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Full Length Research Paper

Cloning and functional analysis in transgenic tobacco of a tapetum-specific promoter from *Arabidopsis*

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The 5'-flanking region of 1174 bp upstream of the translation start point (TSP) of a reported *Arabidopsis* anther-specific gene, *Anther7* gene (*ATA7*), which putatively encodes a protein related to lipid transfer protein, was cloned and functionally analyzed in transgenic tobacco after been fused with β-glucuronidase (GUS) gene reporter. Histochemical GUS staining of the transgenic plants showed that the cloned fragment did drive GUS expression exclusively in the anther, not in any other parts of floral organs, including pollens and nor in any vegetative tissue. Transverse section of the GUS-blue anthers disclosed that the blue cells were present uniquely in the tapetum of the anther. A series of 5'-deletion of cloned fragment indicated that a short segment of 179 bp upstream of the TSP (-155 bp upstream of the transcription start site) retained not only the promoter's driving power, but also its tapetum-specificity. *Cis*-acting element search in this short segment revealed the presence of numbers of organ- and tissue-specific motifs, including pollen-specific LAT52 and SLG13. These results indicated that the tapetum-specificity of *ATA7* gene is mainly conferred by its promoter, and such a promoter, in particular, the core one should be useful both for identification of tapetum-involved genes and for biotechnological applications.

Key words: *Arabidopsis*, *Anther7* gene of *Arabidopsis theliana* (*ATA7*), anther-specific promoter, tapetum-specific promoter.

INTRODUCTION

Anther development is a complicated process and involves large number of gene expression, including anther-specific ones. The anther specific genes make anthers to become different from other plant organs, and play an important role in plant reproductive development. They are associated with anther cell division and differentiation (Nonomura et al., 2003), tapetum development (Jung et al., 2005; Luo et al., 2006; Xu et al., 2006; Wu et al., 2008), male meiosis (Yang et al., 2003; Kapoor and Takasuji 2006), pollen maturation (Park et al., 2005, 2006; Zhao et al., 2006; Gupta et al., 2007), anther dehiscence (Zhu et al., 2004), stamen filament development (Mariani et al., 1990), etc. The anther-specificity of these genes has been found to be regulated mainly by gene's promoter and in particular by the key *cis*-elements in the promoter at the transcription level (Mascarenhas, 1992; Ariizumi et al., 2002). Therefore, a large number of anther-specific promoters and their key *cis*-elements from different plant species have been isolated and functionally analyzed, such as TA29 (Koltunow et al., 1990), NeIF-4A8 (Brander and Kuhlemeier, 1995), Zm13 (Hamilton et al., 1998), LAT52 (Muschietti et al., 1994), Osg6B (Yokoi et al., 1997), PyD3 (Xiao et al., 2006), PsEND1 (Roque et al., 2007) and MdAGP3 (Choi et al., 2010). Some of these anther-specific promoters, such as TA29, LAT 52, PsEND1 and key *cis*-element, for example,

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Abbreviations: TSP, Translation start point; **GUS,** β-glucuronidase; ***ATA7,*** *anther7* gene of *Arabidopsis theliana*; **MS,** Murashige and Skoog; **CTAB,** cetyl trimethylammonium bromide; **PCR,** polymerase chain reaction; **Carb,** carbenicillin; **KanR,** kanamycin resistant; **KanS,** kanamycin -susceptible; **FAA,** formaldehyde : acetic acid : ethanol; **Str,** streptomycin; **TSS,** transcription start site; **PLACE,** plant cis-acting regulatory DNA elements; **ATA7F0,** activity of *anther7* gene of *Arabidopsis theliana* full-length promoter; **CK,** untransformed plants.

"anther-box", have been already used for genetic engineering of male-sterile plants (Mariani et al., 1990; 1992; Van der Meer et al., 1992; Xiao et al., 2000; Roque et al., 2007; Garcı´a-Sogo et al., 2010).

Anther7 gene of *Arabidopsis theliana* (*ATA7*) which putatively encodes a protein related to lipid transfer protein was found to express exclusively in flowers, and its mRNA was more pronounced in immature inflorescences than in mature flowers (Rubinelli et al., 1998). Later, study of the gene by *in situ* hybridization showed that the transcript of *ATA7* was present only in the tapetal cell layer of the anther, beginning at the free microspore stage, soon after meiosis (Yang et al., 2003). In order to elucidate the specificity of *ATA7* gene and the main regulatory elements for the specificity, we cloned and characterized the 5'-flanking region upstream of the translation start site of the gene, and functionally analyzed the cloned fragment with a series of 5'-deletion in transgenic tobacco plants.

