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Pathogen Inducible *cis*-acting Elements of Synthetic Promoters in Plants-review

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ABSTRACT

Synthetic pathogen inducible promoters are used for the improvement and application of transgenic techniques in research and to increase agriculture production. The promoter contains specific cis-regulatory elements (W box, GCC box, Box S and D box) which induce anti pathogen molecular cascades. Insertion of dimerized form of cis acting elements at upstream region of promoter in promoter probe vector drives the expression of resistance gene or reporter gene. The expression indicates that synthetic promoters are responded to fungal elicitors. Expression of resistance restrict to infection sites which boost disease resistance in plants.

Keywords: Promoters; synthetic promoters; cis-actings elements; pathogen inducible promoters.

1. INTRODUCTION

Promoter is a *cis*-regulatory DNA sequence controlling the transcription of a region normally located to its downstream. However, promoter sequences are also found within the transcribed region (gene) [1, 2,3,4]. It contains a TATA box and serving as the start site of transcription [5].

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Transcription factors (TF) and RNA polymerase recognize a promoter by its structural features and associate with it to initiate transcription. In this process, the newly formed complex positions RNA polymerase at the transcription initiation site and activates transcription [6].

The promoter can generally be divided in two parts: a proximal part (3') and a distal part (5'). The proximal part is believed to be responsible for correctly assembling the RNA polymerase II complex at the right position and for drives a basal level of transcription [7,8,9]. This assembling is mediated by elements, such as TATA and initiator boxes through the binding of the TATA box-binding protein, and other general TFs [10,11]. The distal part of the promoter is believed to contain *cis*-acting elements that regulate the spatio-temporal expression [12,13]. In addition to the proximal and distal parts, somewhat isolated, regulatory regions have also been described, both in plants and animals that contain enhancer and/or repressor elements [14,15]. The latter elements can be found from a few kilo base pairs upstream from the TSS, in the introns, or even at the 3' side of the genes they regulate [16,17].

Cis-acting elements found in the distal part of the promoters, generally make up the transcription factor (TF) binding sites. There can be multiple elements each consisting of short sequences (5 to 20 nucleotides), thus representing a modular structure. These elements can be dispersed or can overlap. A constitutive promoter contains elements recognized by basal and upstream activators to initiate transcription in all tissues and at all times. However, inducible promoters are activated by one or more stimuli such as hormones, chemicals, environmental conditions/stresses and biotic stresses, where as tissue-specific promoters control gene expression in a tissue-dependent manner and according to the developmental stage of the plant. Hence the kind of *cis*-acting elements within a promoter decides its nature of expression. The merging of these regulatory elements is often unique for most genes or pathways.

Various stresses induce plants' response in terms of gene expression. Perception of a pathogen by a plant triggers rapid defense responses via a number of signal transduction pathways. A major target of signal transduction is the cell nucleus where the terminal signals lead to the transcriptional activation of numerous genes and consequently to the *de novo* synthesis of a variety of proteins and antimicrobial compounds. Transcriptional activation is brought about by binding of transcription factors to the *cis*-acting elements present in the promoter of pathogen responsive genes. Such *cis*-acting regulatory elements are recognized as W, D, Gst, GCC, S and Myb boxes etc, which are the binding sites for various transcription factors. These *cis*-acting elements can alone mediate pathogen-inducible expression *in planta*. When taken out of their native promoter contexts, they retain pathogen inducibility directing expression that is local and that correlates with the extent of growth of the pathogen.

To design and synthesize pathogen inducible promoters that contain various copies of *cis*-acting regulatory elements fused to a minimal promoter. Effects of varying the number, order, and spacing of such elements on their inducibility have been established using synthetic promoters. The activity of a promoter can be monitored and characterized at the mRNA and/or protein level of the gene it drives. The most common approach to study the activity of a plant promoter, however, is to employ a promoter probe vector wherein the promoter to be tested is fused to a gene coding for reporters, such as the β-glucuronidase (GUS) [18], the luciferase (LUC) [19], the chloramphenicol acetyl transferase (CAT) [20], the green fluorescent protein (GFP) gene [21] or XylanaseA [22,23] for monitoring the expression of reporters in stably or transiently transformed tissues. By this approach, the expression pattern of a promoter can be analyzed qualitatively and/or quantitatively in plant tissues, and its expression pattern in response to environmental conditions can be characterized by exposing the transgenic plants to those conditions.

