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Isolation and Characterization of a Putative Class E Gene from *Taihangia rupestris*

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Abstract

Studies in model plants showed that *SEPALLATA* (*SEP*) genes are required for the identification of floral organs and the determination of floral meristems in *Arabidopsis*. In this paper a *SEP* homolog, *TrSEP3,* was isolated from a China-specific species, *Taihangia rupestrisi* Yü et Li. Phylogenetic analysis showed that the gene belongs to the *SEP3*-clade of *SEP* (previous *AGL2*) subfamily. *In situ* hybridization was used to reveal the potential functional specification, and the results showed that *TrSEP3* expression was first observed in floral meristems and then confined to the floral primordia of the three inner whorls. In the matured flower, *TrSEP3* was strongly expressed in the tips of pistils and weak in stamens and petals. The evolution force analysis shows that *TrSEP3* might undergo a relaxed negative selection. These results suggested that *TrSEP3* may not only function in determining the identity of floral meristems and the primordia of three inner whorls, but also function in matured reproductive organs.

Key words: class E gene; MADS-box; selection pressure; *Taihangia rupestris.*

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Floral homeotic MADS-box genes encoding transcription factors play important roles in determining floral organ identities. The classical ABC model, primarily based on the studies of homeotic mutants of *Arabidopsis* and *Antirhinum*, provides a conceptual framework to explain how homeotic genes control floral organ identities (Coen and Meyerowitz 1991). Class A genes are responsible for the specification of sepals in the first whorl, class A and B genes in combination for petals in the second whorl, class B and C genes together for stamens in the third whorl, and class C genes alone for carpels in the fourth whorl. Later, based on studies on petunia, the ABC model was extended by class D genes, which specify the identity of ovules (Angenent et al. 1995; Colombo et al. 1995). Furthermore, this model has been significantly modified by the introduction of new class E genes (represented by *SEP*-like genes) (Pelaz et al. 2000; Honma and Goto 2001; Ditta et al. 2004). In *sep* single mutant, only subtle phenotype is observed, while in *sep1/2/3* triple mutant, all of the flower organs are converted into sepals and the centers of the flowers lose determinacy, which is strikingly similar to that of *bc* (*ap3 ag* and *pi ag*) double mutants (Pelaz et al. 2000; Honma and Goto 2001). In *sep1/2/3/4* quadruple mutant, flower organs are converted into leaf-like organs (Pelaz et al. 2001; Ditta et al. 2004). These data suggest that four *SEP* genes of *Arabidopsis* are required for the specification of all four whorls of flower organs and for the determinacy of floral meristems. Based on these and other

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experimental evidence, a "floral quartet" model is proposed (Theissen 2001). This model proposes the importance of SEP proteins in the specification of flower organs with predicting the molecular mode of interaction of the different floral homeotic genes at molecular level for the specification of floral organ identities (Theissen 2001).

The *SEP*-like genes form a separate subfamily (previous *AGL2*-like subfamily) within the MADS-box gene family (Becker and Theissen 2003). Some genes with *SEP* functions have been studied in other core eudicots, such as tomato (*Solanum lycopersicum*) (Pnueli et al. 1994; Ampomah-Dwamena et al. 2002), petunia (*Petunia hybrida*) (Angenent et al. 1994; Ferrario et al. 2003), apple (*Malus X domestica*) (Sung et al. 2000), *Gerbera hybrid* (Kotilainen et al. 2000; Uimari et al. 2004). Most of these genes show similar expression patterns to that of *SEP* genes from *Arabidopsis*, indicating the conserved function of this kind of genes in specifying floral organ identities. However, members of the *SEP*-like gene subfamily may have sub- and/or neofunctionalized. For instance, In *Arabidopsis SEP3* has stronger activity than the other three *SEP* genes although they are functionally redundant (Honma and Goto 2001; Ditta et al. 2004). Two *Gerbera SEP*-like genes *GERBERA REGULATOR OF CAPITULUM DEVELOPMENT1* (*GRCD1*) and *GRCD2* have specific whorl functions, with *GRCD2* acting on the fourth whorl and *GRCD1* on the third whorl (Kotilainen et al. 2000; Uimari et al. 2004). Furthermore, *GRCD2* has also been found to play roles in regulating inflorescence development. These results suggest that the functional conservation of class E gene is not very clear, even in eudicots.