MATERIALS AND METHODS

Plant material

A. thaliana Eco-type Columbia used for isolation of genomic DNA were grown in greenhouse and tobacco plantlets (*Nicotiana tabacum* var. NC89) was used for transformation in Murashige and Skoog (MS) medium (Murashige and Skoog,1962) under conditions previously reported (Geng et al., 2009).

Cloning and 5'-deletion of 5'-flanking region of *ATA7* gene

Genomic DNA of *Arabidopsis* was extracted from young leaves with cetyl trimethylammonium bromide (CTAB) method (Dolye and Dolye, 1987). The 5'-flanking region upstream of 1174 bp relative to the translation start point (TSP) of *ATA7* gene, based on genome sequence of *Arabidopisis* shown in TAIR, was polymerase chain reaction (PCR)-amplified with primer pair 5'-AAGCTTCAAATCAG TAAAAGCTGACTC-3'/5'-GGATCCCTCATGTTTCCTTCTTACTCG-3' (the *Hin*dIII and *Bam* HI sites added are respectively underlined) under the PCR program: 94°C for 5 min, then 30 cycles of 94°C for 45 s, 56°C for 1 min and 72°C for 1.5 min, and the final elongation at 72°C for 10 min. The PCR products were cloned into pBS-T vector (Qiagen, China), and sequenced to check the identity by a commercial sequencing company after *Hin*dIII-*Bam* HI digestion verification. The cloned region expected was 5'-deleted in series by using PCR with forward primers and the original reverse primer listed in Table 1, and all fragments obtained were linked into the pBS-T vector and sequenced as described earlier.

Construction of plant expression vector and plant transformation

Basic DNA manipulation, including plasmid extraction, restriction digests and ligation, agarose gel electrophoresis and *Escherichia coli* (strain DH5a) transformation was performed with standard methods (Sambrook and Russell, 2001).

The sequencing-verified 5'-flanking region and its 5'-deleted fragments were isolated from the pBS-T vector with *Hin*dIII-*Bam*HI digestion and then individually fused with the promoter-less β-glucuronidase (GUS) reporter gene in the binary plant expression vector pRD410 (Datla et al., 1992) that contains also a NptII as selectable marker in the T-DNA. The resulting recombinant plant expression vectors were verified first by enzyme-cut and then by sequencing.

Each of the verified recombinant plant expression vectors was introduced into *Agrobacterium tumefaciens* strain LBA4404 using freeze-thaw method (Komari et al., 1996), and the LBA4404 were selected on YEB-agar plates with 50 µg/ml of kanamycine (Kan) and 100 µg/ml of streptomycin (Str). The transformed bacteria were then used to infect tobacco leaf-discs by co-culturing according to Hirsch et al. (1985). Putative tobacco transformants were selected on solid MS containing Kan 50 µg/ml and carbenicillin (Carb) 500 µg/ml, and the resistant ones were then PCR-identified for the presence of the "promoter" cloned. The PCR positive transformants were grown in tissue culture room for rooting at 25 ± 1°C under fluorescent white light in a 16/8 h light/dark cycle. Rooted plantlets were transplanted in pots in greenhouse and the resulting plants were detected for reporter gene expression at different developmental stages. Seeds from independent transgenic lines with strong expression of the reporter gene were collected and germinated on solid MS supplemented with Kan (100 µg/ml). The seedlings of the line that showed a ratio of 3:1 for Kan-resistant (KanR): Kan-susceptible (KanS) in offspring segregation were grown in pots in greenhouse as T1 plants of corresponding transgenic T0 line in each construct.

Histochemical GUS staining and anther transverse-section

Vegetative and floral organs were sampled from transgenic line T0 and T1 plants, and the control (wild-type plants) at different developmental stages were stained for GUS protein presence with 5-bromo-4-chloro-3-indoxyl-β-D-glucuronic acid (X-gluc) as a substrate according to Jefferson et al. (1987). After GUS-staining, the samples were treated with 70% ethanol for clearing chlorophyll and then photographed.

The GUS-blue anthers were fixed at least 24 h in FAA (formaldehyde: acetic acid: ethanol, 1:1:12) and then treated either for paraffin-embedded transverse-sectioning or for frozen one. For the paraffin sectioning, the anthers were transferred from the fixing solution to 70% ethanol for at least 8 h, and then dehydrated as described by Luo et al. (2006). The dehydrated anthers were embedded in paraffin one by one, mounted onto sectioning stubs and transverse-sectioned with rotary microtome (American Optical, USA). Frozen sectioning of the GUS-blue anthers was carried out based on the protocol developed by Knox et al. (1970). The anthers were transferred directly from the FAA solution to Jung Tissue Freezing Medium (Lecia Microsystems, Germany), and frozen at -20°C one hour before sectioning. The frozen anther was then transverse-sectioned at 50 µ and the sections were posted on slide.

