Cloning and functional analysis of the promoter of a stress-inducible gene (Zmap) in maize

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Abstract

The anionic peroxidases play an important role in a variety of plant physiological processes. We characterized and isolated the Zmap promoter (PZmap) at the 5’ flanking region in order to better understand the regulatory mechanisms of Zmap gene expression. A series of PZmap deletion derivatives, termed a1 –a6, at positions −1694, −1394, −1138, −784, −527 and −221 from the translation start site were blended to the β-glucuronidase reporter gene. Agrobacterium-mediated transformation method was used to study each deletion construct in tobaccos. Sequence analysis showed that several cis-acting elements (MYB binding site, Box-II, a TGACG-element, a CGTCA-element and a low temperature responsive element) were located within the promoter. Deletion analysis suggested the sequence between −1,694 and −1394bp may contain cis-elements associated with GUS up regulation. The MYB binding site (-757) might act as a negative drought-responsive element. There might be repressor elements located in the region (−1,694 to −1394bp) to repress Zmap expression under 4˚C. The characterized promoter would be an ideal candidate for genetic engineering for improving the resistance of maize to different stressors.

Introduction

Plants are usually subjected to many hostile environments, such as drought, salinity, and low temperatures, which severely affect plant growth and productivity. A series of complex cell signal transduction processes will occur to limit the damage under these abiotic conditions. Many kinds of defense mechanisms can also be activated under abiotic stressors; among them, the expression of resistance genes can be found under single or multiple stress conditions. Therefore, the expression of stress-inducible genes and promoters play an important role in plant resistance.

Promoters are important DNA sequence signals in gene expression. Constitutive promoters can continuously drive transcription and expression of downstream genes, including exogenous genes. However, gene over expression may hinder the energy required for normal growth and the synthesis of RNA and proteins [1–3]. In contrast, inducible promoters limit gene
expression to specific tissues or organs, or to defined growth stages, such as limited growth conditions or the presence of insults, which could reduce the adverse effects on plant growth. Therefore, the study of inducible promoters will improve our understanding of the molecular mechanisms of signaling pathways [4].

Anionic peroxidases play an important role in a variety of plant physiological processes such as lignifications, suberifications, wound repair, and defense against disease [5]. Anionic peroxidase expression can be divided into constitutive and inducible expression. Inducible expression anionic peroxidases considered some of the most important plant protective iso-enzymes, play an important role in both pathogen infection and abiotic stressors [6]. To understand the expression mechanism of the anionic peroxidase gene, we functionally characterized the promoter region of anionic peroxidase in maize (Zmap promoter). In this report, we investigated the Zmap promoter region inducible activity and identified the response of the 5'-flanking sequence to different stimuli, including methyl jasmonate (MeJA), low temperature and drought. The study could provide valuable insights into the mechanism of the Zmap promoter involved in Zmap gene expression patterns under abiotic stressors.

Materials and methods

Plant materials and growth conditions

Zea mays (B73) plant seeds were collected in the experimental field of our university and the author was not obliged to have any permissions. This work did not involve endangered or protected species and the species Zea mays (B73) is a common plant. Maize plants were propagated within a controlled environment chamber with a photoperiod of 16 h light/8 h dark at 25°C. At the same time, tissue-culture tobacco Nicotianatabacum (NC 89) plants were raised on Murashige-Skoog (MS) medium supplemented with 30 g/l sucrose, 7 g/l agar, 3 mg/l 6-benzyladenine, 0.2 mg/l α-naphthaleneacetic acid and adjusted to pH 5.8. Plants were maintained 16 h light/8 h dark photoperiod at 25°C. Genetic transformation experiments were carried out with fully developed tobacco leaves.

Promoter cloning and sequence analysis

Bioinformatics analysis to identify putative regulatory motifs in the Zmap promoter sequences from maize was performed using the database of PlantCARE [7].

For determination of the structure of Zmap promoter, polymerase chain reaction (PCR) was carried out using the primer pair aP-F/aP-R shown in Table 1 with maize DNA as template. Cycling conditions including 94°C for 5min; 30 cycles of 94°C for 45s, 58°C for 40s, 72°C for 2min; and 72°C for 10min. BamHI and NcoI restriction enzymes restriction sites were represented by underlined letters. Recombinant clones were sequenced following cloning of PCR products into the pMD18-T vector.

