Biotic stress inducible promoters in crop plants- a review
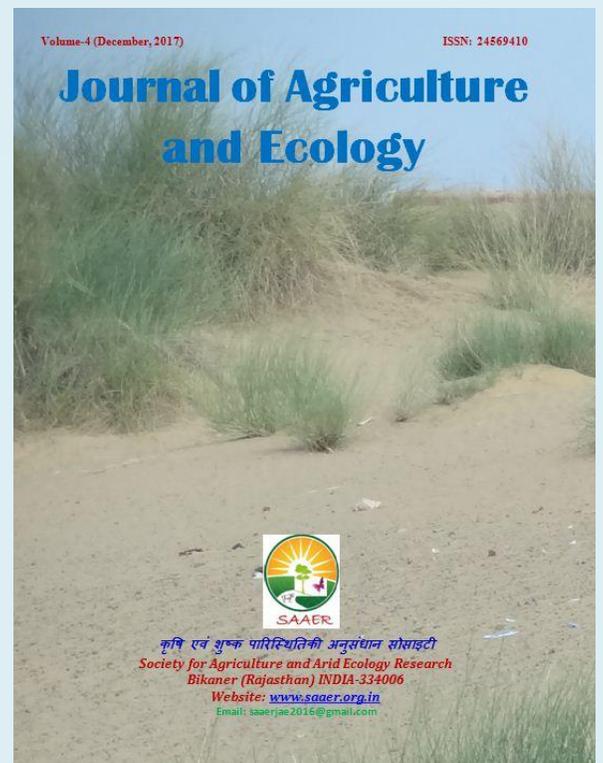
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Biotic stress inducible promoters in crop plants- a review

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Article Info

Abstract

Promoter is a DNA sequence that regulates the expression of a particular gene. They are classified on the basis of their function and spatio-temporal expression into constitutive, tissue-specific or development-stage-specific and inducible promoters. Plant genes associated with defence responses are activated by stress-factors and these genes are known to be regulated by promoters or the upstream elements. Promoters induced by abiotic stress factors in plants are fairly well studied compared to biotic stresses. This review presented information generated on promoters and regulatory elements involved in defence gene expression due to insect damage, pathogen and nematode attack to crop plants, mechanism and their utilization in crop improvement through genetic engineering.

Introduction

Multicellular organisms, including plants maintain their inherited genetic material in most of their cells throughout their life-cycle through different conserved molecular and developmental processes. Regulated selective expression of genes through gene regulation led to development of various specialized tissues and organs during various growth and developmental stages. Regulation at the transcription site plays a major role in regulating the quantity and its spatio-temporal expression pattern. The successful binding of transcription machinery upon the promoter region ensures the expression of the gene. Promoters are the DNA sequences upstream to a gene's coding region that contains specific *cis*-regulatory sequences recognized by the proteins for initiation of transcription (Pilpel

et al. 2001) (Fig. 1). The *cis*-regulatory sequences or "motifs" are functionally important regions of the promoter. The variability in the *cis*-regulatory sequences in the promoter region regulates the protein binding and also the expression pattern of the gene. Transcription factors/regulatory proteins recognize these conserved *cis*-regulatory motifs and their binding is regulated in response to developmental and environmental cues (MacIsaac & Fraenkel 2006). The *cis*-regulatory sequences can be discretized into *cis*-regulatory elements (CREs) that are composed of conserved DNA sequences (typically, non-coding DNA) containing binding sites for transcription factors and/or regulatory proteins that are essential to activate and sustain transcription (Ong & Corces 2011; Gupta et al. 2012). In plants, most of the promoters are located proximally to the transcription start sites (TSS), while some genes contain alternative promoters that can activate transcription from different positions in the gene (Srinivasan & Saha 2009). The core promoter elements are conserved minimal DNA sequences that are necessary and sufficient for initiation for transcription (Roeder 1996). The TATA box, TATA (A/T)A, located -25 to -35 bases upstream to the TSS, is one of the major CREs that are required to mediate the direct binding of the transcription factor TFIID complex to a TSS (Buratowski et al. 1989; Sainsbury et al. 2015). The GC box and CCAAT box are present at -80 to -150 bp operates co-operatively with other conserved motifs (Zuo & Li 2011; Muthusamy et al. 2017). Promoters without the TATA box or the TATA-less promoters are also found, and they are found mostly in photosynthetic plant genes (Bernard et al. 2010).
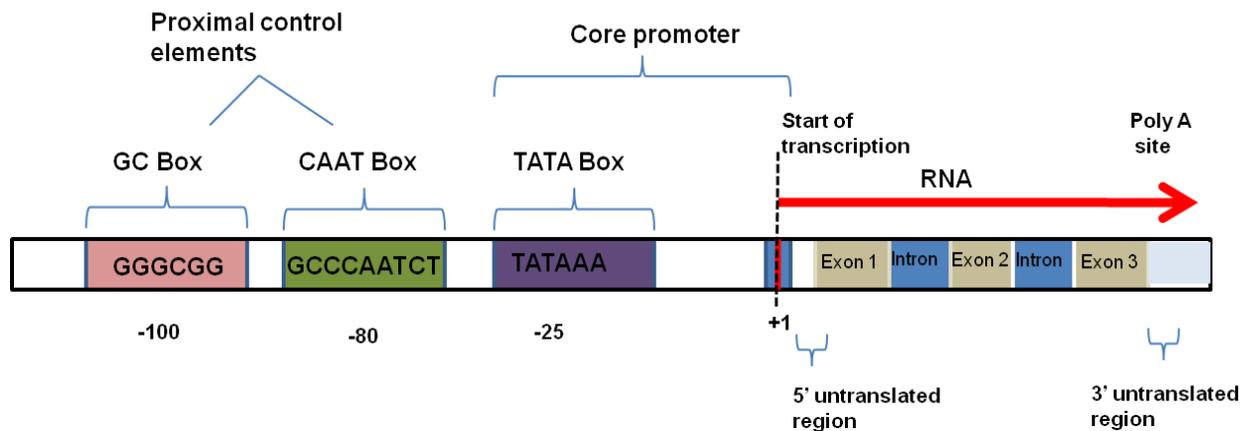

