

## cDNA Cloning and Gene Expression of Anthocyanidin Synthase from *Torenia fournieri*

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### Abstract

The cDNA clone, *TANI*, encoding anthocyanidin synthase (ANS) was isolated by screening of a cDNA library constructed from flower petals of *Torenia fournieri* cv. Summer Wave Blue using a heterologous probe. Nucleotide sequence analysis revealed that it contains an open reading frame encoding a polypeptide of 376 amino acids. The postulated amino acid sequence shows 48 % to 72 % identities with those of previously reported ANSs. Southern blot analysis suggested that there is a single copy of *ANS* gene in the genome of *T. fournieri*. Northern blot analysis indicated that *ANS* gene is expressed in flower bud but not in leaf or mature flower.

The nucleotide sequence reported in this paper has been deposited in GenBank/EMBL/DBJ databases with accession number AB044091.

Anthocyanins, representative secondary metabolite found in most plant species, are formed through flavonoid biosynthetic pathway (Heller and Forkmann, 1993). Enzymes responsible for most steps of the pathway have been described, and their cDNAs have been cloned from various plant species (Dooner and Robbins, 1991; Forkmann, 1993; Holton and Cornish, 1995; Martin and Gerats, 1992). Anthocyanidin synthase (ANS) catalyzes the reaction leading to anthocyanidin from leucoanthocyanidin, the first step for the formation of colored metabolites, and thus an important step in anthocyanin formation. In our previous paper (Saito *et al.*, 1999), we studied the mechanism of ANS reaction catalyzed by the recombinant enzyme from *Perilla frutescens*. In the present study, we have isolated a cDNA encoding *ANS* from the flowers of *Torenia fournieri*, and compared with ANS proteins from various plant species. *T. fournieri* is a commercial ornamental plant and also a suitable experimental model for genetic engineering of flower color (Suzuki *et al.*, in press).

The ANS cDNA, *TANI*, was isolated from *T. fournieri* cv. Summer Wave Blue (Suntory Ltd.) by screening of  $\lambda$ ZAP II cDNA library (Stratagene) constructed from flower petals using the heterologous probe isolated from *Perilla frutescens* (Saito

*et al.*, 1999). Nucleotide sequence analysis was carried out by dideoxy chain termination method using automatic sequencer (model DSQ-2000L, Shimadzu, Japan). The nucleotide sequence of *TANI* was 1348 bp long, and contained open reading frame of 1128 bp encoding 376 amino acids forming a 42,130-Da polypeptide (Fig. 1). The authenticity of the first ATG codon was confirmed by the presence of an in-frame stop codon (TAA) at 18-bp upstream from the first ATG. Sequence comparison of amino acid sequence of *TANI* with those of reported ANSs indicated 48–72 % identity and 60–80 % similarity in amino acid residues (Fig. 2A). His-240, Asp-242 and His-296 are completely conserved in ANSs from several plant species, which are thought to bind with iron at the catalytic center of the iron-containing soluble oxygenases (Britsch *et al.*, 1993; De Carolis and De Luca, 1994; Matsuda *et al.*, 1997; Prescott and John, 1996; Prescott, 1993). The Arg-Xaa-Ser (306–308) motif, reported as the specific binding site of 2-oxoglutarate in flavanone 3 $\beta$ -hydroxylase from petunia (Lukacin and Britsch, 1997; Lukacin *et al.*, 2000), is also conserved in ANSs. From phylogenetic tree (Fig. 2B), the *Torenia* ANS falls into a group of dicot ANSs. The phylogenetic tree also indicated the presence of distinct difference