2. PATHOGEN INDUCIBLE PROMOTERS

The ideal pathogen-inducible promoter would be rapidly activated by a wide array of pathogens, and be inactive under disease-free conditions. Otherwise, the biosynthesis of abundant, unnecessary recombinant proteins controlled by strong constitutive promoters in transgenic plants can represent a high metabolic cost and eventually impact the energy allocated into traits of interest such as yield and biomass [24]. Plant-pathogen interactions can be split into non host, biotrophic and necrotrophic based on the life-styles of pathogens [25]. Plants respond to

pathogens using different signaling pathways. Salicylic acid signaling pathways are usually involved in response to biotrophic pathogens and it has been shown that jasmonic acid and ethylene-related signaling pathways control defense against necrotrophic pathogens. The signaling pathways have many cross-talks and interactions. Wounding shows considerable overlap with jasmonic acid and ethylene signaling pathways [26]. A major target of signal transduction is the cell nucleus where the terminal signals lead to the transcriptional activation of numerous genes and consequently to the *de novo* synthesis of a variety of proteins and antimicrobial compounds [27,28]. Transcription activation of pathogen-responsive genes is mediated by pathogen-inducible promoters. An ideal pathogen-inducible promoter is the one that is activated rapidly to a wide range of pathogens, and inactivated under disease-free conditions. Also it should not lead to spurious defense responses triggered by leaky expression of the transgene [29], which otherwise results into an uncontrolled spread of gene expression; so called “run away cell death”. Several pathogen-responsive transcription factors (TFs) have been identified in recent years [30]. Most prominently, the WRKY TFs are major regulators in plant-pathogen interactions [31]. In Arabidopsis (*Arabidopsis thaliana*), WRKY22 and 29 were described as downstream targets of a flagellin-regulated mitogen-activated protein kinase (MPK) signal transduction pathway involving MPK3 and -6 [32]. MPK4 forms a nuclear complex with MKS (for mitogen-activated protein kinase substrate) and WRKY33. When WRKY33 is released from this complex upon phosphorylation of MKS by MPK4 and it activates the transcription of target genes [33].

3. *Cis*-acting ELEMENTS OF PATHOGEN-INDUCIBLE PROMOTERS

Hitherto, several pathogen-inducible genes and their promoters have been identified in plants. *cis-acting* regulatory elements are essential for transcriptions of gene regulatory units because they control various stress responses. Recent advancements in such experimental techniques as RNA interference, microarrays, RNAseq and others have allowed identification and investigation of promoter regions of target genes but these techniques are expensive and technically challenging. Therefore, computational methods are being used to search the promoter regions for different cis-elements responsible for the regulation of the genes [34]. Different computer programs can also be used to look for known cis-elements and to study their organization. Such web-based tools as PLACE [35], PlantCARE [36], AGRIS [37], TRANSFAC [38] and PlantPAN [39], have been developed for the analysis of cis regulatory elements in plant genes.

3.1 W Box

In parsley genome, PR1 is a pathogenesis-related protein encoded by a family of three genes (PR1-1, PR1-2 and PR1-3). Down and up regulation of function experiments in a transient expression system demonstrated the presence of two fungal elicitor responsive elements in each of the PR1-1 and PR1-2 promoters. W1, W2 and W3 elements contain the sequence (T) TGAC(C) and disrupt of this sequence abolishes the function by mutations. Loss- and gain-of- function experiments showed that W boxes act as important elements required for elicitor responsive expression of PR1-1 and PR1-2. W boxes function independently of each other, indicating a redundancy in function. Box WI and Box W2 both contain TTGACC elements, whereas Box W3 has two TGAC elements. The W boxes are therefore different at the sequence level. However, their similarity in function, nuclear protein binding and binding by WRKY1, 2 and 3 suggests that they are similar elements that are characterized by a TGAC core.

