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EVALUATION OF CIS-REGULATORY ELEMENTS OF DICOT β-TbSt PROMOTER

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ABSTRACT

The eukaryotic gene expression is controlled by a regulatory region called promoter. Many plant promoters have been characterized for regulatory motifs. There are three types of plant promoters i.e. inducible, constitutive and tissue specific on basis of regulatory motifs. Plant sources have been searched for isolation of strong promoters that are being utilized in molecular biology research. The researchers need to address IPR issues for utilizing the strong patented promoters for the expression of their transgenes. The identification and characterization of strong dicot promoter is necessary for the expression of transgenes by native researchers to evaluate their artificial gene. The promoters isolated from viral sources have some limitations. The dicot promoter sequence of *β-tubulin* (*β-TbSt*) was explored and isolated from potato. The *β-TbSt* promoter sequence consists of light responsive, hormonal responsive and stress responsive elements. Motifs having responsive elements were identified in *β-TbSt* promoter. The *β-TbSt* promoter is highly constitutive promoter.

Key Words: *Cis*-acting elements, *β-TbSt* promoter, Dicot, *Solanum tuberosum*

I. Introduction

Transcription factor binding sites (TFBSs) are cis-regulatory sequences that possess maximum transcriptional activity. There are various web-based tools for the analysis of TFBSs in eukaryotic gene promoters such as The Plant Promoter Analysis Navigator (*PPAN*). The online databases provide information about the genes and their regulatory regions for the evaluation of TFBSs in the upstream gene regulatory promoter sequences. There are other databases such as *TRANSFAC* which helps to locate the TFs that could bind to the various domains in the promoter region. Similarly, various plant genes related to plant defense against various factors can be characterized by *PathoPlant* database.

Athen is another tool that specifies the prediction of promoter sequences in *Arabidopsis thaliana* as well as previously characterized TFBSs and information about recurring TFBSs in different promoters. *PLACE* database depicts various motifs and *cis*-acting regulatory elements that have significant roles in the plant vital functions. Furthermore, another online database named *PlnTFDB* deals with the evaluation of the plant specific TFs. (Higo *et al*, 1999). *AGRIS* database entails *Arabidopsis* TFs as well as their DNA binding motifs (Davuluri, 2003). JASPAR database describes eukaryotic TFs (Bryne *et al*, 2008; Sandelin *et al*, 2004). *In silico* analysis of promoter can also be accessed by *PlantCARE* that can provide detail information of promoters' cis acting elements in both DNA strands. For *Arabidopsis*, *AthaMap* is used to search for TFs and their corresponding binding sites (Lescot *et al*, 2002). All these databases provide preliminary information about promoter motifs that can be further validated using various reporter gene systems.

Plants integrally respond to environmental changes such as heat, light intensity, temperature, and chemical substances by various cell signaling cascade mechanisms. A number of studies have been performed at molecular level in order to investigate plant interactions with environmental changes, which lead to the conclusion that the expression of genes directly depends upon environmental changes. The cell signaling mechanisms work under TFs in the regulatory sequences of genes. Promoters of genes control cell signaling that can change the genetic system according to environmental conditions (Priest *et al*., 2009).

There are short conserved motifs of DNA called as *cis*-regulatory elements that are located within the regulatory sequence of a specific plant gene. This region is a binding site for multi-subunit RNA polymerase, which under adverse conditions can further accelerate transcriptional

activity in specific tissues. The TFs can either bind to basal transcriptional machinery or can conduct gene expression from the distal area of the promoter (Rombauts *et al*., 2003).

CREs (*Cis*-regulatory elements) are necessary for genes expression in various climatic changes and stages of plant development (Picot *et al*., 2010). These elements can be found in over expressed promoters in the TATA box (Featherstone, 2002), C box, A box (Song *et al*., 2008) and G box (CACGTG) (Siberil *et al*., 2001). The TATA box lies near to TSS that is actively involved in the establishment of a transcription initiation complex (Pribnow 1975). Similarly, the G-box (CACGTG) has also been studied in numerous plant promoters. It has been seen that the tissue specific gene expression can be regulated by G-box under light and hormone inducing factors (Hudson and Quail, 2003). Furthermore, the bZIP proteins have also been investigated to recognize sequence specific DNA binding factors i.e. G-box binding factor (GBF) (Siberil *et al*., 2001).

