton et al., 1996; Park et al., 2004). Sucrose accumulation was reported to facilitate defence enzymes response to pathogen attack (Moghaddam and Ende 2012). It was implicated to activate numerous signalling pathways by regulating the source-sink metabolism and activating defence responses. Thus enormous availability of sucrose in the extracellular was shown to be an indicator of pathogen infection (Roitsch, 1999). These elements although found at differential amount in the SPS and SPP isozymes, the GT1 was however found to be at a very high frequencies compared to the rest. Thus SPS and SPP isozymes are very likely to be rapid induced during diseases, elicitors or pathogen attack(s) that will require and urgent need of sucrose synthesis and export to the suddenly high energy demanding injured tissues.

Wound responsive element (W-Box)

Wounding; be it by artificial mechanical damage, insect herbivory or pathogens attacks will require sudden metabolic readjustment. This will involve increase carbon and energy requirements towards the affected plant area, not only to meet the cellular need but to provide carbon that will be required in cascades of resistant/defence mechanisms (Smeekens, 2000). The presence of very high frequencies W-Box in all the SPS and SPP isozymes, only points to the fact that SPS and SPP are wound responsive proteins, and their expression will be very highly regulated by wounding. SPS and SPP will be involved in rapid synthesis of sucrose from the source (photosynthetic cells) and export to the injured tissue (Ibraheem et al., 2013). Plant hormones such as ethylene, jasmonic acid and salicylic acid have been shown to participate as a secondary messenger in transduction mechanisms during wounding (Maleck et al., 2000).

Essential nutrient responsive elements (CuRE and SuRE)

Among the vital nutrients required by plants for cellular metabolism and development are copper and sulphur. Copper is an essential co-factor required by proteins involved in electron/oxygen transfer reactions (Hänsch and Mendel, 2009). Copper responsive element (CuRE) has been implicated to be contained in genes that require copper for their cellular expression (Quinn et al., 2000). Among these genes are photosynthetic genes such as cytochrome oxidase, plastocyanin, polyphenol oxidase, etc, which contain CuRE element at their regulatory sites, and researches have shown that this element is very essential for their downstream expression (Quinn et al., 2000). The CuRE elements were found at very high frequencies in the SPS and SPP isozymes, with the exception of few isozymes such as ZmSPSα, ZmSPS3, OsSPS3, OsSPS5, SbSPSγ and SbSPSκ. Thus, SPS and SPP though not photosynthetic proteins, but they utilize or converts photosynthetic products are very likely to be influenced by factors that do have influence on photosynthesis, their expressions are therefore mostly likely to be regulated by copper.

Sulphur is an essential part of the thiol group of methionine and cysteine, where it contributes in the formation of disulphide bonds in protein globular structure. It is essential for plant development and overall productivity, and its deficiency was found to reduce photosynthesis as a result of impairment of chlorophyll synthesis (Hopkins, 1999). Sulphur responsive elements (SuRE) has been reported to participate in the expression and regulation of genes that required sulphur for their cellular activities. The frequencies of SuRE element were found to be very low in the SPS and SPP isozymes with the few exceptions of ZmSPSα and SbSPSκ that have relative high frequencies. Although the frequencies of SuRE may be low, that does not necessary mean SPS and SPP may not be regulated by sulphur, it might

only be infer that the expression of ZmSPSα and SbSPSκ may be well regulated by sulphur than others. Sulphur has been shown to increase photosynthetic machinery leading to increase glucose formation (Fields and Clair, 1984), which eventually will be converted by SPS and SPP to sucrose.

METHODS

Search for Sucrose 6-phosphate synthase (SPS) and Sucrose 6-phosphate phosphatase (SPP) Isozymes

In order to find the SPS and SPP isozymes, the keyword ‘‘sucrose phosphate synthase and sucrose phosphate phosphatase ’’ were used to search and retrieve the complete amino acids sequences of all previously identified SPS and SPP in maize, rice and sorghum using the Entrez Protein Sequence database on NCBI website (http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein).
These sequences were saved as FASTA format and used to search against individual plant proteome by entering the amino acids sequences manually into the basic local alignment search tool (BLASTP); protein query to protein database search query of Phytozome v9.1 (Goodstein, et al., 2011; www.phytozome.com). From the BLASTP results obtained from Phytozome v9.1, the Gene pages were opened and the genomic sequences were obtained. Subsequently, 1500bp upstream of these sequences [5' untranslated region (5'UTR) used as the promoter/regulatory region] starting from the translation start (ATG) of each SPS and SPP were obtained and saved as FASTA format. The amino acids, genomic and regulatory sequences were used for subsequent computational analysis.

Analysis of Cis-acting regulatory elements

Identification of the *cis*-acting regulatory elements was achieved by scanning the 1500bp upstream regulatory sequences of the SPS and SPP isozymes for the presence of putative *cis*-acting regulatory elements identical with or similar to the motifs registered in Genomatix Mat inspector professional (Cartharius, et al., 2005; http://www.genomatix.de/cgi-bin/matinspectorprof/ matfam.p1), PLACE (Higo et al., 1999; http://www.dna. affrc.go.jp/PLACE/ signalscan.html), Plant Pan (Chang et al., 2008; http://PlantPan.mbc.nctu.edu.tw) and Plant CARE (Lescot et al., 2002; http://bioinformatics.psb. ugent.be/webtools/plantcare/cgi-bin/CallMatIE55.htpl); using their default settings.

Prediction of Structural/Evolutionary Relationship

The default settings of Clustal Omega: EMBL-EBI, Multiple Sequence Alignment (Siever et al., 2011; McWilliams et al., 2013; http://www.ebi.ac.uk/ Tools/clustalo/) was used to search for common/possible structural similarities/divergence between the 5' UTR, genomic and amino acids sequences of the SPS and SPP isozymes. Phylogenetic tree that showed their structural/evolutionary relationships was constructed using the amino acids sequences with the default settings program of Constraint Based Protein Multiple Alignment Tool (COBALT; Papadopoulos and Agarwala, 2007; http:www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?Link_loc=BlastHomeLink).

CONCLUSION

This study provides the comparative study of the cis-acting elements identified in the non-coding region of SPS and SPP isozymes present in three different monocot plants; maize, rice and sorghum using bioinformatics approach. The analyzes of these SPS and SPP isozymes gave a better understanding on how and when this isozymes may be expressed and what external stimulus may be involved in their expressions. This may serve as a good platform for future expressional *in-vitro* analyzes to verify the information as presented here. This among others may involve experimental demonstration of tissue specific/stress induced expression and regulatory patterns, and in-depth Phylogenetic, kinetic and functional characterizations of the various monocot and dicot isozymes. Researchers have shown that the identification of the positions of cis-acting regulatory elements not only provide necessary information on the plant genetics, but they may be employed in breeding programmes for the generation of improved crops that will have tolerance or perhaps resistance to environmental stress conditions.

ACKNOWLEDGMENTS

The authors are grateful for the laboratory space provided by Department of Biological Science Landmark University, Nigeria.

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