RESULTS

Cloning and characterization of 5'-flanking region of *ATA7*

PCR amplification of *Arabidopsis* leaf DNA with the primer pair F0/RW (Table 1) produced a fragment of ca. 1170 bp (Figures 1, Lane 1), and the fragment was 1174 bp in length as disclosed by sequencing. Basic Local Alignment Search Tool (BLAST) -compared with the genome sequence of *Arabidopisis* in TAIR, the cloned fragment was identical to the 5'-flanking regions upstream of the translation start point of *ATA7* genes (Xiao et al.,

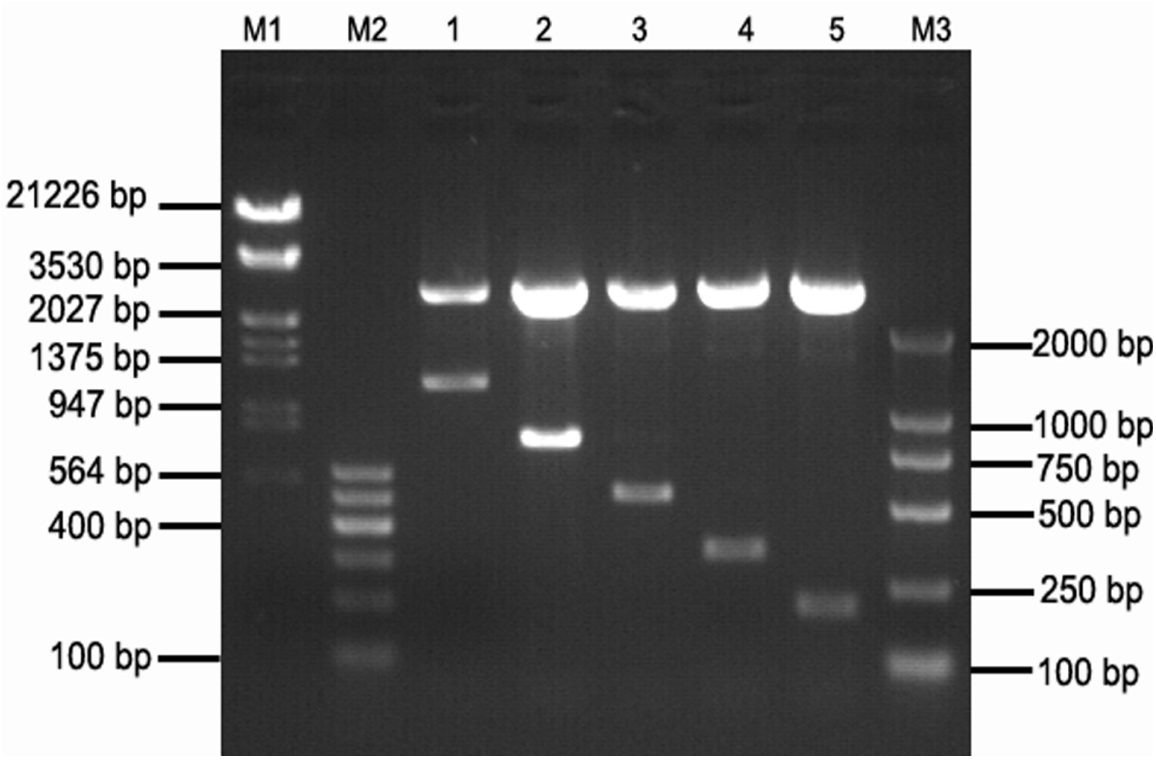

Figure 1. Restriction identification of cloned ATA7 promoter and its 5' deleted fragments. PCR product-carrying vector pBS-T was double-digested with *Hin*d III and *Bam* HI. M1: Molecular marker (λDNA/*Hin*d III + *Bam* HI); M2: 100 bp ladder; M3: 2000 bp marker; Lane 1 to 5: pBS-T + ATA7F0, ATA7F1, ATA7F2, ATA7F3 or ATA7F4, respectively.

2007), as expected. Further comparison with *ATA7* mRNA sequences available in GenBank, such as AF037589, NM118981 and NM118981.2, showed that the cloned fragment likely contained a common transcription start consensus "C*A*TCC*"* and used the "A" as transcription start site (TSS) which is located at 24 bp upstream of the translation start point. Thus, the cloned fragment consisted of a putative promoter region of 1150 bp and a 5'-UTR of 24 bp.