Genetic transformation and construction of expression vectors

The functional regions of the Zmap promoter were investigated by5'-end deletion analysis. A series of Zmap promoter deletions were generated by PCR, named as a1 (1694 bp), a2 (1394 bp), a3 (1138 bp), a4 (784 bp), a5 (527 bp) and a6 (221 bp) using the primers shown in Table 1. CaMV35S promoter was replaced following the cloning of amplicons into the pCMBIA1301 plasmid (Fig 1). The recombinant plasmids were introduced into Agrobacterium tumefaciens strain EHA105. Expression of reporter β-glucuronidase (GUS) gene was measured in order to evaluate promoter activity.
Tobaccos were infected using the Agrobacterium-mediated method. Briefly, the leaves were cut into small pieces and were cultured on MS premedium for 2 days followed by transgenic Agrobacterium tumefaciens strain EHA105 infection. The leaf pieces were cultured on selection medium and then were transferred onto rooting medium following growing sprouting, and finally potted in soil (Fig 2). The second generations of transgenic plants were used for the subsequent study.

In total, 0.1g tobacco leaves were collected from each transgenic plant and the genome DNA were extracted using CTAB method. PCRs were carried out using the Zmap promoter and hygromycin gene contained in plasmid, with water as blank control and wild type tobacco as negative control (Fig 3).

As shown in Fig 3A, the Zmap promoter DNA fragment of 1694 bp can only be detected in seven transgenic tobaccos, while not in water or wild type tobacco controls. Similar results were observed in hygromycin gene plasmid PCR (Fig 3B). These results indicated that the target gene had been successfully transferred into transgenic tobaccos.

### Generation and identification of transgenic plants

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### Table 1. Primers used for polymerase chain reaction (PCR).

| Primer name | Primer sequence (5' - 3') |
|-------------|--------------------------|
| aP-F(a-1)   | CGGGATCCCTGCCGATACCGCTTA |
| a2         | CGGGATCCAACTCACGGAGGCTAC |
| a3         | CGGGATCCATGACCCAGCCACCCAC |
| a4         | CGGGATCCCTGATCCCAGGGCCGTT |
| a5         | CGGGATCCATGACCCACCCACCCAC |
| a6         | CGGGATCCGAGAATGAGATCATCCAC |
| aP-R       | CCGATCTCAGCTTGCTTGCTTGCT |
| ACTIN- F   | CGGAATTCACAATATCCGCTAGGT |
| ACTIN- R   | CCCATGGCTTCATTATCGGAGG |
| GUS- F     | CCGGATCCCTGCCGATACCGCTTG |
| GUS- R     | CCCATGGCTTCAGCTTGCTTGCTTG |

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### Fig 1. Schematic representation of the PZmap: GUS construct.

The insertion position of the Zmap promoter in the vector is indicated with restriction enzyme sites (BamHI and NcoI). LB, left border; RB, right border; 35s-ter, Cauliflower Mosaic virus 35S terminator; 35s Pro, Cauliflower Mosaic virus 35S promoter; GUS, β-glucuronidase gene; HPTII, hygromycin phosphotransferase (II) coding region; NOS-ter, nopaline synthase terminator; Zmap Pro, Zmap promoter.

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Histochemical staining

Histochemical staining was performed as described previously [8]. Samples collected from transgenic tobacco after different stress-related stimuli were incubated in GUS reaction buffer (3 mg/ml X-gluc, 40 mM sodium phosphate pH7, 10mM EDTA, 0.1% Triton X-100, 0.5mM potassium ferricyanide, 0.5mM potassium ferrocyanide, and 20% methanol). Stained samples were bleached ethanol (70% (v/v)) to remove chlorophyll after overnight incubation (37˚C) and observed under white light using a Nikon SMZ1000 microscope. GUS expression patterns in whole plants were visualized by histochemical assay.

Plant treatment

The fourth and sixth leaves of the transgenic tobacco were used to investigate the effects of the different stress-related stimuli on GUS reporter gene expression. For drought stress treatment,
tobacco plant roots were treated with 20% polyethylene glycol (PEG). Tobacco plants were put in growth chamber at 4˚C for low temperature treatment. Tobacco plants were put in growth chamber at 4˚C for low temperature treatment. Untransformed tobacco plants, plants transformed with CaMV35S (pCAMBIA1301 vector), and transgenic plants treated with water in the same areas were provided as controls. All tobacco samples were treated at 1, 3, 5, 10 and 24h. After each treatment, tobacco leaves were frozen in liquid nitrogen, and stored at a temperature of -80°C for total RNA isolation.