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1  GGCACGAGACAAGCAACACGCGTTTCTTAATTTCTCCCAAAGAACATGGTTTCTCCAGCATCTCCGAGCC
      *                               M V S P A S P S P
71  CGGCCCGTGTGCAACTACTGTGCGAACAGCGGAATCAAAGCGATCCCGAAAGAGTACGTAAGGACCGAAGA
      A R V E L L S N S G I K A I P K E Y V R T E E
141 AGAGCTCCGGAGCATAACCGACATCTTCTCGAAAGAAGATGGCGCGAAAACATCGACAGCCCAGATTG
      E L R S I T D I F S K E D G A K N I D S P D L
211 CCCATCATCGACCTATCGAAGATCGACTCGAGCGACGAAGAGACGCGCAAGAAAGGCCACGAGGAGTTGA
      P I I D L S K I D S S D E E T R K K G H E E L K
281 AAGAAGCAGCGATCGAATGGGGCGTCATGCATCTGATCAACCACGGAATATCGGACGAGCTGATCAATCG
      E A A I E W G V M H L I N H G I S D E L I N R
351 GGTCAAGAAAGCGGGCGCGAGTTCTTCGATCTCCCCGTCGAGGAGAAGGAGAAGTATGCCAACGACCAG
      V K K A G G E F F D L P V E E K E K Y A N D Q
421 TCGAGCGGGAACGTTTACGGGGTACGGAAGTAAATTTGGCGAACACGCGGGTGGGATTTTGGAGTGGGAGG
      S S G N V Q G Y G S K L A N N A G G I L E W E D
491 ACTATTTCTTCCATTGCGTGTATCCCGAGGAGAAGAGGGATATGGCTATTTGGCCTAAGGATCCACAAGA
      Y F F H C V Y P E E K R D M A I W P K D P Q D
561 TTACATCCCGCAACGACCGAGTACGCGAAAGAGATACGATCCCTCACGACCAAGATCCTCTCGGTCTCTC
      Y I P A T T E Y A K E I R S L T T K I L S V L
631 TCGCTCGGCCTCGGGCTCGACCAAGACCGTCTTGAGAAAAGAGGTGCGGTGAAAAGACGACCTCACCCCTCC
      S L G L G L D Q D R L E K E V G G K D D L T L Q
701 AAATGAAGATAAACTACTACCCGAAATGCCCTCAACCCGAGCTAGCCCTGGGCGTCGAGGCCACACGGA
      M K I N Y Y P K C P Q P E L A L G V E A H T D
771 CGTGAGCGCCCTCACGTTTCATCCTCCACAACATGGTCCCGGGCCTCCAAGTGTCTACAAAGCGGAGTGG
      V S A L T F I L H N M V P G L Q V L Y K G E W
841 GTCACCGCTTCATGCGTGCCTGCGACTCCATAATCTTGCATGTTGGGGACACGATCGAGATCTTGAGCAATG
      V T A S C V P D S I I L H V G D T I E I L S N G
911 GCATGTATAAGAGCGTGCCTCCATAGGGGTTTGGTGAATCGGGAGAGGGTTAGGGTTTCTTGGGCGGTCTT
      M Y K S V L H R G L V N R E R V R V S W A V F
981 TTGCGAGCCGCCCAAGGAGAAGATCGTGTCTCAAGCCACTGCCCGAGACGGTCCGCGAGGATCGTCCCGCT
      C E P P K E K I V L K P L P E T V G E D R P A
1051 CTGTTTCCGCCTCGGACTTTCCGCGAACACATGAAGCATAAAGTTGTTCAAGAAGAGCGATGATGAAGTGC
      L F P P R T F A Q H M K H K L F K K S D D E V H
1121 ATGATGAAGAGGGTGTAGTAGTTGTTGAGCATGATGATAAGAGTGTGAGTGTGGGTGGAGTTTATTTGT
      D E E G V V V V E H D D K S V E *
1191 TTGATGATGTTTTGGTTTGAATGTTTGCAATTGTGTTTTTCTGTGATTAATGGATGTATAATGTCCG
1261 GATTATTTTAAGAATATATAACCGTATGTTTTATGTACCATGAAAAATAAGTAATGCTTTTAATTGGCT
1331 AAAAAAAAAAAAAAAAAAAA 1348

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**Fig. 1.** Nucleotide sequence and deduced amino acid sequence of *Torenia ANS*. Asterisk indicates in-flame stop codon.

between the sequences of monocotyledoneae and dicotyledoneae.