The I-box (GATAAG) has been identified in numerous Ribulose bisphosphate carboxylase small subunit (RBCS) genes and aforementioned box is found close to chlorophyll a/b-binding (CAB) genes (Gidoni *et al*., 1989). W box (TTGACC/T) has also been detected in the promoters of pathogens responsive genes (Navarro *et al*., 2004). The hormone-inducible promoters have been found with conserved sequence motifs as for example abscisic acid responsive motif (ABRE) (Simpson *et al*., 2003), P box (TGTAAAG) (Kim *et al*., 1992), and the TCA motif (Liu *et al*., 2009). The dehydration response motif has been detected in various stress inducible genes and performs in frosty and water deficit conditions (Yamaguchi and Shinozaki, 1994). Promoter of Pro-dehydrogenase (*ProDH)* contains a unique motif (ACTCATCCT) which responds to Proline (*Pro*) and hypo-osmolarity (Satoh *et al*., 2002). However, abscisic acid responsive promoters consist of ACGTGG/TC motif (Zhu, 2010). The motif CE1 (TGCCACCGG) and CE3 (ACGCGTGCCTC) are found to be involve in ABA-induced gene expression in several plants (Shen *et al*., 1996). Similarly, C repeat (GGCCGACAT) and low-temperature-responsive element (GGCCGACGT) have been found in cold inducible promoters (Yamaguchi and Shinozaki, 2005).

II. Methodology

1. Retrieving of promoter sequences

The sequences of particular genes promoters were retrieved and analyzed in HTGS databases. The sequence of gene was particularly selected on root of strong expression mostly in dicot plants. The sequence of *β-tubulin* was retrieved from NCBI followed by BLAST search against HTGS, the dicot HTGS reported matched from *Solanum tuberosom.* Then matched HTGS coding

sequence of *β-tubulin* was investigated by using BLAST-X for the start codon and the coding sections were eliminated. Gene's upstream region of selected dicot having coding sequence can be particularly selected for depiction of full-length promoter. The sequence was further analyzed by different bioinformatics tools. The IPR contents were accessed via BLAST and FASTA, which are designed to detect biologically relevant sequences. Patent databases, such as the public database PAT (supplied by NCBI's patent sequence division) and the proprietary database GENESEQ provided by Derwion Thomas Scientific, were also used to do patent searches (Dufresne *et al*, 2002).

2. Detecting *Cis*-regulatory motifs in *β-TbSt* promoter

Using various plant promoter databases, the *β-TbSt* promoter sequence acquired from HTGS was examined for cis-acting regulatory motif. *PlantCare* and *PlantPAN* databases were utilized to search for potential regulatory motifs in the *β-TbSt* promoter. The NCBI's patent blast search tool was used to compare the *β-TbSt* promoter sequence to previously patented promoters. To search eukaryotic promoters, TSS (transcription start sites) of the -TbSt promoter sequence was searched using the BDGP database (http://www.fruitfly.org/seq tools/promoter.html).

III. Results

1. BLAST search of *β-TbSt* promoter against patent sequence database

β-TbSt promoter sequence was compared to previously published sequences in NCBI databases using patent BLAST. Table 1 summarizes the patent's findings. The *β-TbSt* promoter sequence has less than 2% homology with previously published patent sequences. According to a patent BLAST search against the patent database, nucleotide sequence for query promoter was considerably altered from the recorded patented promoter sequences in database.

2. Prediction of Eukaryotic promoter

The transcription start site (TSS) sequence detected in *β-TbSt* promoter sequence is mentioned in Table 2. TSS of the whole length promoter is predicted by the bold alphabet in the sequence. In the *β-TbSt* promoter, the actual TSS nucleotide was found to be "A."