**Total RNA extraction and real-time quantitative RT-PCR analysis**

Total RNA from tobacco leaves was extracted by the RNAiso Reagent (Takara, Changchun, China). Total RNA was reverse transcribed into single-stranded cDNA by using M-MLV Reverse Transcriptase and anoligo (T) 18 primer (Takara, Changchun, China). RT-PCR analysis was performed using SYBR Green I (TaKara) on an Applied Biosystems 7500 real-time PCR machine (Applied Biosystems, Foster City, USA). The tobacco actingene (GenBank Accession No.U60491) was taken as endogenous control gene. RT-PCR primers are shown in Table 1. Real-time PCR cycling conditions were as follows: 95˚C for 30sec; and 40 cycles of 95˚C for 5s, 56˚C for 40s. All reactions were performed three times. The data were analyzed via $2^{-\Delta\Delta Ct}$ method [9].
Fluorometric GUS assay

GUS activity was detected by fluorometric assay according to the method of Jefferson et al [8]. GUS extraction buffer (0.1% TritonX-100; 50 mM phosphate buffer, pH 7.0; 10 mM EDTA; 0.1% sodium lauryl sarcosine; 10 mM β-mercaptoethanol; 20% methanol) was used to extract various tissues of tobacco leaves. After centrifugation, the total protein content of extracted supernatants was measured by the Bradford method [10] by using a Bio Rad Protein Assay Kit with BSA as a standard. The assays were performed in triplicates for each sample.

Results

Structure analysis of cis-acting elements in Zmap promoter sequences

Zmap promoter was analyzed by bioinformatics using the PlantCARE database to identify the cis-acting regulatory elements. The 1694bp DNA sequence located upstream of the translation start site (indicated with “+1” at the ATG start codon of the Zmap gene) was considered the putative promoter in this study. Bioinformatic analysis of Zmap promoter allowed us to identify the existence of some putative regions that could modulate gene expression. These putative regions are also known as cis-acting regulatory elements (Table 2). Zmap promoter sequence contains several core fragments shown in Table 3. They consisted of one TGACG-motif (TGACG), one LTR(CCGAAA), one CGTCA-motif (CGTCA), one CAT-box (GCCACT), one box-II (TCCACGTGGC), one G-Box (GTGCAA), one GA-motif(TCATCTTT), three MYB binding sites (MBS, one TAACTG and two CAACTG), and many other cis-acting regulatory elements, such as TC-rich repeats, HD-Zip II and AuxRR-core. Analysis of Zmap expression potentially indicates its regulation and expression by many different stress stimuli [11].

GUS reporter gene expression from Zmap promoter in response to different stimuli

The 1694bp full-length Zmap promoter was transferred into tobacco plants following the fusion of the GUS reporter gene in a plant expression vector in order to determine the regulatory mechanisms of controlling the expression of Zmap gene. Histochemical GUS staining was used to measure the expression levels of GUS gene in transgenic tobacco which showed the inducible activity of the Zmap promoter. The data revealed that a decrease in GUS gene expression after low temperature treatment (4˚C) (Fig 4A, 4C and 4F), but an increase after the other treatments (Fig 4A, 4C, 4D and 4E). Slight GUS staining was observed in untransformed plants, though these background levels were far below those observed in CaMV35S-transformed tobacco plants (Fig 4A, 4B and 4C). GUS gene expression occurred mainly in the aerial parts of the plants rather than the roots.

GUS reporter gene expression was examined quantitatively by real-time RT-PCR using total RNA extracted from the aerial parts of transgenic tobacco between the fourth and sixth leaves at chosen time points after treatment with MeJA, PEG, or low temperature (Fig 5). GUS transcript levels were induced by PEG, with a maximal level at 10 h. MeJA treatment also significantly increased GUS gene transcription at 24 h. In contrast, low temperature treatment decreased GUS transcript levels compared to the untreated control (0 h).