Sequence analysis of the putative promoter region of 1150 bp with Plant cis-acting regulatory DNA elements (PLACE) (http://www.dna.affrc.go.jp/PLACE/index.html, Higo et al., 1999) showed the presence of both common core promoter elements such as "TATA-box" (-48 to -43), "CAAT-box" (-73 to -70) and various organ-specific *cis*-acting elements, such as pollen-specific activation element POLLEN1LELAT52 (Bate and Twell, 1998, Filichkin et al., 2004), pistil- and pollen-specific element SLG13 (Dzelzkalnset al., 1993), root-specific ones OSE1ROOTNODULE and OSE2ROOTNODULE (Vieweg et al., 2004) etc (data not shown). This information suggested that the cloned promoter region displayed common characteristics of the TATA-box-containing promoter, together with some kind of organ specificity, which can be further analyzed functionally with transgenic plants.

Construction of plant expression vector with promoter:GUS fusion and obtaining of transgenic tobacco plants

Insertion of full length (1174 bp) of the cloned 5'-flanking region upstream of the TSP in front of the promoter-less GUS gene of pRD410 gave rise to a recombinant plant expression vector with promoter:GUS fusion (Figure 2), and the fusion was in correct reading frame by sequence-verification (data not shown). This construct, designed as ATA7F0 (-1150), was introduced into *A. tumefaciens* strain LBA4404.

Infection of tobacco leaf-discs with the LBA4404 harboring ATA7F0 regenerated more than 30 independent Kan-R shoots, and most of the shoots (24) were PCR positive for the presence of *ATA7* promoter (figure not shown). The PCR-positive rooted shoots and the transplanted plantlets in greenhouse grew normally as untransformed control.

Activity of *ATA7* full-length promoter in transgenic tobacco T0 plants

The activity of *ATA7* full-length promoter (ATA7F0) in transgenic tobacco plants was detected by histochemical

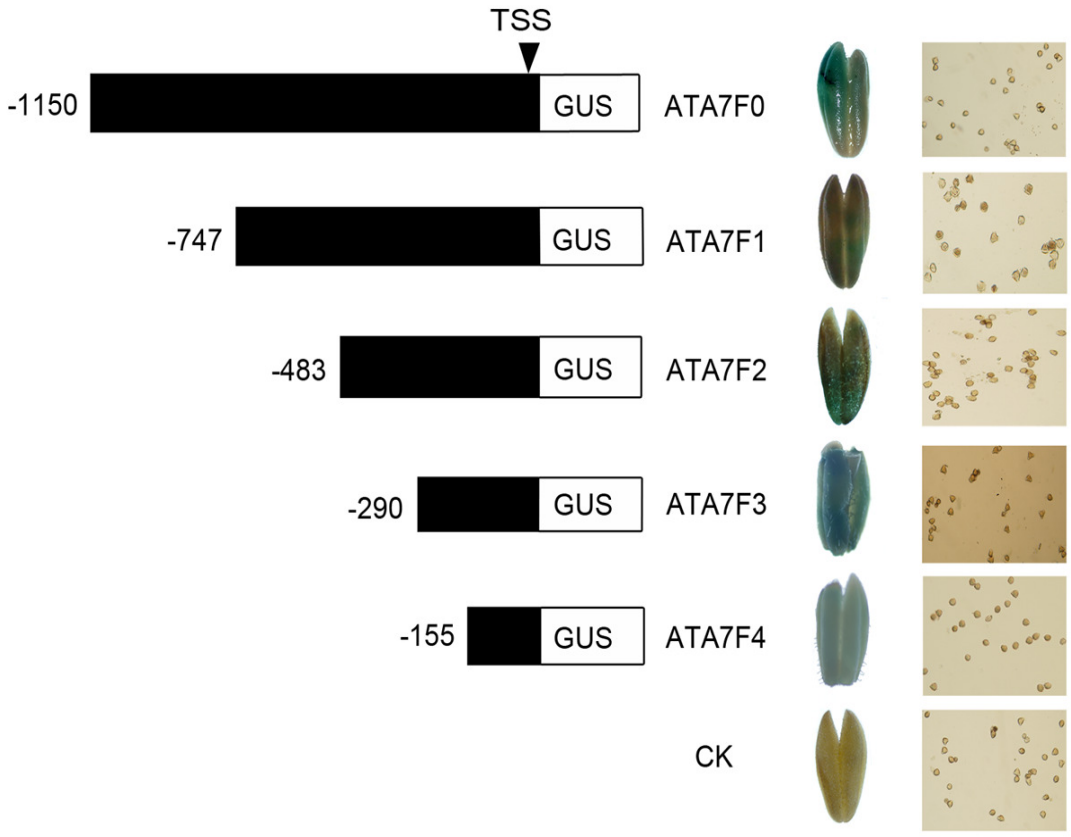

Figure 2. Expression constructs of full-length and 5'-deleted *ATA7* promoters and histochemical analysis of GUS activity in the anther and pollen of transgenic tobacco T0 plants conferred by the constructs. Solid rectangle and the data at its left side: promoter and its length (bp) relative to TSS (Transcription Start Site); ATA7F0: full-length ATA7 promoter; ATA7F1~ATA7F4: four 5'-deleted *ATA7* promoters. Anther and pollen taken from T0 transgenic tobacco plants transformed with corresponding constructs at their left side or from untransformed plants (CK), and stained for GUS activity.