Not all W box–containing synthetic promoters behave similarly. For example, both transient expression experiments and results from transgenic plants show that box W2 is much stronger than box W1, even though both contain the same TTGACC core element. W-box present in the promoter of *PcCMPG1* gene in *Petroselinum crispum* does not respond to wounding, but respond to pathogen, therefore bear potential for new approaches to diseases resistance breeding in crop plants [40]. WRKY1,-2 and -3 proteins bind specifically *in vitro* to functionally defined elicitor-response elements of the W box type [(T)TGAC(C)], designated W1, W2 and W3, present in *PR1* promoters [41]. Park et al. [42] reported that CaWRKY-a protein had W-box binding activity and also involved as a transcription factor in plant defense-related signal transduction pathways [40].

There is increasing evidence that W boxes are a major class of *cis*-acting elements responsible for the pathogen inducibility of many plant genes [43,44]. The significance of W boxes was

illustrated by studies of the Arabidopsis transcriptome during systemic acquired resistance (SAR) [45,46]. In some cases, clustering of W boxes may be associated with inducibility by pathogens. In parsley, three WRKY proteins bind specifically to functional W boxes in the PRI-1 and PRI-2 promoters [44]. The binding site for members of the WRKY family transcription factors is W box [(T)TGAC (C/T) [44].

3.2 GCC Box

Biochemical analysis revealed that JERF1 bound not only to the GCC box but also to the DRE sequence. Expression of the JERF1 gene in tomato was induced by ethylene, methyl jasmonate (MeJA), abscisic acid (ABA) and salt treatment, indicating that JERF1 might act as a connector among different signal transduction pathways. Further studies with transgenic JERF1 tobacco plants indicated that over expression of JERF1 resulted in activated expression of GCC box-containing genes such as *osmotin, GLA, Prb-1b* and *CHN50* under normal growth conditions and later resulted in increased tolerance to salt stress. The three (*Pti4, Pti5, Pti6*) Pti proteins belong to the EREBP family of transcription factors and bind *in vitro* to GCC box and expressed specifically during stress but not abiotic or hormonal stresses, suggesting a specific role of *Pti* in plant defense against pathogens [47]. In addition, the GCC-box is implicated in ozone-pathogenesis-related PR1 protein gene via ethylene-dependent signaling [48].

MeJA treatment and fungal elicitors rapidly induce transcript levels of Orca2 and Orca3.The ORCA2 and ORCA3 proteins regulate overlapping but distinct sets of genes associated with secondary metabolism via specific binding to promoter elements called the jasmonate and elicitor-responsive elements, which contains a core GCC-box [49,50] reported that the GCC-box plays a key role in conferring jasmonate responsiveness to the PDF1.2 promoter. However, deletion or specific mutations introduced in to the core GCC-box sequence did not completely eliminate the jasmonate responsiveness of the promoter, suggesting that the other promoter elements present downstream from the GCC-box region may contribute to expression of jasmonate responsiveness.

Ohme-Takagi and Shinshi [51] revealed that ethylene-inducible pathogenesis-related protein genes has GCC box, which is a 11 bp sequence (TAAGAGCCGCC) was conserved in the 5‘ upstream region of in *Nicotiana* spp and in some other plants. A number of proteins that bind to GCC boxes have been isolated and found to be members of the ethylene-responsive element binding proteins (EREBP). These transcription factors also bind to dehydration-responsive element (DRE) present in the promoters of drought related genes. Zhang et al. [11] by using yeast one-hybrid assay isolated a cDNA coding for the transcription factor Jasmonate and Ethylene Response Factor 1 (JERF1) from tomato. Biochemical analysis revealed that JERF1 bound not only to the GCC box (Brown *et al,* 2003) but also to the DRE sequence. Expression of the JERF1 gene in tomato was induced by ethylene, methyl jasmonate (MeJA), abscisic acid (ABA) and salt treatment, indicating that JERF1 might act as a connector among different signal transduction pathways. Further studies with transgenic JERF1 tobacco plants indicated that over expression of JERF1 resulted in activated expression of GCC box-containing genes such as *osmotin, GLA, Prb-1b* and *CHN50* under normal growth conditions and later on resulted in increase tolerance to salt stress. In rice promoter regions, G-box, GCC-box, and H-box of which, 53.5% were up- or down-regulated when pathogens attack. The PICEs in the promoters are critical for rice response to pathogen infections. They are also useful markers for identification of rice genes involved in response to pathogen infections [52].