Table 1: Patent BLAST *β-TbSt* promoter (GenBank HTGS: AC233624)

Accession No	Description	Max score	Total score	Query coverage In %	E value	Max ident in %
FW370726.1	Novel compositions and methods for cancer	46.4	46.4	1	0.24	94
FV536295.1	Modified Microbial Nucleic Acid	46.4	46.4	1	0.24	85
FV536294.1	Modified Microbial Nucleic Acid	46.4	46.4	1	0.24	85
GN008256.1	Sequence 697 from Patent EP2014669	46.4	46.4	1	0.24	94
FV536331.1	Modified Microbial Nucleic Acid	44.6	44.6	1	0.85	84
FV536330.1	Modified Microbial Nucleic Acid	44.6	44.6	1	0.85	84

3. *Cis*-regulatory elements in the *β-TbSt* promoter

Sequence study of *β-TbSt* promoter exhibited that most frequent motifs present were light responsive elements, stress induced motifs and motifs related to hormones. These three types of motifs were present throughout the promoter region.

Table 2: Predicted TSS sequences in *β-TbSt* promoter

Promoter name	Predicted TSS
β-TbSt	AACATATGTCTATATATGCGTTACCTAACTTTTAAGATTT**A**TTCATCCCC

3.1. Light responsive motifs

The *β-TbSt* promoter has a lot of light response motifs as shown in Table 3. It includes following matrix scores; AE-box (0.9), AT1-motif (0.9), ACE motif (0.9), Box 4 (1), ATCT-motif (0.9), Box II (1), G-box (1), CATT-motif (1), GAG-motif (0.8), GATA-motif (0.8), GT1-motif (1), TCT-motif (0.8), Sp1, TCCC-motif (0.8), and Lamp box (0.8). G-box can be found in three places in the *β-TbSt* promoter. The *β-TbSt* promoter has 15 light responsive motifs.

Table 3: Light responsive motifs detected in *β-TbSt* promoter

Motif name	Sequence	β-*TbSt* promoter
AT1-motif	AATTATTTTTTATT	1
GAG-motif	AGAGATG	1
GATA-motif	GATAGGG	1
I-box	GATAGGG TATTATCTAGA	2
3-AF1 binding site	TAAGAGAGGAA	1
Box 4	ATTAAT	1
Box I	TTTCAAA	1
G-box	CACGTC	1
GT1-motif	GGTTAA	1
ACE	CTAACGTATT	1
AE	AGAAACAT	1
TCCC-motif	TCTCCCT	1
Box II	GTGAGGTAATA	1
Sp1	CC(G/A)CCC	1
Total motifs		15

3.2. Defense and stress responsive motifs

The *β-TbSt* promoter contains a number of defense and stress response motifs as shown in Table 4. Among the defense and stress motifs; there are two ABRE (ABA responsive), one box-W1 (as fungal elicitor responsive), two MBS (as drought inducible), three TC-rich repeats (for defense and stress responsive), two TCA-elements (salicylic acid responsive), and single MBSII (flavonoid biosynthesis) with different matrix score ranges from 1-0.8.

Table 4: Defense and stress responsive motifs

Motif Name	Sequence	B-TbSt Promoter
MBS	TAACTG CAACTG	2
TC-rich repeats	ATTTTCTCCA ATTTTCTTCA	2
TCA-element	CCATCTTTTT GAGAAGAATA CAGAAAAGGA	3
TGACG-motif	TGACG	1
CGTCA-motif	CGTCA	1
LTR	CCGAAA	1
ABRE	CACGTG	1
W box	TTGACC	1
Box III	atCATTTTCACt	1
Box-W1	TTGACC	1
Total motifs		14

3.3. Leaf morphology motifs in *β-TbSt* promoter

The leaf morphology motifs found in *β-TbSt* promoter were HD-Zip 1 and HD-Zip 2. Table 5 shows the diverse leaf morphology TFBS in the *β*-TbSt promoter.