GUS staining of the vegetative and reproductive organs of the plants at various developmental stages, with untransformed wild type as control. From seedling to maturity, the untransformed wild type plant did not show GUS activity in vegetative organs, nor in the reproductive ones. For 11 vigorous independent ATA7F0-transgenic plants tested, the GUS activity was present only in the anther (Figure 2), but not in any other floral organ such as sepal, petal, stamen filament, stigma and ovary, nor in any vegetative organ (figure not shown). For further analysis of the timing of the anther-specific GUS expression, the anthers were GUS-stained at developmental stages of -4 to 1, 2, 3, 4, 6 to 7, 8 and 12, which corresponded to the flower bud size (in mm) 6 ± 2, 11 ± 2, 14 ± 2, 17 ± 2, 25 ± 2, 34 ± 2 and 38 ± 2 mm to open flower, respectively, according to Koltunow et al. (1990). The GUS expression became visible in the anther at stage -3 ~ stage 1, and continued to stage 8 (Figure 2), which corresponded to the anther bud sizes of 6 ± 2 to 11 ± 2 and 34 ± 2 mm, respectively. For the pollens, the GUS expression was not observed from the anther at stage 8 - 12, nor from those dehisced (Figure 2).

Active site of *ATA7* full-length promoter in the anther of transgenic tobacco T0 plants

For locating specific tissue sites of GUS-expression in the anther, the strong GUS-blue anthers, after FAA fixation, were transverse-sectioned with paraffin-embedment. The anther transverse-section showed that the *ATA7* full-length promoter-driving GUS gene expressed exclusively in the tapetum and the tapetal cells were GUS-stained blue (Figure 3). In other tissues of the anther, such as connective, epidermis, vascular bundle, no GUS-blue cells were visible. In the lobes of the anther, some pollen-like cells looked like blue-colour, but the pollens isolated were GUS negative (Figure 2). No visible background GUS-staining was detected in anthers from untransformed plants (CK) under the experiment conditions used (Figure 2).