3.3 S Box

In parsley, ELI7 gene family members were expeditiously transcriptionally activated by an elicitor derived from the phytopathogen *Phytophthora sojae*. Several cDNA and genomic clones of ELI7 were isolated [53]. The deduced amino acid sequences revealed close similarity to fatty acid denaturases/hydroxylases, however, the precise functions are still unknown. Analysis of the promoters of two strongly elicitor-induced family members, ELI7.1 and ELI7.2, indicated a novel, independently acting regulatory region (S box). *In situ* RNA/RNA hybridization using an ELI7.1 gene-specific probe demonstrated that expression of this gene is rapidly and locally induced around infection sites *in planta* as well [53].

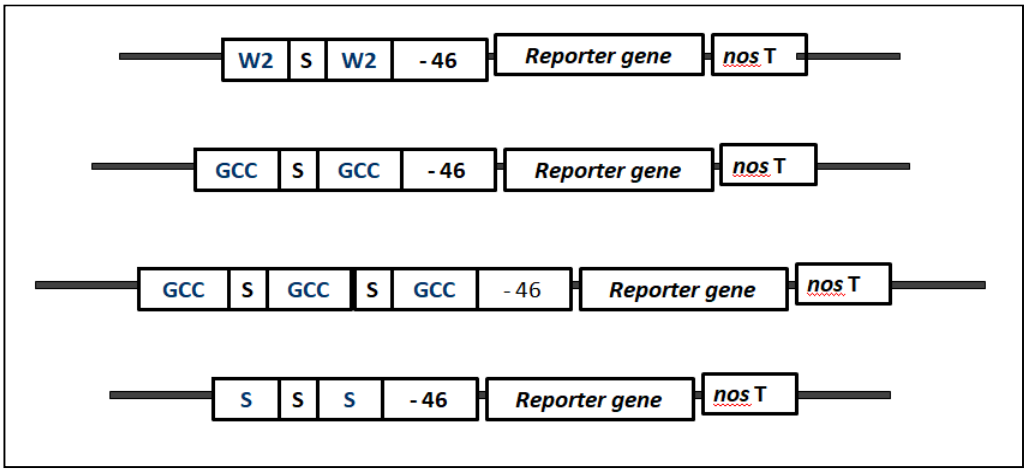

Fig. 1. Synthetic pathogen inducible plant promoters

3.4 D Box

Box D was discovered as a DNase1 footprint from approximately -76 to -52 in the parsley *PR2* promoter [54]. This region (box D short) had high elicitor inducibility but was weak. Because the exact extent of the element was unclear, a longer version (box D) containing the next six bases from the *PR2* promoter at the 3' end was constructed. Box D longer was almost 30 times stronger than 4x D short but inducibility was reduced.

3.5 GST1

Interestingly, although more than one type of *cis*-acting element is not required for pathogen inducibility, some pathogen-inducible promoters contain elements of more than one type. An example is the Gst1 box, which contains both a W box and an S box. This places the *gst1* gene under the control of WRKY and APETALA2 (AP2)/ethylene-responsive factors (ERF). The potato *gst1* promoter has been shown to be activated transcriptionally in response to pathogens [55]. It may be common that signaling pathways operating via different transcription factors can target the same gene. Similarly, promoter of the parsley *WRKY1* gene contains a W box and a GCC box [41].

