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In Silico Analysisof Constitutive dicot TubulinGene Promoter

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Abstract: *Gene transformation is a valuable application used to introduce foreign genes into plants. The expression of transgenes is regulated by the promoter attached upstream to the gene. Promoters are non coding DNA sequences which occur upstream of the coding region of gene. They are transcription control modules that control overall expression profile of the gene. A variety of promoters is necessary at all levels of genetic engineering in plants from basic research to the development of economically viable crops and plant commodities.Plant gene promoters are categorized into constitutive, inducible, tissue-specific and synthetic/ hybrid promoters. The study involves identification and analyses of constitutive dicot gene promoter isolated through High throughput Genomic Sequences (HTGS). High throughput Genomic Sequences (HTGS) in the GenBank provide one of the information databases for utilizing bioinformatics approaches to identify promoter regions of different genes. The regulatory regions of a particular gene detected on an HTGS can be further screened by different bioinformatics tools to detect cis- regulatory elements, transcription start sites and transcription factor binding sites.*

Keywords: *Transformation, Promoter, Cis regulatory elements, transcription factor binding sites*

I. Introduction

Genetic engineering can be used to introduce foreign genes into plants. It is a powerful and interesting application used to study gene expression in plants. It will not replace conventional breeding but can add efficiency to crop improvement. It involves alteration and modification of genetic material by scientist. It is a technology that combines genes from totally dissimilar species in combinations that are not achievable using old methods. Each gene is identified as being related to the expression of a specific trait. The desired gene is isolated, and transferred to another plant using methods such as injection by needles or Particle bombardment method. It involves shooting a piece of DNA into the recipient plant tissue. This is carried out using a gene gun. One of the major concerns in genetic engineering is to ensure that the gene which is inserted in one plant will be passed on from one generation to the next. Plants and crops are engineered to express a resistance to herbicides and specific pests. Scientists promise that genetically modified plants will have better texture and higher nutritional value than wild varieties of the same crops. Better crop yields may be achieved using genetically modified plants, meaning that land use will become more efficient.

The expression of transgenes is regulated by the promoter attached upstream to the gene. A variety of promoters is necessary at all levels of genetic engineering in plants, from basic research, to the development of economically viable crops and plant commodities. The plant promoters fall into various categories including constitutive, tissue specific, inducible and differentially regulated. Moreover, different promoters in the same category may have different strengths for the expression of a gene being regulated by them. The constitutive overexpression of transgenes that interferes with normal processes in a plant underscores the need for refinement of transgene expression. The development of tissue-specific promoters to drive transgene expression has helped fulfill that need. Currently, the most widely used plant promoters for gene expression in plants include, 2X35S, Ubiquitin and RolC etc. The 35S promoter of the cauliflower mosaic virus is commonly used in dicots, while the maize ubiquitin promoter is the monocot promoter of choice. These promoters are very useful but have property rights. A large number of constitutive, tissue-specific and inducible promoters have been characterized and reported in dicot plants. Hence, these plant-derived promoters can be characterized and used for developing transgenic plants (Rushton et al., 2002). An example of constitutive dicot gene promoter is polyubiquitin promoter (Gmubi). This promoter from soybean (Glycine max) showed high levels of constitutive expression and was used as an alternative to viral promoters (CaMV35S; Potenza et al. 2004) for driving gene expression in soybean (Hernandez-Garcia et al., 2009).

II. Plant Promoters and their types

Depending on the activity and type of gene expression plant promoters can be classified as constitutive promoters, tissue-specific promoters and inducible promoters.

Constitutive promoters

Constitutive promoters express the genes constitutively and represent major proportion of promoters that drive the expression of genes in plants. Examples of Strong constitutive promoters include maize, tobacco ubiquitin promoters(Christensen and Quail, 1996;Genschik et al., 1994**).** Although, the 35S promoter and its derivatives can drive high levels of transgene expression in dicotyledonous plants (Battraw and Hall, 1990; Benfey et al., 1990) yet their activities are substantially lower in monocotyledonous plants (Christensen et al., 1992; Gupta et al., 2001; Weeks et al., 1993).