Activity of 5'-deleted *ATA7* promoters in transgenic tobacco T0 plants

In order to identify putative *cis*-elements that control the

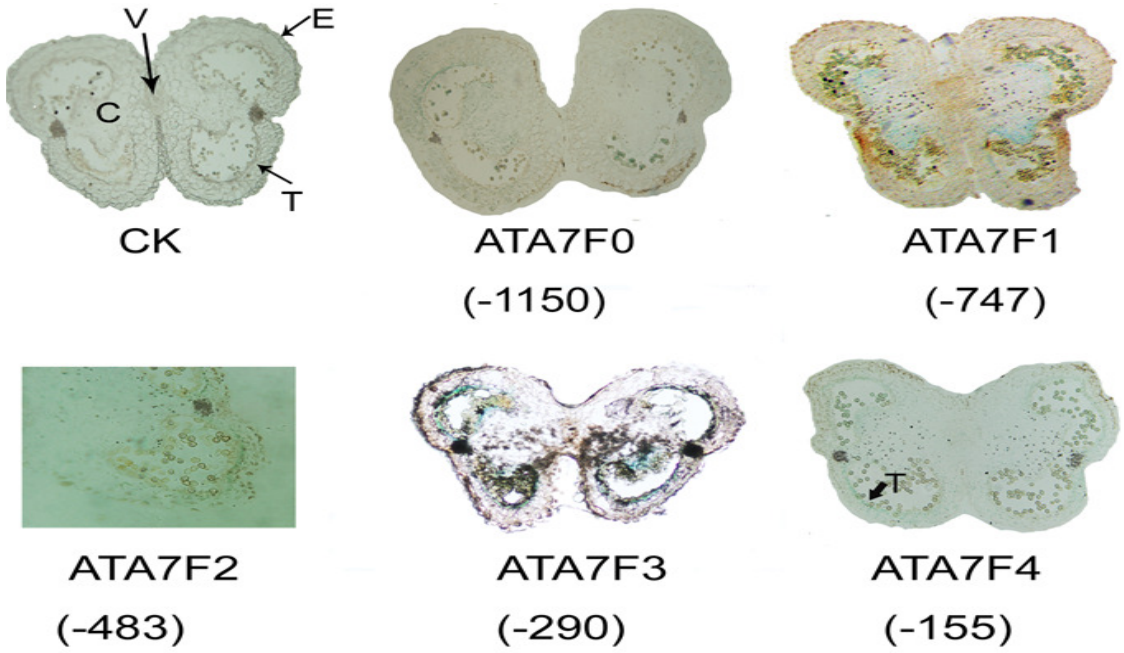

Figure 3. Transverse section photography of GUS-blue anthers from T0 transgenic tobacco plants transformed with full-length or 5'-deleted *ATA7* promoters. ATA7F0: Full-length ATA7 promoter; ATA7F1~ATA7F4: 5'-deleted ATA7 promoter fragments; C: connective; E: epidermis; T: tapetum; V: vascular bundle. The data in parentheses are promoter length in base pair (bp) relative to the transcription start site. Anthers from ATA7F0, ATA7F2 or ATA7F4-tansgenic plants and CK (untransformed control) are paraffin-embedded section; anthers from ATA7F1 and ATA7F3 are the frozen section.

tapetum-specificity of the promoter and to elucidate the core promoter, functionally analyzed *ATA7* full-length promoter was 5'-deleted with the forward primer F1 to F4 and a common reverse primer used for the full-length *ATA7* promoter cloning (Table 1). This series of deletion gave rise to 4 promoter fragments of length (in bp) 771, 507, 314 and 179 (Figure 1; Lane 2 - 5) which corresponded to -747, -483, -290 and -155 bp relative to the transcription start site, respectively (Table 1). The resulting fragments sequencing-verified were separately fused with GUS gene in pRD410 as described for the full-length *ATA7* promoter, and this produced 4 expression vectors, namely, ATA7F1 (-747), ATA7F2 (-483), ATA7F3 (-290) and AA7F4 (-155) , respectively (Figure 2). These constructs were then introduced into tobacco by *A. tumefaciens*-mediated transformation as described earlier, and each constructs produced more than 30 independent transgenic plants for GUS expression analysis as conducted for the full-length *ATA7*.

As for the full-length of *ATA7* promoter, all 5'-deleted *ATA7* promoter fragments did drive GUS gene to express in the anther of the transgenic tobacco plants, but not in pollens (Figure 2), nor in any other floral or vegetative organs tested (data not shown). Among 4 deleted *ATA7s*, a slight difference was observed mainly in the strength and the timing of GUS expression in the anther. In ATA7F1-transgenic anthers, the strong GUS expression appeared at the developmental stage -2~ stage 6, while for ATA7F2, the GUS blue anthers became visible at the stage -3- stage 6. The anther of ATA7F3-transgenic plants had high level of GUS expression from stage -2 to stage 6, and it was the same for the anthers transformed by ATA7F4, the shortest fragment after 5'-deletion (Figure 2).

The GUS-blue anthers from the transgenic plants transformed by each of the 4 deleted promoters were also transverse-sectioned in paraffin-embedment and/or in frozen. The sections showed that the GUS-blue cells were present only in the tapetal cells, not in any other tissues of the anther (Figure 3), and there were no difference among the 4 deleted promoters, ATA7 F1, ATA7F2, ATA7F3 and the shortest one, ATA7F4.

Activity of full-length and 5'-deleted *ATA7* promoters in transgenic tobacco T1 plants

The T0 transgenic lines with stronger GUS-blue anthers in each construct were chosen for producing seeds by selfing, and the T1 offspring used for GUS analysis came from the seeds which gave rise to a 3:1 seedlings segregation of KanR: KanS in the seed germination medium supplemented with the 100 µg/ml of Kan. More than 10 T1 individuals were detected for GUS activity in each of the 3 independent T0 lines per construct.

All T1 individuals displayed a similar anther-specific

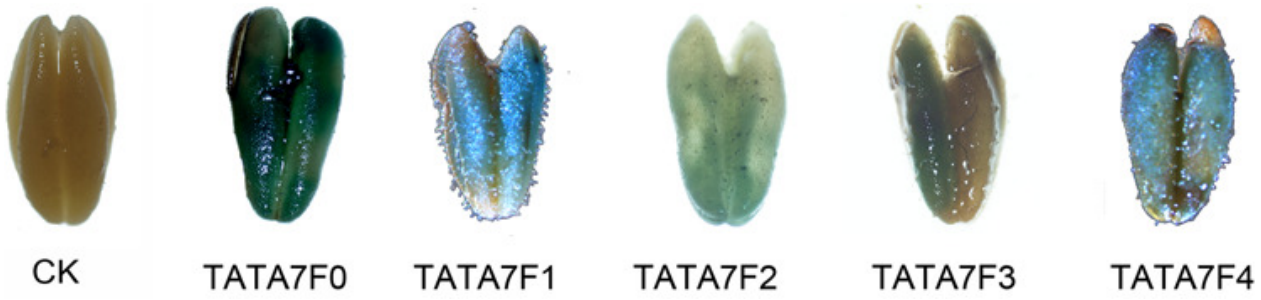

Figure 4. GUS-blue anthers from T1 offspring of T0 transgenic tobacco transformed with full-length or 5'-deleted *ATA7* promoters. TATA7F0 ~ ATA7F4: Anthers of T1 progeny of ATA7F0 ~ ATA7F4-transgenic tobacco plants, respectively; CK: untransformed control.