For Southern blot analysis, 2.5  $\mu$ g of genomic DNA from leaves of *T. fournieri* isolated by the described method (Murray *et al.*, 1980) was digested with several different restriction enzymes, which do not cut within the *ANS* cDNAs. Then the digested DNAs were separated by electrophoresis on a 0.8 % agarose gel, transferred onto Hybond-

N+ membrane (Amersham) and hybridized with a  $^{32}$ P-labeled cDNA probe at 60 °C. As a probe, coding region of *ANS* cDNA was labeled by  $^{32}$ P-dCTP using Random Primer DNA Labeling Kit (Takara Shuzo, Japan) and purified by NAP5 Column (Pharmacia Biotech). The membrane was finally washed in 0.1  $\times$  SSPE, 0.1 % SDS at 60 °C for 10 min. Hybridization signals were detected with a BAS-2000 Image Analyzer (Fuji Film,

(A)

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torenia      11..MVSPASPSPARVELLNSGILKATPKKEYRFEELRLSTIDFKEDGAKNIQFDPIIDLKIDSSQELT...KKCHBELKRAA   WGVVHL NH : 93
snapdragon  11...MAPAIAPPSRVVELLKSGLQSPKKEYRFEELRLSIGDFAEE...KNN GPO PVIDLAAINSDEVE...KKCH ELKRAA   WGVVHL NH : 89
perilla     11..MVTSAMGPSRVVELLARGSDTIPK YRPEELRLSIGNTLAE...KSS GPO PTIDLKIDSRNEG...KKCH BELKRAA   WGVVHL NH : 91
petunia     11..MVNAVVTTFSPRVESLARGSGIQAIPKEYRFEELRLSIGDFAEE...KRD GPO PTIDLKIDSDKEIT...EKCH LKRAA   WGVVHL NH : 91
grape       11...MVTSVAPRVESLARGSQSPKKEYRFEELRLSIGDFAEE...KRD GPO PTIDLKIDSDKEIT...EKCH LKRAA   WGVVHL NH : 87
forsythia   11...MAKVNPRVELLARGSQSPKKEYRFEELRLSIGDFAEE...KNN GPO PTIDLKIDSDKEIT...EKCH BELKRAA   WGVVHL NH : 89
apple       11...MVSDDSVMSRVELLSGDTIPKKEYRFEELRLSIGDFAEE...KRD GPO PTIDLKIDSDKEIT...EKCH BELKRAA   WGVVHL NH : 87
carrot      11...MVNATLESRVELLAGSGDLIPKEYRKH ELISITDFEE...KSD GPO PTIDLKIDSDKAVR...EKCH BELKRAA   WGVVHL NH : 89
chinaaster  11...MVISANTRVELLAGSGDLIPKEYRKH ELISITDFEE...KSD GPO PTIDLKIDSDKAVR...EKCH BELKRAA   WGVVHL NH : 87
arabidopsis 11...MVAVERVELLARGSGIQAIPKEYRFEELRLSIGDFAEE...KKE GPO PTIDLKIDSDKEIT...EKCH BELKRAA   WGVVHL NH : 85
commonstock 11...MVAVERVELLARGSGIQAIPKEYRFEELRLSIGDFAEE...KKE GPO PTIDLKIDSDKEIT...EKCH BELKRAA   WGVVHL NH : 85
sweetpotato 11..MVTTISATVPSRVELLAGSGDLIPKEYRFEELRLSIGDFAEE...MGGGPO PTIDLKIDSDKEIT...EKCH BELKRAA   WGVVHL NH : 85
maize       11.MESSPLLQLPAAVRVELLSCSALIPKEYRFAERAGSGEALDLAR...THANDHTARIPVDSPPFLDSSSQQQRKCAAA   WGVVHL AGH : 98
rice        11...MTDAELRVELLAGSASALIPKEYRFEELRLSIGDFAEE...AASDDATARPVDSPPFLDSSSQQQRKCAAA   WGVVHL AGH : 69

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torenia      94.GISDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:193
snapdragon  90.GPDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:189
perilla     92.GIPDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:191
petunia     90.GISDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:191
grape       92.GISDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:191
forsythia   88.GISDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:186
apple       90.GISDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:189
carrot      90.GISDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:189
chinaaster  88.GISSLRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:187
arabidopsis 86.GIPALNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:185
commonstock 86.GIPALNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:185
sweetpotato 92.GIPDELNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:191
maize       99.GIPALNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:198
rice        90.GIPALNRVYKAGQEFFLPVEEKEKYANDQAG...QGYGSKLANNASGQLEWEDYFFHCVPEKRDLPKPKDQDIYPATSEYAKRLSLTR   LS:189