Fig. 1 Typical structure of eukaryotic promoter of a gene

Types of promoters

Constitutive promoters

These promoters are constitutive and the direct the constant expression of genes in most of the tissues and developmental stages. Constitutive promoter's expression is not conditioned by endogenous, environmental and developmental factors. These promoters are usually functionally active across species and even across kingdoms. Most of the plant housekeeping genes and plant virus genes are expressed under constitutive promoter. The CaMV35S promoter derived from the cauliflower mosaic virus is one of the most widely utilized constitutive promoters for basic research and the development of transgenics (Odell et al. 1985). These promoters express constitutively and drive high level of transgene expression in both monocots and dicots. The presence of multiple tissue-specific *cis*-elements with additive function enhances the expression of CaMV35S promoter (Hernandez-Garcia & Finer 2014). Constitutive promoters are preferred for evaluating the transgenes as transgene effects may be easier to score if the introduced gene can be expressed in most of the tissues under many different conditions (Abdeeva et al. 2012). It is often used as a promoter for expressing selectable marker genes because constitute expression of markers is necessary for efficient selection of transgenic plants (Abdeeva et al. 2012). However, constitutive expression of the transgene where it's function if not normally required competes for the plant energy and lead to decreased growth. On the other hand, overexpression of transgene using constitutive promoters may activate the endogenous post-transcriptional gene silencing pathway which can lead to silencing of transgene expression.

Tissue-specific or development-stage-specific promoters

These promoters are not constitutive and direct the expression of a gene only in specific tissue (s) or at certain development stages. These promoters have a tightly regulated pattern of expression which can be very handy in the sense that the expression of the transgene is achieved only under a certain condition, tissue or developmental stage (Srinivasan & Saha 2009; Muthusamy et al. 2016). The selective spatio-temporal expression of genes conserves the plant energy efficiently and also plays vital role in formation of specialized tissue in plant growth and development (Muthusamy et al. 2016, 2017). Tissue-specific or development-stage-specific promoters can be used to express the transgene at desirious spatio-temporal pattern. For example tomato *LeEXP1* gene was overexpressed using fruit-specific promoter *LeACS4* to enhance the fruit texture of transgenic tomato (Kaur et al. 2010).

Synthetic promoters

Synthetic promoters are developed *in-vivo* by bringing together the primary elements of a promoter region from diverse origins. The strength and specificity of the promoter can be tailored by manipulating its *cis*-elements by the use of recombinant DNA technologies (Dey et al. 2015).