Table 1. Oligonucleotide primers used for PCR-cloning and 5'-deletion of *ATA7* promoter.

Primer name	Primer sequence	Expected length (bp) of PCR product	Relative distance (bp) to TSS*
Forward			
F0	5'-AAGCTTCAAATCAGTAAAAGCTGACTC	1174	-1150
F1	5'-AAGCTTGAGCTTACCGTGGATTTTTCGC	771	-747
F2	5'-AAGCTTTACCTAATGAAAGTGAGAGACCTC	507	-483
F3	5'-AAGCTTTCTTCTTCAGCCGGACAG	314	-290
F4	5'-AAGCTTAGGCAATGATATCGGCTCATG	179	-155
Reverse	5'-GGATCCCTCATGTTTCCTTCTTACTCG		

*TSS: Transcription start site. Underlined in the forward and reverse primers are *Hin*d III and *Bam* HI recognition sites added, respectively.

GUS expression pattern, just as their parent lines, no matter the length of the *ATA7* promoters used to drive GUS gene in the parent lines (Figure 4). Figure 4 showed a visible difference in GUS-staining strength of the T1 anthers between those that came from the full-length and 5'-deleted *ATA7* parent lines, and the former looked more pronounced than the later, while this kind of difference was not obvious among those from four deleted *ATA7* parent lines. Transverse sections of the GUS-blue anthers from representative T1 individuals in each construct also revealed similar GUS expression pattern in the anther, and only the tapetal layer of cells appeared to be GUS-blue (data not shown), as their parent lines.

DISCUSSION

ATA7 was originally identified by Rubinelli et al. (1998) to be anther-specific at the mRNA level, and the mRNA was then shown to be present only in the tapetum of the anther by *in situ* hybridization (Yang et al., 2003). In this report, we isolated a 1174 bp fragment of the 5'-flanking region upstream of the TSP of *ATA7* which corresponded to 1150 bp upstream of the TSS, and demonstrated that the fragment could direct GUS reporter gene to express exclusively in the anther tapetum of transgenic tobacco plants, both in T0 plants and in their T1 progeny (Figures 2 - 4). This suggested that the tapetum-specificity of *ATA7* mRNA was conferred mainly, if not completely, by its gene promoter.

In order to elucidate putative *cis*-acting elements that may regulate or control the tapetum-specificity in *ATA7* promoter, we performed DNA sequence analysis of the cloned 1150 bp fragment upstream of the TSS with PLACE (http://www.dna.affrc.go.jp/PLACE/index.html). We found that the fragment contained, besides the core promoter element TATABOX5 (Tjaden et al., 1995) at site -48 ~ -43, common *cis*-acting element CAATBOX1 at -73 ~-70 and other 6 sites scattered from -941 to -192, a large number of organ-specific *cis*-regulatory elements, such as pollen-specific POLLEN1LELAT52 (Bate and Twell, 1998; Filichkin et al., 2004), 6 copies, root nodule-specific OSE1ROOTNODULE, 6 copies, and OSE2ROOT NODULE (Vieweg et al. 2004), 1 copy, etc (data not shown). Based on this analysis, we narrowed the cloned fragment by sequential 5'-deletion and got 4 shorter fragments, -747, -483, -290 and -155 relative to the TSS (Figure 1 and Table 1).

We found that the -155 region of *ATA7* promoter was sufficient for conferring tapetum-specific expression of the GUS in transgenic tobacco T0 and T1 plants (Figures 2 - 4), and the longer region, such as -747, -483 and -290,