Collectively, GUS expression levels were examined by histochemical GUS staining (Fig 6). The results confirmed our observations above, with GUS expression about 4-fold higher after PEG treatment. Expression levels were lowest in plants treated with low temperature, corroborating results showing inhibition of GUS gene expression in cold-treated transformed tobacco plants. MeJA-treated plants had 2-fold higher GUS expression levels compared to untreated
| Position | Sequence                                      | Functiona l analysis of Zmap promoter in maize |
|---------|----------------------------------------------|-----------------------------------------------|
|        |                                              |                                               |
| -1693  | TGGCGTGTATA CCGACCTGAG TCCGAAGGTA CATTATA CTT CCAGAAATAC |                                               |
|        |                                              |                                               |
| -1633  | TGGTATACCC TTGTATGCTAT GACAGCAAT ATATTGAAG CCGACCCTTC ACGTATATTGT |                                               |
| -1573  | AGTTAGGCATG TGGCTGGCAA TTTTTTTTAA TTTTCTTCAT TTTTTGCTA TAAACGCTGC |                                               |
| -1513  | AGTTAGGCATG AGCCTGATTA TAATTTTTAA GGTGGTATGT TGAATGAGAT TGGACCTGTC |                                               |
| -1453  | GCTCAGGTGCA ATGCCGGTAT ATATTCACGT AGATTTGCTA TAAACGCTTA TGGAGTCCCA |                                               |
| MBS     |                                              |                                               |
| -1393  | ACTCAGGCAA CCTACGGCAA TCTACAATAAAAAAAGACAATGCTG ACTGAGTCGA |                                               |
|        |                                              |                                               |
| -1333  | CATGATATTC CCTTGACTAC CCACAGTGCA AGAACAGGCA CATCTGACTA CAGTATATTGT |                                               |
| -1273  | TAGTACAGCA ACTATATCC CATTCAGGAA TGACAGTGTG AAGGCATTATA TAATGGGCGC |                                               |
| TATA-box |                                              |                                               |
| -1213  | ACAATGGCAT ACCCATACAT ATTACGAGAA AATAGCTGAC CCACGGGTA CGGATGTCAC |                                               |
| -1153  | AACATCGCTCA TATCATACAC CACACGACCC ACCACGGGTA GTGGGTATAT TACGATACCC |                                               |
| CGTCA-motif |                                              |                                               |
| -1093  | CATATATAC ACACATATAC ACAATAGACA CATCTGACTA CAGTATATTGT |                                               |
| -1033  | CATCATACAA GCCATGATG AGAGAAAGA AGCCCTTATA TCTGGACTCA TATGATTATAT |                                               |
| -973   | GTTCAGGCGG ATCTTGACGA TGAGCGGGTA TTTGGCAAA AAGGCATGCA ACCATCATCAT |                                               |
| -913   | CATACCCAAAT GATAATAAAC AATGTCTCAC CCACTACATC GTGGATACAA |                                               |
|        |                                              |                                               |
| -853   | [ATA]ATACATG TGCACTAAATA GGAACAGCTA GCTATCAGAT ATCGGGTTTC AGGTAATCGAC |                                               |
| TATA-box |                                              |                                               |
| -793   | GTTGGTCAAC CCCCCTGCC TGGTCACTAC ATCTGGTTGG CAGTTGCTG |                                               |
|        |                                              |                                               |
| -733   | AACATGCATG CATTTTGAGG TAATATACAA TGGATGCTTG CATTCATGTG GTGGGTCTTC |                                               |
|        |                                              |                                               |
| -673   | TCCATGCCAC CTCTGACTC GTGAGACAG AGAGCATGCA GATGCTAAGT |                                               |
| CAT-box  |                                              |                                               |
| -613   | GCAGCAACAT CAGTCCACAC CCCCAGGAT CACATTGATG ACTCCAGGGA GCCATACAAA |                                               |
| -553   | GACGCTTCCGC CACCTGCGCA TGCTCCAATA GCCAGTTGTCG |                                               |
|        |                                              |                                               |
| -493   | GCCAATACAT GGGTGTCGA AAAAAAAC CTATCAAGCC CGGAG ATTAAAGACCA |                                               |
| HD-Zip 2 |                                              |                                               |
| -433   | TTCTATGCTT CGACGGCCGC AGCCGGCGGT CACCGACTAG CTACGCGCGC ACCGAAATTAG |                                               |
| -373   | CCTACACAGA GTATGATAAA AGCTGTTTAG TATGAGAAATG GATCTGATTC A[CATATT] |                                               |
| GA-motif |                                              |                                               |
| -313   | CACTCCCTCAC TTTTTTTTGT TGGGTGTGTG GAATATTTG AGTGGTATCA TACTACACCTC |                                               |
| -253   | ATCTTTTATA GTATTATTG TAGTACTAAT ATGAGAAATTG AGATCAGCTT ACCAAGTTTG |                                               |
| -193   | AGATATGGAG ATAGTACCGA CACATATATT TGGGATAAGA TGATTTCTCA AACCACCAAA |                                               |
| AuxRR-core |                                              |                                               |
| -133   | CCCATATATTC CGGACGGGAC GATCAGCTT TACCAGGCTA AAAACCAACG ATGCAAAGAAA |                                               |
| TATA-box |                                              |                                               |
| -73    | CTGTGCTGAG CGCTGCAA GCTCAAGGC AACGAGGAGA TGGGACGCA TGCAAGCA |                                               |
| G-box   |                                              |                                               |
| -13    | CAAACGCTA GAAATG +1 |                                               |