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torenia      194:LSGLG.....LQDRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:284
snapdragon  190:LSGLG.....LEPRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:280
perilla     192:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:282
petunia     190:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:280
grape       192:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:277
forsythia   187:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:280
apple       190:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:280
carrot      190:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:278
chinaaster  188:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:276
arabidopsis 186:LSGLG.....LEPRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:276
commonstock 186:LSGLG.....LEPRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:276
sweetpotato 192:LSGLG.....LEQRLEKEVGG...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:282
maize       199:LSGLG...LPBETLERRRGGHLAGV...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:286
rice        190:LSGLG...LPBETLERRRGGHLAGV...LLOMKINYYPKCQPELALGVEAHTDVSALTFILHNNVPGQLQFYVGGKRWVTAKCVPDSIIMHIGDT   E:286

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torenia      285:ILSNGKYSILHRGLVNEVVRSWAVFCPPPKK...LKPLEPTEVGEADR...PALFPFRFQA...KHLF...ESDDEVHDEEGVVVVEHDDKSVE...   :375
snapdragon  281:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPEV...EAL...PAMFPFRFQA...KHLFR...EGEGEVKDGDDGGVVDIGKQDEKVE...   :373
perilla     283:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...EVE...PFRFPFRFQA...KHLFR...TDDGDEKETY...   :362
petunia     281:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...EAL...PFRFPFRFQA...KHLFR...DDKDAAEVHKVFNEDLDTAAEHKVLKRDN...   :379
grape       283:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLAHG...RCLR...L...LSH...S...AFFPNIF...SSSSGPRRFLYSPNEL...   :362
forsythia   278:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...EAL...PFRFPFRFQA...KHLFR...S...S...S...S...S...S...   :357
apple       281:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...EAL...PFRFPFRFQA...KHLFR...S...S...S...S...S...S...   :379
carrot      281:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...EAL...PFRFPFRFQA...KHLFR...S...S...S...S...S...S...   :355
chinaaster  279:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...VES...AKFPFRFQA...KHLFR...S...S...S...S...S...S...   :356
arabidopsis 277:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPEM...VES...AKFPFRFQA...KHLFR...S...S...S...S...S...S...   :356
commonstock 277:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPEM...VES...AKFPFRFQA...KHLFR...S...S...S...S...S...S...   :356
sweetpotato 283:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPET...EAD...PFRFPFRFQA...KHLFR...T...D...G...A...D...T...P...D...   :364
maize       299:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPEL...E...S...L...P...L...E...L...V...E...G...H...P...R...T...P...R...F...Q...   :395
rice        287:ILSNGKYSILHRGLVNEKVRISWAVFCPPPKK...LKPLEPEL...E...S...L...P...L...E...L...V...E...G...H...P...R...T...P...R...F...Q...   :375

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torenia      :
snapdragon  :
perilla     380:QDAVAENKDIKEDEQCGPAEHKDIKEDGGQAAAENKVFKENNQDVAAEESK :430
petunia     :
grape       :
forsythia   :
apple       380:KTESNPKIELKPGEEASSP... :402
carrot      :
chinaaster  :
arabidopsis :
commonstock :
sweetpotato :
maize       :
rice        :

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(B)

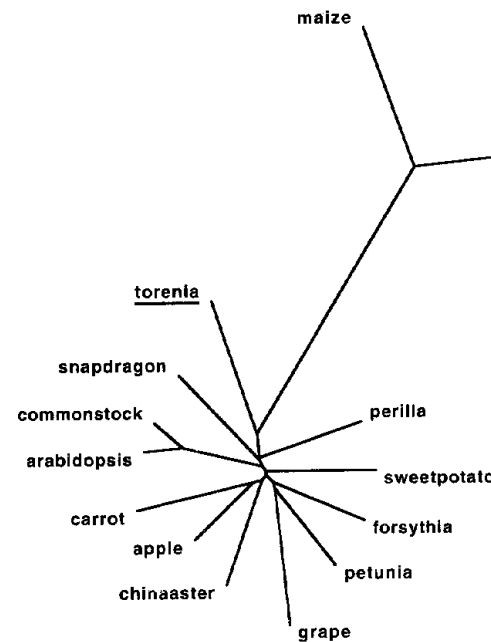
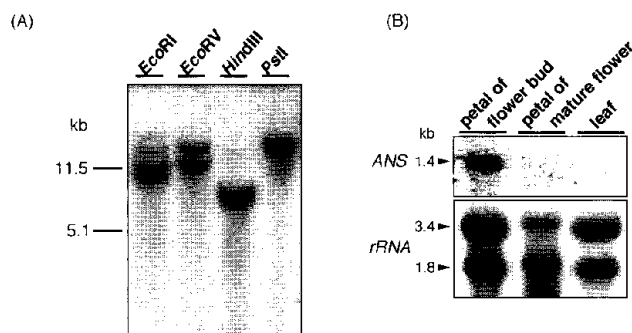


