Promoter Types and Their Applications in Crop Biotechnology: A Review

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Abstract

Plant genetic engineering is an incredibly important tool to study the gene regulation, plant development and produce resistant varieties against various abiotic and biotic stresses. Genetic transformation of plants in addition to target gene also requires a highly specific promoter for the specific and temporal expression of the target gene. In crop biotechnology, transgene is commonly driven by, constitutive promoters, such as CaMV 35S (cauliflower mosaic virus), or its derivatives. Such promoters are in use for long, although they efficiently drive the expression of genes but are associated with a number of unwanted problems such as homology-dependent gene silencing, altered plant development or morphology and are constitutively expressed at high levels throughout the plant even in the absence of the inducers (abiotic or biotic stress). To overcome this burden, tissue or organ specific and inducible promoters can be used to drive transgene expression. Various tissue specific promoters such as leaf-specific promoter, phloem-specific promoter**,** root-specific promoter, fruit-specific promoter, specific promoter and flower specific promoter have been isolated and characterized earlier. The variations in the expression of stress inducible genes are a result of the architecture of the promoters. Expression of transgene under the control of stress-inducible promoters is in demand and is preferred to produce transgenic plants having resistance to multiple stresses. This review highlights the advantages and disadvantages of constitutive promoters and the need for inducible promoters.

Keywords: Genetic engineering, 35S promoter, salicylic acid and jasmonic acid

Introduction

Promoters are DNA sequences usually upstream of transcribed gene and play a central role in the regulation of gene expression determining when, where and to what extent a gene is expressed. The structure of eukaryotic promoter is modular, comprising distal (upstream activation sequence; UAS) and proximal region (core promoter) containing TATA element. RNA polymerase binds to the TATA region of the core promoter and initiates transcription of the gene. Core promoter region provides the binding site for recruiting general transcription factors (GTFs). The GTFs consist of TATA-binding proteins (TBP), RNA polymerase II and other associated factors as well transcription factors for basal transcriptional activity of the linked gene. Majority of plant gene promoters contain highly conserved sequence TATA box which is normally located at -25 to -35 regions in the eukaryotic promoter. The consensus sequence of TATA box is 'TATAAT' however mismatch of one or two nucleotides have been reported from several promoters which do not alter the function of the promoter (Butler and Kadonaga, 2002). Although TATA box is important constituent of promoter, several promoters are also known in plants which lack TATA box and are commonly classified under TATA- less promoter. Such promoters are mostly found in photosynthetic genes (Nakamura *et al.*, 2002).

Eukaryotic promoters comprise of multiple elements, some of which are found in nearly all promoters. These include, CAAT box which is a consensus sequence close to -80 bp from the start point (+1), playing an important role in promoter efficiency, by increasing its strength, and function in either orientation. The consensus CAAT sequence found in conserved eukaryotic promoter is GGCCAATCT. In plants an analogous sequence called AGGA box is present (Roa-Rodriguez, 2003).TATA box a sequence usually located around 25 bp upstream of the start point. The TATA box binds to RNA polymerase II and a series of transcription factors to an initiation complex (Smale and Kadonaga, 2003) **(Figure 1).** GC box, the sequence rich in Guanidine (G) and Cystidine (C) surround the TATA box in the promoter region. The consensus GC box sequence found in conserved eukaryotic promoter is GGGCGG (Roa-Rodriguez, 2003). CAP is the site at which the transcription process actually starts; it is designated as +1. The consensus CAP site sequence found in conserved eukaryotic promoter is TAC. RNA polymerase II, the enzyme that transcribes a gene into mRNA, and the relevant transcription factors recognize the promoter region. RNA polymerase binds on the TATA box and scans along the DNA till it finds the CAP site, latter is the actual site of RNA synthesis. The transcription process only takes place in the downstream direction, from 5' (left) to 3' (right) (Smale and Kadonaga, 2003).