Inducible promoters

Inducible promoters direct the expression of a gene only at certain conditions *i.e* presence of inducers chemical/ environmental factors. Their performance is not conditioned to endogenous factors but to environmental conditions and external stimuli that can be artificially controlled (Tang et al. 2004). Within this group, there are promoters modulated by abiotic factors (light, oxygen levels, heat, metal and cold) and biotic factors (insects, fungi, bacteria, nematode, *etc*.). Overexpression of defense genes with a strong constitutive promoter probably exert load on plant cellular machinery can led to stunted growth and reduction of yield in transgenic plants(Gurr & Rushton 2005; Mazarei et al. 2008; Lin & Chen 2017). Identification and characterization of tissue-specific stress-inducible promoters would be of larger practical value as they avoid the unnecessary physiological burdens associated with constitutive expression of transgenes on the host plant by restricting their expression to specific time and the site of infection (Hernandez-Garcia & Finer 2014). Therefore, inducible promoters, which are expressed only when exposed to stresses, are of importance for developing transgenic plants (Ferry & Gatehouse 2010; Sanghera et al. 2011). Engineering defense genes under inducible promoter may provide a new strategy for the control of diverse insects/pathogens (Biłas et al. 2016). It is highly imperative to identify and characterize the stress responsive promoters for development of transgenic for biotic stress tolerance (Ferry & Gatehouse 2010; Sanghera et al. 2011). So far, only a few pathogen responsive promoters were identified and functionally characterized in plants. The details of the identified biotic stress responsive promoters are shown in table 1.

Table 1. Biotic stress induced promoters in crops

Promoter	Crop	Pathogen	Reference
R2329 and R2184	Rice	Blast	(Sasaki et al. 2007)
Pi54rh	Rice	Blast	(Das et al. 2012)
OsWRKY53	Rice	Broad spectrum (Fungus)	(Chujo et al. 2007)
PRPI	Rice and wheat	Broad spectrum (Fungus)	(Kovalchuk et al. 2010)
AGO18	Rice	Broad spectrum (virus)	(Wu et al. 2015)
GhMPK7	Cotton	Fungus and virus	(Shi et al. 2010)
PBZ1	Rice	Fungus (*Magnaporthe grisea*)	(Mei et al. 2006)
Pita	Rice	Blast fungus	(Ramkumar et al. 2014)
CYP76M7	Rice	Blast fungus	(Vijayan et al. 2015)
CMPG1	Parsley	Fungus	(Kirsch et al. 2001)
Hahsp17.7G4	Sunflower	Nematode	(Escobar et al. 2003)
cry1Ab	Broccoli	Insect	(Cao et al. 2001)

Biotic stress inducible promoters

Insect-inducible promoters

In potato, Insect attack or other severe wounding induces the expression of potato *proteinase inhibitor II* (*pinII*) gene. Transgenic *Arabidopsis* lines carrying GUS gene containing potato *pinII* promoter displayed induced expression under wound- and insect-attack (An et al. 1989; Godard et al. 2007). The potato *proteinase inhibitor II* (*pinII*) promoter was induced in most of the plants and considered as an ideal promoter for expression of *defensin* gene (Yang et al. 2008). The expression of *mannopine synthase* (*mas*) (Langridge et al. 1989) and *nopaline synthase* (*nos*) (An, 1990) promoters were induced under wound- and insect-attack in leaf and stem tissues. Transgenic peanut (*Arachis hypogaea* L.) expressing transgene *Cry1EC* from an inducible promoter *PR1-a* confers enhanced resistance to the insect *Spodoptera litura* (Zhu-Salzman et al. 2004; Tiwari et al. 2011). Insect-inducible *PR1-a* promoter is considered as a ideal promoter for developing transgenic for aphid resistance genes, because the expression of the genes under these promoter were only induced during aphid attack (Will & Vilcinskas 2013). Tomato *Lipoxygenase D* (*TomLoxD*) promoter displayed induced expression under wound- and insect-attack (Yan et al. 2013). Transgenic broccoli expressing insecticidal trangene *cry1Ab* under inducible promoter *PR-1a* display resistance to insect diamondback moth (Cao et al. 2001).

Nematode-inducible promoters

Plant parasitic nematodes cause severe yield loss in major crops all over the world. Fewer efforts have been made to isolate the nematode inducible promoters. In Arabidopsis, *Pdf2.1*, *Pdf2.2* and *Pdf2.3* promoters displayed induced expression under beet cyst nematode *Heterodera schachtii* infestation (Siddique et al. 2011). The *GUS* reporter gene fused with the nematode-responsive-root-specific promoter (AT1G26530) displayed induced expression under root-knot nematode *Meloidogyne incognita* infection (Kumar et al. 2016). Development of transgenics using RNAi based strategy along with indicuble promoters against plant parasitic nematodes can be a ideal strategy to combact the plant parasitic nematodes (Banerjee et al. 2017).