3.6 MYB Recognition Elements

Two motifs, MYBPLANT (A/CACCA/TAA/CC) and MYBPZM (CCA/TACC) are the candidate target *cis*-elements of MYB (myeloblastosis) transcription factors in the anthocyanin pathways in *Antirrhinum* [56,57] and maize [58]. In Arabidopsis, Myb mRNA was induced by dehydration and disappeared upon rehydration. It also accumulated upon salt stress and with the onset of treatment with abscisic acid. AtMyb2 bound specifically to a consensus sequences, TAACTG found in maize bronze 1 and Arabidopsis rd22 promoter [59].

Recent studies on transient transactivation experiments with *Arabidopsis* leaf protoplasts have confirmed the function of *Arabidopsis* MYC (*rd22BP1*) and MYB (*ATMYB2*) proteins acting as transcriptional activators in ABA and dehydration inducible expression using a 67 bp region of the *rd22* gene promoter containing the myc and myb DNA recognition elements [60].

3.7 G Box

Environmental cues such as ABA, light, UV radiation and wounding as well as pathogen signals regulates G box (CACGTG) function of diverse gene [61]. G box is a member of the family of ACGT-containing *cis*-acting elements that respond to pathogen attack [62]. G box functions in concert with H box in the promoter of bean chalcone synthase (*CHS*) gene to regulate floral and root-specific expression [63].

3.8 Ocs-element

The expression of pathogen genes in infected plants and plant defense genes requires group of promoter's i.e Ocs-elements. They were first identified as 20 bp (CTGACGTAAGGGA TGACGCA) sequences located less than 200 bp

from TATA of promoters of several opine synthase genes of *Agrobacterium tumefaciens* [64,65]. The promoters of three different DNA viruses in the Caulimovirus group also contain ocs-element [66]. Lam et al. [67] called it as AS-1 element in case of 35S promoter of cauliflower mosaic virus, and identified the activating sequence factors (ASF) binding to this element. However, ocs-element was very rare among plants though found in the promoter of soybean heat shock gene [68].

One class of bZip proteins that is linked to stress responses comprises the TGA/*octapine synthase (ocs)*-element-binding factor (OBF) proteins. These bind to the *activation sequence-1 (as-1)/ocs* elements, which regulates the expression of some stress-responsive genes such as the *PR-1* and *GLUTATHIONE S-TRANSFERSE6 (GST6)* [69]. The ocs-element functions as an enhancer in dicot (tobacco) and monocot cell (maize) [70].

Apart from these well known *cis*-elements, several other elements or sequences that are pathogen-inducible have been identified. Xu et al. [71] isolated the genomic stilbene synthase gene from Chinese wild *Vitis pseudoreticulata.* Stilbene synthase transcripts were expressed in the grape–powdery mildew interaction. Upstream region of the *VpSTS* gene required for promoter activity were recovered using deletion analysis.

Transgenic rice and wheat were developed with defensin promoter fused to GUS reporters to study the promoter activity [24]. Defensin promoter was found to be active in various tissues at different time of development. It was strongly induced by wounding in leaf, stem and grain of transgenic rice plants.

Pathogen and salt stress inducible promoter (GmCaM-4) has been identified from soybean. The expression *cis*-acting elements that regulate the GmCaM-4 gene were identified, between -1,207 and -1,128 bp and between -858 and -728 bp in the GmCaM-4 promoter. Assay with transformed Arabidopsis protoplasts showed higher GmCaM-4 promoter than did the CaMV35S promoter after pathogen or NaCl treatments [72].