Tissue-specific promoters

Tissue-specific promoters control gene expression in a tissue-dependent manner and according to the developmental stage of the plant. They not only increase transgene expression in specific organs or developmental stages, but can also avoid the loss of unnecessary energy that is utilized by the gene expression in undesired plant organs. The isolation, identification and characterization of such promoters, e.g. seed and fruit specific promoters, have become an important aspect in transgenic plant research (Yutao et al., 2003).

Inducible promoters

Inducible promoters are another important type of promoters, which are activated by one or more stimuli such as hormones (auxin, abscisic acid, gibberellic acid, ethylene and salicylic acid). Yet other kind of inducible promoters can be regulated by chemicals like alcohol, steroids and physical factors e.g. light or temperature and environmental conditions (Zhu et al., 2010).

Synthetic Promoters

Synthetic promoters represent another class of artificially designed promoters. These promoters are synthesized by joining different modules from the regulatory regions of promoter sequences. Syntheses of these promoters, particularly, focus on cis- and trans- arrangement of respective regulatory elements (Venter M, 2010).In the present study, the constitutive promoter having no IPR issues was selected from dicot plant. The promoter was named according to its associated gene. Therefore, the *Beta* tubulin gene promoter from *Brassica rapa* was selected in this study to figure out, if it really represents a constitutive promoter and can substitute the equivalent promoters for construction of multiple gene expression cassettes.

III. Materials and Methods

The motive of this research was to analyze the promoter sequence isolated from High throughput genome sequence (HTGS) database. The regulatory sequence was isolated from highly expressed gene in dicots. The studies were carried out to analyze one of the selected promoter from tubulin family through various bioinformatics tools.Different experimental and bioinformatics software's can be used for identifying potential cis-regulatory elements within the selected beta tubulin gene promoter.

Transcriptional regulatory networks that drive organ-specific and cell-specific patterns of gene expression also mediate interactions with the environment and represent one aspect of plant cell signaling. Essentially, the transcriptional regulation of gene expression in eukaryotes is mediated by the recruitment of transcription factors (TFs) to cis regulatory elements. TFs interact with specific DNA elements and the basal transcriptional machinery to regulate the expression of target genes.Multiple cis-elements comprise cis-regulatory modules (CRMs) which integrate signals from multiple TFs, resulting in combinatorial control and highly specific patterns of gene expression. Therefore, identifying and understanding the functions of cis-elements and their role in CRMs is essential for elucidating the mechanisms by which cells perceive and correctly respond to their environment and participate in the development of an organism.

Promoter analyses through Bioinformatics tools

Bioinformatics provides many tools and software's to analyze highly expressed dicot gene promoters.

Promoter sequence analysis through PlantCARE

The cis-regulatory elements within the selected promoter sequences were analyzed using PlantCARE software (http://oberon.rug.ac.be:8080/ PlantCARE/index.html).

Identification of transcription start site through BDGP

The transcription start site (TSS) indicates the transcription starting position in a sequence. It was obtained by using BDGP software.

Identification of transcription factor binding site through PlantPAN and PLACE

PlantPAN (www.plantpan.mbc.nctu.edu.tw/) identifies the transcription factors that are key regulators of gene expression.

IV. Results

Bioinformatics tools provide a convenient and efficient way to identify promoter sequences. PlantCARE software (http://oberon.rug.ac.be:8080/ PlantCARE/index.html) was used for the evaluation of the cis-regulatory elements in selected promoter sequences. Several types of motifs were found dispersed over the entire promoter sequences. The most frequent motifs observed in Beta tubulin gene promoters were identified to be light responsive elements.