Putative cis-acting regulatory elements, detected in the promoter fragment using the PlantCA RE database, are indicated within grey shaded boxes. The translation start site is indicated with “+1”.

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Table 3. Putative cis-acting elements and their positions in the Zmap promoter.

| Cis element   | Sequence   | Position | Function                                      |
|---------------|------------|----------|-----------------------------------------------|
| TC-rich repeats | GTTTTCTTAC  | -832     | defense and stress responsiveness element     |
| TGACG-motif    | TGACG      | -1359    | MeJA-responsiveness element                   |
| box II         | TCCACGTGGC | -798     | part of a light responsive element             |
| LTR            | CCGAAA     | -1333    | low temperature responsiveness element         |
| HD-Zip 2       | CAAATCTTGGTTT | -490   | leaf morphology development control element    |
| AuxRR-core     | GGTCCAT    | -189     | auxin responsiveness regulatory element        |
| CAT-box        | GCCACT     | -667     | meristem expression regulatory element         |
| CGTCA-motif    | CGTCA      | -1148    | MeJA-responsiveness regulatory element         |
| G-Box          | GTGCAA     | -57      | light responsiveness regulatory element         |
| GA-motif       | TCCATTTTT  | -315     | part of a light responsive element             |
| MBS            | TAACTG     | -1407    | MYB binding site involved in drought-mediated induction |
|                | CAACTG     | -514; -757 |                                                |

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Fig 4. Histochemical staining of GUS activity in six-week-old transgenic tobacco plants. β-glucuronidase (GUS) expression in (A) wild-type; (B) CaMV35S-transformed tobacco plants; (C) untreated transgenic tobacco plants; and transformed tobacco plants treated with 20% polyethylene glycol (D), 100 μM methyl jasmonate (E) and low temperature (4˚C) (F).

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plants. Differences in overall expression levels between the different treatments are probably due to differential rates of gus mRNA or protein turnover.

**Analysis of Zmap promoter deletion mutants**

To further study stress-inducible expression from the Zmap promoter, a series of 5′ promoter deletion-GUS constructs were transferred into the tobacco plant by transient expression. GUS expression in transformed plants was then measured by a fluorometric GUS assay. Deletion
promoters were named a1 (1694 bp), a2 (1394 bp), a3 (1138 bp), a4 (784 bp), a5 (527 bp) and a6 (221 bp), respectively (Fig 7). Fluorometric GUS activity assay was used on the leaves of stress-treated transgenic tobaccos. It has been noted that GUS activity of a1-promoter plants was higher than other deletion promoter plants treated with phytohormone (MeJA) (Fig 8). Comparing with untreated controls, GUS activity of a1 and a2 deletion promoter plants increased significantly, suggesting that MeJA-responsive elements (TGACG-motif and CGTCA-motif) might play important roles on driving GUS expression in a1 and a2 plants. However, a1-mediated GUS activity was reduced significantly after low temperature treatment compared with untreated plants, while GUS expression increased in a2 plants (Fig 8). There was no obvious difference in GUS activity mediated by other deletion promoters. GUS activity increased in all plants after PEG treatment (Fig 8), with a1 plants increasing the most and a5 plants also showing a large increase.

Discussion

Studies demonstrated that the expression of many plant genes such as, metabolic, regulatory and structural genes [12–15] were induced by light. In this study, the activity of the Zmap promoter was investigated by a fusion reporter construct (PZmap: GUS) after transformation into the tobacco genome. GUS activity from transgenic plants provided a detailed pattern of Zmap promoter function. Leaves and stems of transgenic tobacco plants exhibited blue staining, but roots not showed any signs of blue staining (Fig 4). It was found that light could interfere with Zmap promoter, and several light-responsive elements such as the GA-motif, box-II and the G-box [16–18] were also found in the promoter sequence (Table 3).