In addition to the core promoter and proximal promoter sequence, other *cis*-acting DNA sequences that regulate RNA polymerase II transcription positively or negatively are also present, these include the enhancers, silencers and boundary/insulator elements (Blackwood and Kadonaga, 1998; West *et al.*, 2002). Enhancers and suppressors constitute the distal regulation machinery which can exert their effect from considerable distance and are often capable of modulating expression of adjacent genes (Blackwood and Kadonaga, 1998). Enhancers are found to be located at variable distances from the promoter 'itself' in either of the directions (upstream or downstream), they bind to the transcription factors and enhance the activity of a promoter.

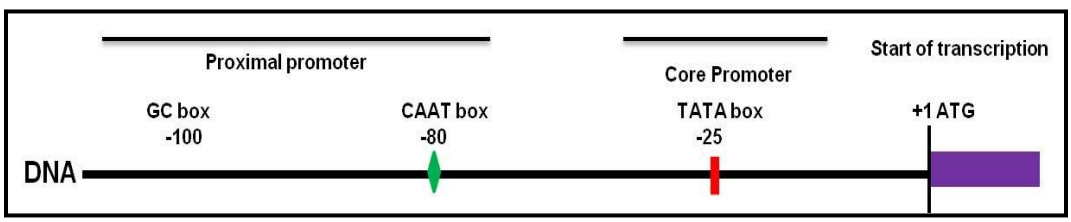

Figure 1: Schematic representation of plant promoter.

Promoter diversity

In plant biotechnology promoters are traditionally grouped into three categories, constitutive (active continuously in most or all tissues), spatiotemporal (tissue specific or stage-specific activity and inducible (regulated by both biotic and abiotic stresses and the application of an external chemical or physical signal) (Potenza *et al.*, 2004). In general the activity of a promoter depends on the availability and activity of the transcription factors. Those binding to constitutive promoters are available and active all the time, whereas those binding to spatiotemporal and inducible promoters are themselves rationed and made available only in certain tissues or developmental stages, or in response to external signals. In recent years, various promoters have been isolated from different sources (plant, viral and bacterial) and characterised to drive the transgene expression in plant systems (Yoshida and Shinmgo, 2000; Muller *et al.*, 2004).

Constitutive promoter

Constitutive promoters (are active in all tissues and at all time) are the most common promoters used to drive the transgene expression in plant biotechnology. These promoters have been isolated from both viruses as well as from plant housekeeping genes. Among plant virus promoters, the most common is the Cauliflower mosaic virus 35S promoter, which controls the synthesis of the 35S major transcript (Odell *et al.*, 1985). Despite widely used, the CaMV 35S promoter has a number of potential drawbacks, such as its poor performance in monocots, its suppression by feeding nematodes (Goddijn *et al*., 1993), homology dependent gene silencing (Vaucheret *et al.*, 1998) and the intellectual property issues. Plant housekeeping genes are another important source of constitutive promoters. Among these are genes encoding actins and tubulins. The rice actin1 promoter drives strong transgene expression in rice protoplasts transiently expressing gusA (McElroy *et al.*, 1990) and in most tissues of transgenic rice plants (Zhang *et al*., 1991). The ubiquitins are another highly conserved family of housekeeping genes, some of them are constitutively expressed (Kawalleck *et al*., 1993) while others are responsive to stress (Christensen and Quail, 1996).

Inducible promoters

These promoters are activated by one or more stimuli and often direct the expression of genes in certain plant tissues. They are generally modulated by both biotic and abiotic factors such as microbes, insects, nematodes, wounding, hormones, cold, salt and chemicals (Tyagi, 2001; Tang *et al*., 2004) **(Table 1).** Inducible promoters are broadly classified into two groups namely physically-regulated and chemically regulated based on the nature of the stimuli that triggers their expression. These promoters are widely known to be very important in plant biotechnology to drive transgene expression.