Pathogen-inducible promoters

Viral, bacterial and fungal pathogens are a major threat to crop production worldwide. Plants has evolved various complex mechanism to express pathogen responsive proteins e.g. PR proteins, anti-viral etc., to resist the pathogen infection. Transgenic expression of defensin, PR, anti-viral genes etc., increased the tolerance of transgenic plants to pathogenic infections (Gurr & Rushton 2005; Sanghera et al. 2011). *Phenylalanine ammonia-lyase* promoter (*PAL1*) expression was induced under bacterial pathogen *Pseudomonas syringae* infection (Giacomin & Szalay 1996). In parsley, a pathogen responsive *CMPG1* gene is induced immediately after pathogen attack. Promoter analysis of *CMPG1* showed the

pivitol role of *cis-regulatory* sequences W box and elicitor-responsive element in gene induction. (Kirsch et al. 2001). The *cis-regulatory* sequence W box, elicitor-responsive element, TC-rich repeats and TCA-element were frequently found in the promoters of pathogen-induced genes in plants (Muthusamy et al. 2016, 2017). The conserved *cis*-elements present in the pathogen-inducible promoters were given in table.2. The expression of *GhMPK7* promoter induced under the treatments with defence signaling molecules and phytohormones Transgenic tobacco plants overexpressing *GhMPK7* gene displayed induced resistance to fungus *Colletotrichum nicotianae* and Potato virus Y (Shi et al. 2010). Several fungal inducible promoter have been clone in plants (Table 1). However minimal efforts have been made to identify bacterial and virus inducible promoters. Next-generation sequencing technologies are providing new ways to mine the biotic-stress inducible promoters in the complex genomes (Muthusamy et al. 2017). The availability of crop genomic resources are increasing rapidly in the public databases (Srinivasan & Saha 2009; Mochida &

Shinozaki 2010). These genomic resources can be efficiently utilized through computational tools for mining biotic-stress inducible promoters. Several omics strategies are available for mining promoters. RNASeq/microarray datasets can be utilized to mine biotic-stress responsive genes (Kumar et al. 2016). The upstream promoter sequences of the corresponding gene can be mined from crop genome database and the conserved biotic-stress responsive *cis*-acting regulatory elements can be predicted by *insilico* analysis of promoter sequences in PlantCARE database (Lescot et al. 2002; Muthusamy et al. 2017). Identified putative promoters can be further functionally characterized using transgenic approach (Sanghera et al. 2011; Abdeeva et al. 2012). The functionally validated promoters can be utilized efficiently in crop improvement programs (Srinivasan and Saha, 2009; Kumar et al. 2016). The regulatory protein binds to the *cis-regulatory* sequence present in the biotic stress-inducible promoters and regulates the expression of stress-inducible genes. List of biotic stress-responsive *cis-regulatory* sequences identified in plants were shown in table. 2.

Table 2. *Cis-* regulatory elements in the biotic stress-inducible promoters in plants*

cis-regulatory elements	*cis-regulatory* sequence	Function
W box	TTGACC	Fungal elicitor responsive element
TC-rich repeats	ATTTTCTTCA	*cis*-acting element involved in defense and stress responsiveness
TCA-element	CCATCTTTTT	*cis*-acting element involved in salicylic acid responsiveness
CGTCA-motif	CGTCA	*cis*-acting regulatory element involved in the MeJA-responsiveness
SARE	TTCGACCTCCTT	*cis*-acting element involved in salicylic acid responsiveness
WUN-motif	AAATTTCCT	Wound-responsive element

EIRE	TTCGACC	Elicitor-responsive element
ELI-box3	AACCAATT	Elicitor-responsive element
box S	AGCCACC	Wounding and pathogen responsiveness
box E	ACCCATCAAG	*cis*-element for induction upon fungal elicitation
GCC box	AGCCGCC	Elicitor-responsive element, wounding and pathogen responsiveness
JERE and JASE1	CGTCAATGAA	Jasmonic acid responsive element
NPR1-motif	TTGACTTGAC	Salicylic acid responsive element

*Cis-regulatory sequence information adapted from PLANTCARE data base (Lescot et al. 2002; Muthusamy et al. 2017)

Conclusion

Constitutive expression of transgenes has been shown to exert load on plant cellular machinery resulted in stunted growth and reduction of yield in transgenic plants. Constitutive promoters are useful for high-level expression of selectable marker genes, efficient for selection and generation of transgenic plants. However, these promoters are not always desirable for generating transgenic plants because the constitutive expression of the transgene competes for energy that is required for plant growth and development. Using a stress-inducible promoter induces the transgene expression only during stress conditions. Unlike constitutive promoters, the biotic stress-inducible promoter doesn't compete for energy, expressed only during infection. Fewer efforts have been made to identify insect, nematode and pathogen responsive-inducible promoters in horticulture crops. Identification and characterization of biotic-stress inducible promoters would be of larger practical value in horticulture crops as they avoid the unnecessary physiological burdens associated with constitutive expression of transgenes on the host plant by restricting their expression to specific time and the site of infection. Engineering defense genes under inducible promoter may provide a new strategy for the control of diverse biotic stresses. Therefore, isolation and characterization of biotic stress inducible promoters suitable for plant genetic engineering are highly desirable.

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