Bioinformatic analysis revealed adversity stress elements (one low temperature element, two MeJA-responsive elements, and three putative drought-responsive elements) in the Zmap promoter region. These elements were hypothesized to have a strong effect on gene expression. To further understand the expression level of stress-induced transcriptional activity of the Zmap promoter at the protein level, real-time RT-PCR analysis was performed (Fig 5). GUS transcript levels decreased in response to low temperature treatment. In contrast, transcript levels increased in response to MeJA and drought treatment. These results were corroborated by histochemical GUS staining analysis (Fig 6).
Deletion analysis showed that the Zmap promoter possess adversity stress cis-regulatory elements that could allow maize to respond to stress. Agrobacterium-mediated leaf-disc was used to transform deletion promoter constructions into tobacco plants. In this study, GUS activity decreased with decreasing Zmap promoter length (a1—a6) in untreated plants. Interestingly, highest activity was shown the full-length Zmap promoter (a1) among all deletion promoters. It is speculated that cis-elements were found in the sequence between −1,694 and −1394bp of the Zmap promoter involved in up regulation of GUS expression.

Compared with untreated controls, the GUS activity of the a1 and a2 deletion promoter plants increased more significantly compared to other deletion promoter plants treated with MeJA (Fig 8). This result showed that the MeJA-responsive elements (TGACG-motif and CGTCA-motif) played a crucial role in enhancing the GUS activities of a1 and a2 [19–22]. Otherwise, a1-mediated GUS activity declined significantly under low temperature treatment, while a2-mediated activity increased in response to the same stimulus. No significant differences in GUS activity were found in other groups. It can be concluded that the low temperature responsive element has a positive regulatory role under low temperature stimuli [23–24] and that there may be other still unidentified negative elements in this 300bp fragment (−1,694 to −1394). Sequence analysis showed that there are three drought responsive elements (MBS) in the Zmap promoter. Transcription factor MYB could bind to MBS, which could act as a target for other regulators [25–28]. After PEG treatment, a1 plants had the highest activity, followed by a5 plants. Therefore it can be obtained that these elements (-1407 and -514) play
important roles under drought stimulation, while the (-757) element might act as a negative drought-responsive element.

In conclusion, the results revealed the activity patterns of the Zmap promoter, and thus could provide better understanding of the complex regulatory mechanisms and functional regions of Zmap promoter. A fluorometric GUS assay and qRT-PCR results indicated that Zmap promoter-mediated activity increased after MeJA and drought treatment but decreased after low temperature treatment. These data will support further studies of the role of adversity-inducible promoters in maize defense response and offer a foundation for improving the resistance of maize to different stressors.

Author Contributions

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References

1. Vaucheret H, Beclin C, Elmeyan T, Feuerbach F, Godon C, Morel JB, et al. Transgene-induced gene silencing in plants. Plant J 1998; 16: 651–659. https://doi.org/10.1046/j.1365-313x.1998.00337.x PMID: 10069073
2. Malnoy M, Reynoard JP, Borejsza-Wysocka EE, Aldwinckle HS. Activation of the pathogen-inducible Gsf1 promoter of potato after elicitation by Venturiain aequalis and Erwinia amylovora in transgenic apple (Malus × domestica). Transgenic Res 2006; 15: 83–93. https://doi.org/10.1007/s11248-005-2943-7 PMID: 16475012
3. Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnology 1999; 17: 287–291. https://doi.org/10.1038/7036 PMID: 10096298
4. Mehrotra R, Gupta G, Sethi R, Bhalothia P, Kumar N, Mehrotra S. Designer promoter: an artwork of cis engineering. Plant Mol Biol 2011; 75: 527–536. https://doi.org/10.1007/s11103-011-9755-3 PMID: 21327513
5. Teichmann T, Guan C, Kristoffersen P, Muster G, Tietz O, Palme K. Cloning and biochemical characterization of an anionic peroxidase from Zea mays. Eur J Biochem 1997; 247: 826–832. https://doi.org/10.1111/j.1432-1033.1997.00826.x PMID: 9288904
6. Baker CJ, Orlandi EW, Mock NM. Harpin, an elicitor of the hypersensitive response in tobacco caused by Erwinia amylovora, elicits active oxygen production in suspension cells. Plant Physiol 1993; 102: 1341–1344. https://doi.org/10.1104/pp.102.4.1341 PMID: 12231911
7. Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Vande PY, et al. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res 2002; 30: 325–327. https://doi.org/10.1093/nar/30.1.325 PMID: 11752327
8. Jefferson RA, Kavanagh TA, Bevan MW. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 1987; 6: 3901–3907. https://doi.org/10.1073/pnas.1411926112 PMID: 3272686
9. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the2-ΔΔct method. Methods 2001; 25: 402–408. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt Biochem 1976; 72: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3 PMID: 942051