According to an angiosperm phylogeny inferred from multiple genes, Rosaceae family and Brassicales family belong to eurosid I and eurosid II, respectively. Both of them are members of eudicots (Soltis et al. 1999). *Taihangia rupestris*, a particular species found only in China, belongs to the Rosaceae family within the Dryadeae tribe. Its flowers are bisexual or unisexual, with five sepals, five petals, numerous stamens or pistils (Yu and Li 1983). Compared with other genera of this tribe, such as *Dryas*, *Geum*, *coluria* and *waldsteinia*, *Taihangia* has advanced features and primitive features. It has unisexual flowers and an always herbaceous habit, and the unisexual flower is regarded as more advanced than the bisexual. In this respect, study on functions of MADS-box genes in *Taihangia*, might provide some valuable information for illustrating the function of *SEP* genes.

Here we isolated a *SEP3*-like gene from *Taihangia*, termed *TrSEP3*. We performed phylogenetic analysis, evolution force analysis and *in situ* hybridization, to characterize its functions. The difference between this gene and other *SEP3*-like genes were also discussed.

Results

TrSEP3 is a *SEP3*-like MADS-box gene

TrSEP3 (GenBank accession no. DQ372071) was isolated by the rapid amplification of cDNA ends method (RACE) from young floral buds of *T. rupestris* using degenerate primers. The nucleotide acid sequence of the *TrSEP3* clone was 900-bp long and potentially encodes 249-AA (amino acid) protein. The putative AA sequence of *TrSEP3* comprises four regions of typical MIKC-type MADS-box genes: the MADS (M) domain (57 AA), intervening (I) domain (41 AA), keratin-like (K) domain (73 AA), and the C-terminal (C) domain (78 AA) (Figure1). The putative TrSEP3 protein had a high sequence identity to those of DEFH72, DEFH200, FBP2, LeMADS5 (ranging from 81% to 82%) and SEP3 (70%) (Table 1). These proteins shared an identical M-domain and highly conserved K-domains (Table 1).

In the K-domain and C-terminus of the TrSEP3 protein, the previously described putative amphipathic alpha-helices (Ma et al. 1991) formed by heptad $(abcdefg)_n$ repeats can be found where **a** and **d** positions are occupied by hydrophobic AAs (Yang et al. 2003b). The AA residues at the **a** and **d** positions were highly conserved among the selected genes (TM5 and GRCD1 are exceptions) (Figure 1). TrSEP3 AA sequence contains the conserved residues at the C-terminus, namely the SEPI (EPTLQIG in most orthologues of SEP3 clade) and SEPII motifs (GWLP in SEP3 clade) (Johansen et al. 2002; Vandenbussche et al. 2003; Zahn et al. 2005), which is typical for the SEP subfamily proteins (Figure 1, Table 1).

To establish the phylogenetic relationship between *TrSEP3* and the other *SEP*-like genes, a phylogenetic tree was constructed using the deduced AA sequences of 27 MADS-box genes at full length. The result reveals that TrSEP3 protein falls into the SEP3 subclade and is more closely relative to FBP2, DEFH72 and DEFH200 than to SEP3 (Figure 2).

Spatial and temporal expression of *TrSEP3* in *T. rupestris*

In order to understand the function of *TrSEP3* in *Taihangia*, *in situ* hybridization was performed to examine the temporal and spatial expression pattern of *TrSEP3* during flower development. The 3'-end of the cDNA including the I-, K- and C-domains was used as the probe to avoid cross-hybridization. A strong *TrSEP3* expression was firstly observed at the central domain of floral meristems before the emergence of floral organ primordia (Figure 3A). The *TrSEP3* transcripts were mainly confined to the perianth-tube (composed of the sepal and petal) primordia emerging on the flanks of the floral meristems (Figure 3B). Subsequently, the *TrSEP3* transcripts were detected in