Swpa4 peroxidase gene is induced by a variety of abiotic stresses and pathogenic infections in sweet potato To elucidate its regulatory mechanism at the transcriptional level under various stress conditions, a promoter region (2374 bp) of swpa4 was isolated and characterized with a transient expression assay in tobacco protoplasts with deletions from the 5'-end of SWPA4 promoter fused to the beta-glucuronidase (GUS) reporter gene. The -1408 and -374 bp deletions relative to the transcription start site (+1) showed 8 and 4.5 times higher GUS expression than the CaMV 35S promoter, respectively [73]. In addition, transgenic tobacco plants expressing GUS under the control of -2374, -1408 or -374 bp region of SWPA4 promoter were generated and studied in various tissues under abiotic stresses and pathogen infection. Nuclear proteins from sweet potato cultured cells specifically interacted with 60-bp fragment (-178/-118) in -374 bp promoter region was disclosed by gel mobility assay. *In silico* analysis indicated that four kinds of *cis-acting* regulatory sequences, reactive oxygen species-related element activator protein 1 (AP1), CCAAT/enhancer-binding protein alpha element, ethylene-responsive element (ERE) and heat-shock element, are present in the -60 bp region (-178/-118), suggesting that the -60 bp region might be associated with stress inducibility of the SWPA4 promoter.

4. SYNTHETIC PROMOTERS

Technological advances in plant genetics integrated with systems biology and bioinformatics has yielded a myriad of novel biological data and insights into plant metabolism. This unprecedented advance has provided a platform for targeted manipulation of transcriptional activity through synthetic promoter engineering, and holds great promise as a way to further understanding of regulatory complexity. The challenge and strategy for predictive experimental gene expression is the accurate design and use of molecular 'switches' and modules that will regulate single or multiple plant transgenes in direct response to specific environmental, physiological and chemical cues. In particular, focusing on *cis*-motif rearrangement, future plant biotechnology applications and the elucidation of *cis*- and *trans*-regulatory mechanisms could greatly benefit from using plant synthetic promoters [74]. Synthetic promoters provide an efficient and flexible strategy to regulate transgene expression in a desired spatial and temporal manner at the site and time of plant-pathogen interaction and reduce the complexity of the expression pattern of natural promoters [74,75,54]. Two main methods are applied during synthetic promoter composition and analyses. They include

modification of distal promoter comprising cis regulatory blocks and creation of two-gene set including one gene encoding trans factor and another gene, being influenced by this trans factor [76].

The realization that pathogen-inducible promoters contain *cis*-acting elements and largely they are conserved across species [41], has led to attempts on precise promoter tuning by selectively including such elements that contribute significantly to promoter strength and activity. Randomizing these elements from various sources could be done by synthetic promoters. A range of pathogen-inducible *cis*-acting elements can alone mediate pathogen-inducible expression. When taken out of their native promoter contexts and framed as components of synthetic promoters, they retain pathogen inducibility. Of the several *cis*-acting elements known to be pathogen-inducible [25], W, GCC, S, D and Gst1 boxes have been used in synthetic promoters [54].

Synthetic promoter shuffling represents a fast and efficient method for exploring the spectrum of complex regulatory functions that can be encoded by complex promoters. From an engineering point of view, synthetic promoter shuffling enables the experimental testing of the functional properties of complex promoters that cannot necessarily be inferred *ab initio* from the known properties of the individual genetic components. Synthetic promoter shuffling may provide a useful experimental tool for studying naturally occurring promoter shuffling [77]. Zhang et al. [78] have developed a more efficient approach, in which a library of synthetic promoters for *Lactococcus lactis* is obtained by randomization of the spacer sequence that separates the consensus sequences of the promoter. In this library, a wide range of promoter activities is covered in small steps. A consensus promoter sequence for *L. plantarum* was derived by aligning its rRNA promoters, and this sequence used as the basis for constructing a synthetic promoter library for *L. plantarum* [79]. Investigations in tobacco have shown that the use of synthetic promoters with minimal sequence similarity could serve as a valuable tool to overcome homology-dependent gene silencing (HDGS) in plant transgenic strategies [80]. The bidirectionality of plant promoters is a phenomenon noticed in nature (e.g. oleosin promoter) and applied into studies [81]. It allows obtaining increased and more stable expression of two genes in multigene constructs. This ability is used mainly to study plant disease resistance and gene silencing [82]. Synthetic plant bidirectional promoters could be obtained by ligation of an opposite oriented promoter with the 5′ end of the other promoter [81,76]. It was proved that the orientation of core promoter elements is essential for bidirectionalization and should be consistent with the direction of a given gene [82].