Table 1

Cis-regulatory element	Organism	Sequence	Function
Box 4	Petroselinum crispum	ATTAAT	part of a conserved DNA module involved in light responsiveness
Box I	Pisum sativum	TTTCAAA	light responsive element
CAAT-box	Brassica rapa	CAAAT	common cis-acting element in promoter and enhancer regions
G-Box	Pisum sativum	CACGTT	cis-acting regulatory element involved inlight responsiveness
G-Box	Solanum tuberosum	CACATGG	cis-acting regulatory element involved in light responsiveness
TC-rich repeats	Nicotiana tabacum	ATTTTCTTCA	cis-acting element involved in defense and stress responsiveness
TCA-element	Brassica oleracea	GAGAAGAATA	cis-acting element involved in salicylic acid responsiveness
TGACG-motif	Hordeum vulgare	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness
5UTR Py-rich stretch	Lycopersicon esculentum	TTTCTTCTCT	cis-acting element conferring high transcription levels
ACE	Petroselinum crispum	CTAACGTATT	cis-acting element involved in light responsiveness

Reported functions of putative elements:

The search of regulatory elements by the Signal Scan resulted in number of noteworthy elements with diverse functions.

Detection of Transcription Start Site (TSS) in Beta Tubulin promoter sequence

Transcription start site is a sequence in a promoter that functions as the RNA polymerase binding site. Its identification assures that the sequence is truly a promoter region. The BDGP promoter analysis revealed following sequences with a probability of 95% for BT promoter.

Start	End	Score	Promoter Sequence
1920	1970	0.81	TCTTAAAAAATGGTGTTAGTACCTTAATGTGTG**A**GAAGTTCGA

The capital A indicates the predicted transcription start site of promoter sequence.

Identification of transcription factor binding site through PlantPAN

PlantPAN software (www.plantpan.mbc.nctu.edu.tw/) identifies the transcription factors that are key regulators of gene expression. The putative transcription factor binding sites(TFBs) inBeta Tubulin(BT)gene promoters are illustrated in table 3.2 below:

Transcription Factor Binding Sites in "BT promoter"
Sequence Length=2500

Factor	Site	Strand	Seq	Species	Source
AGL3	881	-	ctagaaaacTATGGtttt	Arabidopsis	TRANSFAC
AGL3	986	-	gtatttgagTATGGacca	Arabidopsis	TRANSFAC
AGL3	1087	-	acgtattcaTATGGatac	Arabidopsis	TRANSFAC
AGL3	1582	-	acttattttTATGGttcg	Arabidopsis	TRANSFAC
AGL3	2092	+	tgaaCCATAatttttttt	Arabidopsis	TRANSFAC
AG	887	-	aactatggTTTGGggtt	Arabidopsis	TRANSFAC
AG	1364	+	agatCCAAAaaacaatcg	Arabidopsis	TRANSFAC

V. Discussion

The present study was focused on analyses and identification of constitutive dicot beta tubulin gene promoter. The promoter is an important component to study gene expression and is generally patented after its discovery and usefulness. Many cis-regulatory motifs were detected in the BT promoter along with CAAT-box and TATA-box ACE motif include Box I, G-Box involved in light responsiveness. TC-rich repeats are important cis-acting element involved in defense response. CAREs, another class of cis-acting regulatory elements, are short conserved motifs of approximately 5 to 20 nucleotides expected to occur at random every few hundred base pairs and are hard to differentiate from other usual sequences (Blanchette and Sinha, 2001). Comparison with the detection of unknown motifs and that of known motifs is fairly straightforward and is performed by the scanning of DNA sequence with a given motif via specialized databases such as TRANSFAC (Wingender et al., 1996).

Analysis of beta tubulin gene promoter depicted manytranscription factor binding motifs. Transcription factors are highly specific in nature for interacting with proteins in order to modulate transcription.Transcription factor binding sites (TFBSs) are short sequences located near genes transcription start sites (TSSs) and recognized by respective transcription factors (TFs) for gene regulation. Promoter sequence of selected gene was enriched with conserved Transcription binding site motifs AGL3, AG, Athb-1, ANT. These motifs are located in sense (+) and (-) strands of promoter sequences.

VI. Conclusion

It is advantageous to have the choice of a variety of different promoters so that the most suitable promoter may be selected for a particular gene expression, according to cell, tissue, plant or environment. Isolation and identification of useful plant promoters is routinely required for genetic manipulation of plants and is important in achieving controlled gene expression in transgenic plant development programs.The over-expression of the desired genes usually results in gene silencing. Hence, it is proposed that the promoter identified through this study may be utilized to overcome the gene silencing problem by reducing the foreign gene expression to a lower level than the 2X35S.

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