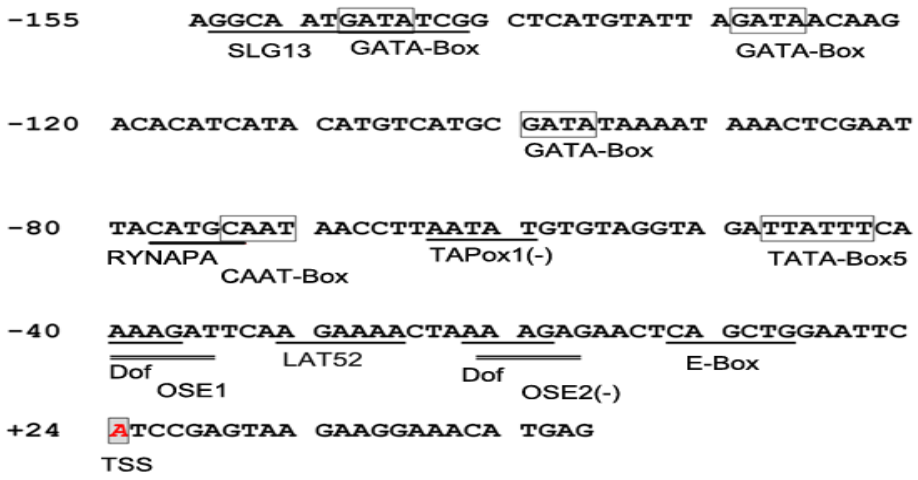

Figure 5. Nucleotide sequence of 179 bp ATA7 promoter fragment and major *cis*-acting elements in its -155 region. Dof: DOFCOREZM; LAT52: POLLEN1LELAT52; E-Box: EBOXBNNAPA; OSE1: OSE1ROOTNODULE; OSE2: OSE2ROOTNODULE; RYNAPA: RYREPEATBNNAPA; TAPOX1: ROOTMOTIFTAPOX1; SLG13: BNSLOCGLYPTN13.

retained also the tapetum-specific expression pattern, but did not increase visibly the driving power. These results suggest that the *cis*-acting element(s) that control or confer the tapetum-specificity of the promoter, if existing, should be within the -155 region. In order to identify this kind of *cis*-elements, we analyzed in detail the DNA sequence of the -155 region (Figure 5) with Plant CARE (http://bioinformatics.psb,ugent.be/webtools/plantcare/html) and SOFTBERRY (http://linuxl.softberry.com/berry.phtml), in addition to PLACE.

We noted that the -155 fragment retained not only the core promoter elements such as TATA-Box, CAAT-Box and GATA-Box, but also numbers of the organ-specific *cis*-elements: LAT52 pollen-specific “AGAAA” at -31 ~ -27, OSE1 root nodule-specific “AAAGAT” at -40 ~ -35 and OSE2 at -21 ~ -16 (-), root-specific ROOTMOTIFTAPOX1 “ATATT” (Elmayan and Tepfer, 1995) at -64 ~ -59 (-), seed-specific RYREPEATBNNAPA “CATGCA” (Ezcurra et al., 1999) at -78 ~ -73 and pistil- and pollen-specific SLG13 “GGCAATGATATCG” (Dzelzkalns et al., 1993) at -154 ~ -142 (Figure 5). In addition, it contained also 2 E-Box “CANNTG” (Stalberg et al., 1996; Sorteberg et al., 2004) at -12 ~ -7 and -12 ~ -7 (-) and 2 Dof binding motifs “AAAG” (Yanagisawa and Schmidt, 1999) at -40 ~-37 and -22 ~ -19. However, we did not observe in this -155 region any tapetum-specific *cis*-element or “motif” conserved in well-known tapetum-specific promoter such as TA29 (Koltunow et al., 1990) and TPD1 (Yang et al., 2003). This result implies that the tapetum-specificity of the -155 promoter was not controlled or conferred by a single or more so called “tapetum-specific *cis*-element” as in the case of TA29. It may be controlled or conferred by some kind of combination and/or interaction of the organ-specific *cis*-elements with certain specific binding site(s) of transcription factor(s). In such kind of combination and/or interaction, the organ-specific *cis*-elements, such as root-, seed- and pollen-specific ones, in single or associated form, may contribute mainly, if not entirely, to the “exclusiveness” (that is, no expression in certain type (s) of organs), but not to any specific organ or tissue, while the specificity to a given tissue or organ may be the major role played by one or more specific binding site(s) of particular transcription factor(s), such as E-Box and Dof binding motifs “AAAG”, etc. The E-Box, named also MYC recognition site, is recognized by the transcription factor bHLH and was presented in some flower-specific promoters (Hartmann et al., 2005). In our previous work, internal deletion of one E-Box in a 294 bp floral organ-specific promoter fragment from *Brassica napus*, Bnfs294, almost abolished the petal-preference and significantly decreased anther-preference of the promoter (Geng et al., 2009). We are clearly aware, that the assumed combination and/or interaction need further investigation, and this is under progress.

In conclusion, we cloned, characterized and functionally analyzed a 1174 bp of *ATA7* which corresponded to 1150 upstream of the TSS, and provided evidence that the cloned fragment could confer tapetum-specific expression of GUS reporter gene in the transgenic tobacco. This result indicated that the tapetum-specificity of *ATA7* mRNA reported was determined by the promoter. We also found that a 179 bp upstream of the translation start point which corresponded to 155 bp fragment upstream of the TSS of *ATA7* promoter was sufficient for

conferring the tapetum-specificity without obviously reducing the driving power of the promoter. The tapetum-specific promoter cloned and the much short one elucidated may be directly used in the creation of male-sterile crops, besides the study of anther-developmental molecular biology.

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