Physically-regulated promoters

These promoters are activated by both biotic and abiotic factors. There are a large number of known pathogen-inducible genes, promoters of some of them have been characterised in plants (Rushton and Somssich, 1998; Singh, 1998; Venter and Botha, 2004; Roychoudhury and Sengupta, 2009; Kovalchuk *et al.*, 2010). In plant genetic engineering, an ideal pathogen-inducible promoter should strongly and rapidly drive the expression of the specific transgene in response to a wide range of plant pathogens. Interestingly it should be able to express plant resistant genes which are commonly used by different research groups (Anand *et al.*, 2009; Guerra-Guimaraes *et al.*, 2009) temporally and locally during plant-pathogen interactions (Gurr and Rushton, 2005). In *Arabidopsis*, pathogen inducible promoter (CMPG1) was not only induced by pathogen attack but also by wounding (Heise *et al*., 2002). *Cis*-acting regulatory elements of plant pathogen-inducible promoters are classified based on their interaction with defense signalling molecules such as salicylic acid, methyl jasmonate and ethylene or signals based on the core sequences which they possess, such as the GCC or W boxes (Mazarei *et al*., 2008). Several plant promoters known to be induced in response to abiotic stresses like rd29 (dehydration inducible promoter) consists of a dehydration responsive element (DRE) that respond to water stress (Yamaguchi-Shinojaki and shinijaki, 1994), Hahb4 promoter from sunflower was found to be induced by water stress, high salt and ABA in tissue-specific manner (Dezar *et al*., 2005)

Table 1: List of pathogen inducible promoters identified in plants

Source and gene promoter	Stimuli reported to cause induction	Reference
Arabidopsis PR1	Salicylic acid	Lebel *et al*., 1998
Arabidopsis VSP1	Jasmonic acid	Guerineau *et al*., 2003
Potato GST1	*Phytophthora* elicitor, oomycetes, fungi, bacteria	Rushton *et al.*, 2002
Tobacco *PR2*-d	Salicylic acid	Shah *et al*., 1996
Tobacco chitinase	Ethylene, *Phytophthora* elicitor, oomycetes, fungi, bacteria	Rushton *et al*., 2002, Ohme and Shinshi 1995, Brown *et al*., 2003
Parsley ELI7	*Phytophthora sojae* elicitor, fungal elicitor, oomycetes, fungi, bacteria	Rushton *et al*., 2002, Kirsch *et al*., 2000
Arabidopsis NPR1	Salicylic acid, *Pseudomonas syringae* pv. *Tomato*	Yu *et al.*, 2001
Periwinkle Str	Jasmonic acid, yeast derived elicitors, *Phytophthora* elicitor, oomycetes, fungi, bacteria	Rushton *et al*., 2002, Menke *et al*., 1999
Arabidopsis OPR1	Jasmonic acid	He and Gan, 2001
Parsley PR1	Fungal elicitor, oomycetes, fungi, bacteria	Rushton *et al*., 2002
Rice NPR1	Salicylic acid	Hwang and Hwang, 2010
Tobaco *tpoxN1*	Vascular tissues, petioles, veinlets, stem epidermal cells	Sasaki *et al*., 2002

Wound induced promoters

Several wound-inducible promoters have been cloned and characterised from different plant species which shows dynamic expression (**Table 2).** It has been reported that the spatial expression patterns of several defensive genes such as chitinases, protein inhibitors and ascorbate free radical reductase, were reported modulated from wounding. The wound inducible expression of FAD7 gene promoter was reported. Many putative wound-responsive elements have been identified, such as the AG-motif (Sugimoto *et al*., 2003), DRE (dehydration responsive element; Yamaguchi- Shinozaki and Shinozaki 1994; Rushton *et al.*, 2002), Gbox (Delessert *et al.*, 2004; Kawaoka *et al.*, 1994), GCC box (Suzuki *et al.*, 1998; Nishiuchi *et al.*, 2004), GST1 box (Strittmatter *et al.*, 1996; Rushton *et al*., 2002), JERE (jasmonate/elicitor responsive element (Menke *et al.*, 1999; Rushton *et al.*, 2002), S box (Rushton *et al.*, 2002), PAL-box (Kaothien *et al.*, 2000), W-box (Eulgem *et al.*, 2000; Rushton *et al.*, 2002), and 13-bp/L-box (Takeda *et al.*, 1999).

Table 2: List of promoters known to be wound-inducible in plants.