The combination of short directly-repeated cassettes producing the strongest enhancement of reporter activity were used to create two synthetic promoters (SynPro3 and SynPro5) that drive leaf reporter activities at levels comparable to the CaMV 35S promoter. Characterization of these synthetic promoters in transformed tobacco showed strong reporter expression at all stages of development and in most tissues [83].

5. FUNCTIONAL ANALYSIS OF *cis*-acting SYNTHETIC PROMOTERS

The putative promoter sequences are generally validated for the activity. Transcriptional fusions where putative promoter fragments are used to drive reporter genes are generally employed for functional analysis. The construction of such fusions is greatly facilitated with the development of promoter-probe vectors. These vectors have a promoter less reporter gene encoding an easily assayable protein, present downstream of one or more restriction sites [84,85]. Putative segments to be characterized for promoter activity are ligated into these restriction sites, and the expression of the reporter gene can then be quantified under various conditions. Promoter probe vectors, in order to be of the greatest use, should (i) function in as many taxa as possible, (ii) show a high degree of sensitivity to detect promoters that are of weak to moderate strength, and (iii) be stable enough to be *in vivo* without antibiotic selection.

Initial promoter-probe vectors used l*acZ* reporter gene for use in *Escherichia coli* [86,87,88]. In order for promoter-probe vectors to be more versatile, they need to contain broad-host-range origins of replication. Several such vectors have been described [89,90,91]. Additionally, many organisms are naturally resistant to varying levels of one or more antibiotics [92]. Therefore, promoter-probe vectors with wide variety of antibiotic resistance genes prove useful. Some promoter-probe vectors have been described in which interference by read-through transcription is largely reduced by the addition of one or more

transcriptional terminators upstream of the multiple cloning site (MCS) [88]. Although elimination of upstream transcription increases the overall sensitivity of the vector, it is also important to consider the sensitivity of the reporter gene. The level of expression of some reporter genes that have been fused to weakly transcribed promoters may fall below the level of detectability. Some reporter genes, such as *inaZ*, which encodes a bacterial ice nucleation protein, are substantially more sensitive than *lacZ* or *gfp* (green fluorescent protein) [93]. The sensitivity of the reporter gene is also an important consideration when a low-copy-number plasmid, such as a broad-host-range vector, is used. A series of integrative and versatile broad-host-range promoter-probe vectors carrying reporter genes encoding GFP, catechol 2,3-dioxygenase (XylE) or beta-galactosidase (LacZ) were constructed and found promising for use in methanotrophs. Vickers et al. [23] reported a novel reporter for quantitative expression analysis. Though synthetic, codon-optimized xylanaseA gene (*xynA*) encoding xylanase enzyme (endo-1,4-glucanase) was developed as a reporter system in plants for accurate and sensitive quantification in transformation studies; the first effort on its use in promoter-probe binary vector was reported by Miller et al. [94].

Medi et al. [95] isolated *Sau*3A digested DNA fragments from tobacco and checked for promoter activity using a promoter probe vector, pGVL120 having a promoterless *nptII* reporter gene. Mixture of recombinant plasmids containing these fragments upstream of the reporter was mobilized to Agrobacterium, and used transformed tobacco protoplasts. By kanamycin selection, transformed plant cell lines containing *nptII* T-DNAs were isolated; eight of these cell lines were regenerated and analyzed for the levels of NPTII activity in stem, root, midrib, and leaf. NPTII expression in various tissues demonstrated novel tissue specific promoters.

6. CONCLUSION AND FUTURE PERSPECTIVE

The dimerized forms of the cis acting element alone and in combination have high potential for the use of biotrophic and necrotrophic sensitive elements within pathogen inducible promoter structure. The synthetic promoters are considered as useful tools to control more specifically the expression of resistant genes in transgenic plants. This technology has the potential to play an important role in plant biotechnology applications in the future.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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