Fig. 2. Multiple alignment (A) and phylogenetic tree (B) of deduced amino acid sequences of ANSs from several plant species. (Continued on back page)

Multiple alignment of amino acid sequences was carried out by clustal W program (Thomopson *et al.*, 1994). Black shading indicates identical amino acids and gray shading shows similar amino acids. Dashes indicate gaps in the sequences for best alignment. Arrowheads show conserved His and Asp residues postulated as required for ferrous-ion coordination. Asterisks indicate the Arg-Xaa-Ser motif conserved in flavanone 3 $\beta$ -hydroxylase from petunia, assumed to be a binding site of 2-oxoglutarate (Lukacin and Britsch, 1997; Lukacin *et al.*, 2000). Phylogenetic tree was generated by neighbor-joining method (Saitou and Nei, 1987). *torenia*, *Torenia fournieri* (this study); snapdragon, *Antirrhinum majus* (Martin *et al.*, 1991); perilla, *Perilla frutescens* (Saito *et al.*, 1999); petunia, *Petunia hybrida* (Weiss *et al.*, 1993); grape, *Vitis vinifera* L. (Sparvoli *et al.*, 1994); forsythia, *Forsythia x intermedia* (Rosati *et al.*, 1999); apple, *Malus* sp. (Davies, 1993); carrot, *Daucus carota* (Hirner and Seitz, accession number AF184273); chinaaster, *Callistephus chinensis* (Henkel and Forkmann, accession number AF015885); arabidopsis, *Arabidopsis thaliana* (Pelletier *et al.*, 1997); commonstock, *Matthiola incana* (Min and Forkmann, accession number AF026058); sweetpotato, *Ipomoea batatas* (Shiokawa *et al.*, accession number AB023786); maize, *Zea mays* (Messen *et al.*, 1990); rice, *Oryza sativa* (Reddy, accession number Y07955).



**Fig. 3.** Southern blot (A) and northern blot (B) analyses of *ANS* gene from *Torenia fournieri*. (A) Genomic DNA (2.5  $\mu$ g) isolated from leaves were digested with *EcoRI*, *EcoRV*, *HindIII* and *PstI*, separated on an agarose gel (0.8 %), transferred onto a membrane, and then hybridized with  $^{32}$ P-labeled *TANI* coding region as the probe. (B) Total RNA (10  $\mu$ g) isolated from petals of flower buds, mature flower and leaves were electrophoresed on an agarose gel (1.2 %), transferred to a membrane, and then hybridized with the probe. The probe was  $^{32}$ P-labeled *TANI* cDNA (*ANS*) or rice DNA encoding ribosomal RNA (*rRNA*) for a load control.

Japan) (Fig. 3A). The hybridization pattern suggested the presence of a single-copy *ANS* gene in *T. fournieri*. Two apparent bands upon digestion by *EcoRI* and *EcoRV* are presumably due to the presence of these restriction sites in intron region or partial digestion of DNA.

For RNA blot analysis, total RNA was extracted using RNeasy Plant Mini Kit (Qiagen) from petals of flower bud and mature flower, and leaf, according to manufacturer's instructions. Ten micrograms of RNA was separated under a denaturing condition on a 1.2 % agarose gel containing formaldehyde and

then transferred onto a Hybond-N+ membrane. Hybridization of the membrane was performed by the same probe under the same condition for Southern blot analysis. To verify equivalent loading of RNA on the blot, the membrane was also probed with  $^{32}$ P-labeled rice DNA for ribosomal RNA, pRR217 (Takaiwa *et al.*, 1985). As shown in Fig. 3B, *ANS* transcript (1.4 kb) was detected only in petals of flower bud and no evident signal of mRNA expression was observed in mature flower petal and leaf. This expression pattern is well consistent with the timing of coloring in petal of *T. fournieri*. The similar developmentally regulated expression of *ANS* gene was reported for the flower of *Forsythia x intermedia* (Rosati *et al.*, 1999).

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