Isolated promoter	Expression in tissues	References
*Tpox*N1	Vascular tissues, petioles, veinlets, stem epidermal cells	Sasaki *et al*., 2002
PR10	Apical meristem of leaves and stem tissues	Liu *et al.*, 2005
BV-XTH1	Roots, leaves	Dimmer *et al*., 2004
BV-XTH2	Trichomes, flowers	Dimmer *et al*., 2004
RNS1	Seedlings and leaves	Hellwig *et al*., 2008
AtTPS12,AtTPS12	Roots, hydathodes and stigma	Ro *et al*., 2006
BjCH1J	Young seedlings and leaves	Wu *et al*., 2009
FAR1, FAR4 and FAR5	Leaves and stem	Domergue *et al*., 2010
RNaseLE	Phloem tissues	Kock *et al*., 2004

Chemically -regulated inducible promoters

Phytohormones like salicyclic acid and jasmonic acid have been explored as effector molecules to regulate the expression of stress inducible genes in plants. Salicylic acid (SA) is one of the important phytohormone signal molecules involved in disease resistance in plants (Alvarez, 2000; Desveaux *et al*., 2004). Many promoters have been identified which are induced by salicylic acid, such as soybean *IFS* promoter, tobacco *PR-1a* and *PR-2d* promoters, *Gastrodia elata GAFP-2* promoter and *Arabidopsis GST6* promoter (Yin *et al*., 2004). These promoters contain the SA responsive *cis*-acting element TGACG, which belongs to the family of activation sequence-1 elements, is reported to function as a transcriptional enhancer conferring SA inducibility to reporter genes in transgenic plants (Subramanian *et al*., 2004). Jasmonates (JAs) are vital regulators of abiotic and biotic stresses in plants, JA also plays important roles in physiological and developmental processes, including root growth, senescence, trichome formation, cell cycle progression, and flower development (Wasternack, 2007; Pauwels *et al*., 2008). MeJA-responsive *cis*-acting elements have been identified in the promoters of several JA-regulated genes (Kim *et al.*, 1992; Ruíz-Rivero and Prat, 1998; Guerineau *et al.*, 2003).

Tissue or organ specific promoters

These promoters show restricted expression to particular cells, tissues, organs or developmental stages of a plant. They are also called spatiotemporal promoters. Many promoters have been identified that drive tissue or organ dependent expression of the target gene specifically to the seed, or to a particular region of the seed. Storage proteins such as corn zein (Schernthaner *et al*., 1988), rice glutelin (Takaiwa *et al.*, 1991), barley hordein (Marris *et al*., 1988), rice prolamin (Qu and Takaiwa, 2004) and wheat glutenin (Colot *et al*., 1987) have been rich sources of seed-specific promoters, predominantly directing expression to the endosperm (Wobus *et al*., 1995). Additional promoters have been shown to direct gene expression to the embryo and aleurone (Furtado and Henry, 2005). Many anther-specific and pollen specific promoters have been identified in a variety of plants, including the TA29 promoter from tobacco (Koltunow *et al*., 1990), the A9 promoter from *Arabidopsis* (Paul *et al*., 1992) and the RA8 promoter from rice (Jeon *et al.*, 1999).

Synthetic promoters

In plant genetic engineering, the availability of a range of defined synthetic plant promoters that can drive the gauged expression of genes would be advantageous, because, such promoters can be exploited to study various signalling pathways and also engineer plants with disease resistant genes that can be expressed only when needed. These promoters can be artificially designed by three ways: (a) By combining defined *cis*-regulatory element with strong constitutive promoter (Rushton *et al.*, 2002; Gurr and Rushton, 2005) or by duplicating the upstream enhancer domains in conjunction with strong promoter (Maiti *et al*., 1997); (b) By combining *cis*-regulatory elements from different promoters (Sawant *et al.*, 2001); (c) By fusing two strong constitutive characterised promoters to develop hybrids that allow both the promoters to be active in either direction or by developing bidirectional promoters (Comai *et al.*, 1990). The best approach to fine tune and restricted gene expression was developed by (Jensen and Hammer, 1998; Hammer *et al.*, 2006).