Table 5: Divers leaf morphology motifs

Motif name	Motif sequence	β-TbSt promoter
HD-Zip 1	CAAT(A/T)ATTG	1
HD-Zip 2	CAAT(G/C)ATTG	1
Total		2

3.4. Hormone responsive motifs

Several hormone responsive motifs were detected in *β-TbSt* promoter as shown in Table 6.

Table 6: Hormone responsive motifs in *β-TbSt* promoter

Motif name	Motif sequence	β-TbSt promoter
GARE-motif	AAACAGA TCTGTTG	1
AuxRR-core	GGTCCAT	1
P-box	CCTTTTG	1
Total		3

3.5. Species specific motifs

In this study, *β-TbSt* promoter possess three species specific motifs having one *auxin* response element binding site, two sites for guard cell specific motif and two binding sites (Table 7) for MYBST1 (novel DNA binding protein).

Table 7: Species specific motifs

Motif name	Motif function	Motif Sequence	No of motifs in β-TbSt promoter
MYBST1	Novel DNA binding protein	TATCC GGATA	2
TAAAGSTKST1	Guard cell specific	CTTTA TAAAG	2
SEBFCONSSTPR10A	Auxin response element	GAGACAA	1

3.6. Seed and endosperm specific motifs

These motifs reported in *β-TbSt* promoter are listed in Table 8. Skn-1-motif (GTCAT), an endosperm-specific motif, is located in the *β-TbSt* promoter. The Skn-1-like motif has been detected in the *β-TbSt* promoter at two different locations. Skn-1 motif has a matrix score of 1 in the *β-TbSt* promoter.

Table 8: Endosperm specific motifs in *β-TbSt* promoter

Motif name	Motif sequence	*β-TbSt* promoter
Skn-1 motif	GTCAT	2

IV. DISCUSSION

The genetic engineering of economic crops like wheat, maize, cotton and rice etc. can improve living standards of human population throughout the world. The genetic engineering can be achieved by using efficient genes and highly expressed promoters. There is much potential of growth in developing countries in the field of biotechnology and bioinformatics tools, which are available online, that can be utilized for the characterization of regulatory sequences which can be further isolated and exploited for the improvement of agronomic traits. The dicot promoter (*β-TbSt* promoter) isolated from potato was characterized through bioinformatics tools for *cis*-acting regulatory motifs. To avoid the patented promoters and IPR issues the sequence of *β-TbSt* promoter was searched in BLAST against patent sequences showed less than 30% homology (Table 1) with already patented sequences.

The *β-TbSt* promoter sequence was searched in *PlantCare* database for the identification of transcription factors (TFs). The *β-TbSt* promoter was found to consist of GARE-motif, P-box, and *Aux*RR-core. *Auxin* response factors bind to promoter elements i.e auxin-responsive element (*Aux*RE) (Hongyan *et al*., 2011). *Aux*RR-core and GARE-motif, hormone response motifs, were found to involve in *gibberellin* responsiveness and these motifs have already been described in *snakin-1* promoter of potato (Natalia *et al*., 2010).

The light response motif (Box4) identified in *β-TbSt* promoter (Table 3) has already been described in the *PAL* gene promoter (Hamlet *et al*., 2012). Motif SP1 has also been located in the P105 promoter, and this motif has been identified as a key element for viral machinery replication (Workman & Buchman., 1993). Motif Sp1 was discovered to be required for unwinding the DNA molecule, and this motif may improve binding of TATA box binding protein (Demer *et al*., 1994). However, phosphorylation of the Sp1 motif can speed up transcription (Mason *et al*., 1998). The GT1-motif was discovered in the *β-TbSt* promoter and GT-1 protein can bind with this motif, which is located in plant nuclear genes and have a principal part in plant transcription (Niharika *et al*., 2011). In eukaryotic promoters, the I-Box found in *β-TbSt* promoter has already been identified as a light response element (Annanda *et al*., 2002). The light response motifs (AT1-motif, ACE, AE-box, Box 4, Box-I, Box-II, CATT-motif, G-box, GAG-motif, GATA-motif, GT-1 motif, I-Box, Sp1, TCCC-motif) have previously been reported in the photoperiod-responsive gene regulatory sequences (Chareerat *et al*., 2009).