11. Zhou YL, Xu MR, Zhao MF, Xie XW, Zhu LH, Fu BY, et al. Genome-wide gene responses in a transgenic rice line carrying the maize resistance gene Rxo1 to the rice bacterial streak pathogen, Xanthomonas oryzae pv. oryzae. BMC Genomics 2010; 11:1471–2164. https://doi.org/10.1186/1471-2164-11-78 PMID: 20122142

12. Eckes P, Rosahl S, Schell J, Willmitzer L. Isolation and characterization of a light-inducible, organ-specific gene from potato and analysis of its expression after tagging and transfer into tobacco and potato plants. Mol Genet 1986; 20: 14–22. https://doi.org/10.1016/0168-9525(86)902427

13. Simpson J, Schell J, Van Montagu M, Herrera-Estrella L. Light-inducible and tissue-specific pea lhcb gene expression involves an upstream element combining enhancer- and silencer-like properties. Nature 1986; 323: 551–554. https://doi.org/10.1038/323551a0

14. Kaldenhoff R, Kölling A, Richter G. A novel blue light- and abscisic acid-inducible gene of Arabidopsis thaliana encoding an intrinsic membrane protein. Plant Mol Biol 1993; 23:1187–1198. https://doi.org/10.1007/BF00042352 PMID: 8292783

15. Sheen J. Protein phosphatase activity is required for light-inducible gene expression in maize. EMBO J 1993; 12, 3497. https://doi.org/10.1046/j.1037-3138.1993.00016.x PMID: 12045275

16. Nishiuchi T, Nakamura T, Abe T, Kodama H, Nishimura M, Iba K. Tissue-specific and light-responsive regulation of the promoter region of the Arabidopsis thaliana chloroplast u-3 fatty acid desaturase gene (FAD7). Plant Mol Biol 1995; 29: 599–609. https://doi.org/10.1007/BF00020987 PMID: 8534855

17. Rouster J, Leah R, Mundy J, Cameron-Mills V. Identification of a methyl jasmonate-responsive region in the promoter of alipoxygenase 1 gene expressed in barley grain. Plant J 1997; 11: 513–523. https://doi.org/10.1039/b700609c PMID: 9107039

18. Dunn MA, White AJ, Vural S, Hughes MA. Identification of promoter elements in a low-temperature-responsive gene (blt4.9) from barley (Hordeum vulgare L.). Plant Mol Biol 1998; 38: 551–564. https://doi.org/10.1023/A:1006908132352 PMID: 9747801

19. White TC, Simmonds D, Donaldson P, Singh J. Regulation of BN115, a low-temperature-responsive gene from winter Brassica napus. Plant Physiol 1994; 106: 917–928. https://doi.org/10.1104/pp.106.3.917 PMID: 7824659

20. Dubos C, Stracke R, Groteveld W, Weisshaar B, Martin C, Lepiniec L. MYB transcription factors in Arabidopsis. Trends Plant Sci 2010; 15: 573–581. https://doi.org/10.1016/j.tplants.2010.06.005 PMID: 20674465

21. Tao Y, Wang FT, Jia DM, Li JT, Zhang YM, Jia CG, et al. Cloning and functional analysis of the promoter of a stress-inducible gene (ZmRXO1) in maize. Plant Mol Biol Rep 2015; 33: 200–208. https://doi.org/10.1007/s11105-014-0741-1

22. Hou LX, Zhu D, Ma Q, Zhang DD, Liu X. H2S synthetase AID-CDs involves in ethylene and drought regulated stomatal movement. Sci Bull 2016; 61: 1171–1175. https://doi.org/10.1007/s11434-016-1128-5