Characterisation and *Insilco* analysis of promoters

The characterization of promoter can be done by first analyzing the DNA sequences through BLAST search of the flanking region against EMBL database such as NCBI (www.ncbi.nlm.nih.gov/) and followed by *in silico* analysis. The *in silico* based promoter prediction tool basically works on two approaches, one of the methods involves search by structural content; like Gene2 Promoter (http://potal.O.genomatrix.de/products/GFene2Promoter), Promoter scan (Pretridge, 1991), which utilizes the information of specific structural features of a promoter based on the actual three-dimensional structure adapted by a promoter element during gene expression process *in vivo*. The other approach of promoter prediction tools like PLACE (Higo *et al*., 1999; www.dna.affrc.go.jp/PLACE/), PlantCare Lescot *et al*., 2002; http:// bioinformatics.psb.ugent.be/webtools/plantcare/html/), (http://www.ppdb. gene.nagoyau.ac.jp), At*cis*Db (http://*Arabidopsis*.med. ohiostate.edu/At*cis*DB/index.jsp) performs 'search by signals' in which the algorithms aim to identify regulatory regions and promoters based on sequence composition. The signal based promoter predictions involve detection of *cis*-acting regulatory elements (CAREs), which are very short stretch of conserved nucleotides and define transcriptional specificity. However detection of CAREs based on *in silico* approaches does not always find functionally relevant. Since the sequence is very short stretches of nucleotides, there is always a random chance of finding such sequences in any stretch of DNA (Blanchette and Sinha, 2001). One of the few approaches to overcome the limitation is to carry out a phylogenetic foot printing to find conserved regulatory elements among functionally related promoters of diverse species or between co-expressed genes. The identity of such elements can only be confirmed by experiments through transgenic studies.

Functional characterization of the plant promoters

In order to test the newly isolated promoter a suitable host is required to understand their role in gene expression. Although, significant progress has been made in many species but most commonly used host plant model system is *Arabidopsis thaliana* (Meyerowitz and Somerville, 1994). Many promoters from different plant species were functionally analysed in *A. thaliana* using transgenic approach. For example anther-specific and pollen specific promoters like TA29 promoter from tobacco (Koltunow *et al*., 1990), and *LAT52* from tomato (Twell *et al.*, 1990) , seedling and flower specific promoter, the A9 promoter from *Arabidopsis* (Paul *et al*., 1992) and the RA8 promoter from rice (Jeon *et al.*, 1999). OrysaEULS2, OrysaEULS3, and OrysaEULD1A from rice (Al Atalah *et al*., 2014), OsPHY1 from rice (Guo *et al.*, 2013). Another approach for the functional validation of plant based promoters is agroinfiltration. Deletion studies of promoters have led us in identification of the important *cis*-elemnts or regulatory

motifs that are essential for conferring specificity. For example, deletion of ABA-responsive *cis*-element in plant stress inducible promoters has shown the functional significance of commonly found consensus sequence elements like 'ACGT' box mediates ABA induction (Shen *et al.*, 1996). There are various approaches for promoter deletion to generate deletion fragments which includes restriction endonucleases (Yang *et al.*, 1995), or sequential deletion of the promoter fragments using exonuclease III enzyme (Leyva *et al.*, 1992; Meister *et al.*, 2004) or PCR amplification of a promoter region by sequence specific primers. PCR based approach of the promoter deletion is commonly utilised method for functional validation of the promoter as well as their sequence motifs (Srinivasan and Saha, 2010).

Conclusion

The present review highlights the importance of stress inducible promoters in plant genetic engineering and also the disadvantages of constitutive promoters. One of the greatest challenges in the plant genetic engineering is the identification of stress inducible promoters which should replace constitutive promoter like 35S promoter. The use of constitutive promoters for developing disease resistant or stress tolerant crop varieties is not always desirable, because constitutive overexpression of transgenes may compete for the building blocks that are required for plant growth under normal conditions. Therefore, stress or pathogen-inducible promoters are expected to be optimal for driving transgenes.

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