The *β-TbSt* promoter contains the Box-II motif (GGTTAA), which has been found in photosynthesis-related genes promoter regions (Yang *et al*., 2009). The interactivity of G-box and phytochrome interacting factors (PIFs) plays an important role in photoperiod response gene transcription (Menkens *et al*., 1995). The Box-II motif (G-box (CACGTG) gives response to Jasmonic acid and further transcription of *Arabidopsis* genes (Guerineau *et al*., 2003). The *snakin-1* promoter of potato was found to consist of light response motifs (CATT, GA, GATA, GT1, I-box, Sp1, TCT). The *snakin-1* promoter shows effective part in potato development can also be persuaded by high temperature and injury (Natalia *et al*., 2010). *Lhcb* promoter having GATA motif was contemplated to be associated with plant genes transcription. For definite transcription activity, the combined action of specific transcription factors is important. *Cis*-acting elements are found in all eukaryotic promoters. The motifs related to light response (GATA, ATCT, TCT, GAG, Box-I, I-box, Box-II, G-box) have already been reported in *Pt-RbcS* (Like *et al*., 2013).

Some promoters that have already been defined have defense and stress response elements. ABRE, Box-W1, which stands for fungal elicitor responsive motif, MBS, which stands for drought inducible, and MBSII, which stands for flavonoid biosynthesis responsive motif, are all stress-related motifs. The androgen responsive element (ARE) detected in *β-TbSt* promoter has already been reported in *hPar1* promoter (Salah *et al*., 2008). In the *β-TbSt* promoter, *MeJA*-responsive motifs with nucleotide sequences CGTCA and TGACG, have also been anticipated in DREB promoters (Amrita, 2010). Now, W-box (stand for fungal elicitor response motif) isolated from sunflower found in the *β-TbSt* promoter has also been studied in *HAHB4* promoter (Manavella *et al*., 2008).

The defense and stress responsive *cis*-acting elements reported in potato *snakin-1* promoter (TC-rich repeats, ARE, MBS, HSE, TCA-element and CGTCA-motif) were also present in *β-TbSt* promoter (Natalia *et al*., 2010). The regulatory motifs related to defense and stress located in CYP71A2 promoter (ABRE, CGTCA TCA-element, and HSE, TC-rich element) have also been found in *β-TbSt* promoter. In conjunction with the as-1 element, the TGACG motif has been discovered in constitutive root promoters (Krawczyk *et al*., 2002).

Specific TFBS (Table 7) SEBFCONSSTPR10A (Boyle & Brisson, 2001) and guard cell specific TF (TAAAGSTKST1) can be found in the *β-TbSt* promoter for binding auxin responsive TF (Plesch *et al*., 2001). MYBST1, a new DNA binding protein (Baranowskij *et al*., 1994), binds to the *β-TbSt* promoter, which has two TFBS (GGATA and TATCC).

The defense and stress motifs show expression under severe conditions such as dehydration, heat stress, and insect attack. Light-regulated transcription of plant genes are regulated by light responsive motifs. The *β-TbSt* promoter motifs is responsive towards hormones transcribed hormone regulated genes. In development of plants, motifs of *auxin* and *gibberellin* were found in *β-TbSt* promoters showing regulation of gene expression. On the basis of the regulatory motifs in *β-TbSt* promoter, it is detected that this promoter is constitutive dicot plant promoter with other beneficial characteristics. The promoters consist of functional TFBS for binding with various transcription factors that may regulate plant growth as well as other developmental and defense related activities.

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