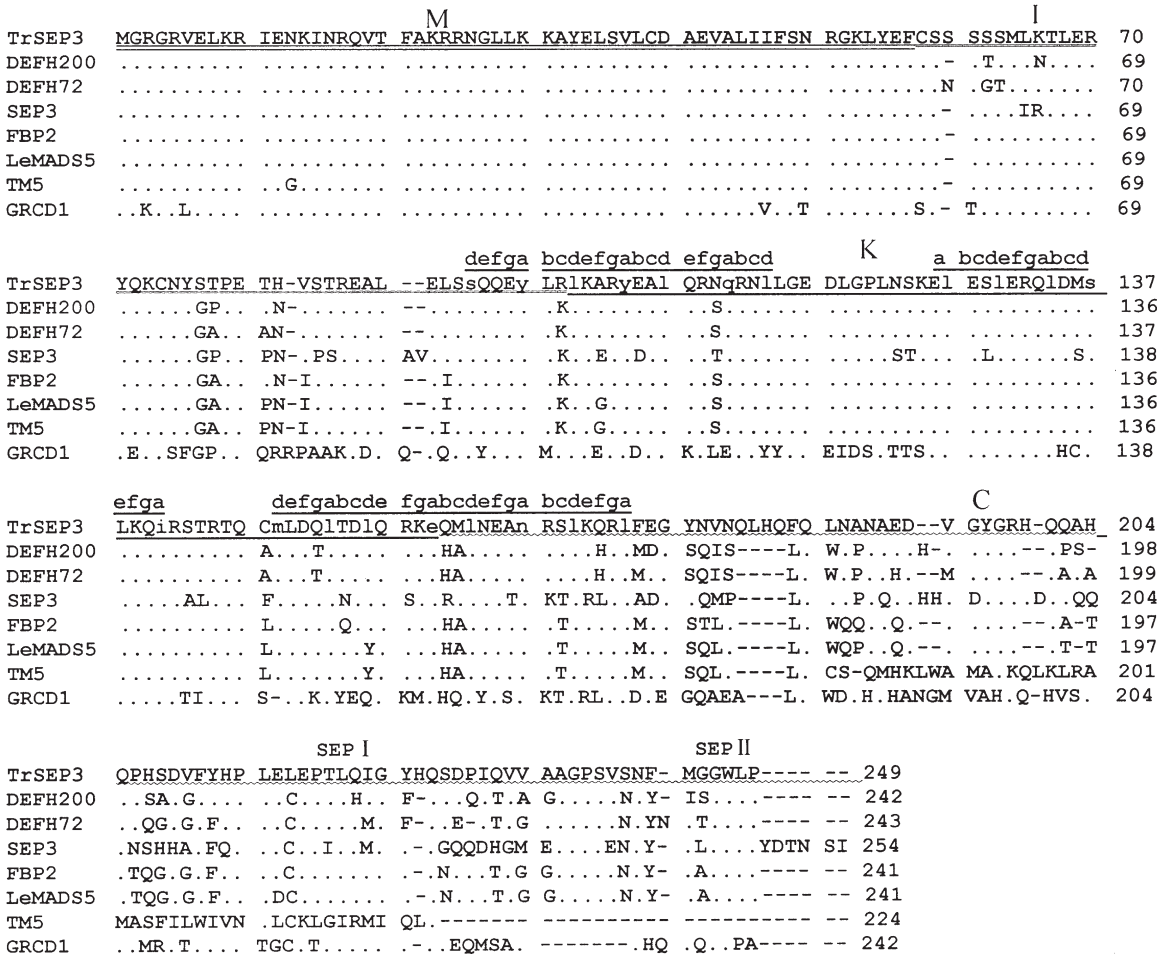

Figure 1. Multiple alignment of TrSEP3 with other selected SEP3-like proteins.

Compared with SEP3, TrSEP3 contain M-domain (57 AA, indicated by the M and double line), I-intervine (41 AA, indicated by I and double wave-line), K-box (73 AA, indicated by K and single line) and C-terminus (78 AA, indicated by C and wave-line). The K1, K2, and K2 motifs are indicated with heptad $(abcdefg)_n$ repeats above the sequence. Dots indicate identical residues and dashes represent gaps introduced to maximize the alignment. The accession numbers of the proteins are the same as those in Table 1.

Table 1. Amino acid identity of the TrSEP3 protein, the M and K domains with the other SEP3-like proteins

	Protein (%)	M (%)	K (%)	I (%)
DEFH72	81.2	100	91.78	83
DEFH200	81.6	100	91.78	85.4
SEP3	70	100	75	74.4
FBP2	82	100	90.4	76.1
LeMADS5	81	100	91.8	83
TM5	65	98	80	83
GRCD1	57	93	54	53.5

Accession no: LeMADS5 (AAP57413), TM5 (Q42464). The others are the same as those in Table 2.

the emerging primordial of stamens and carpels, and were strongly expressed in stamen primordia (Figure 3C). When the three inner whorls of floral organs matured, *TrSEP3* expression was mainly confined to the reproductive organs (stamens and ovules), while very weak (Figure 3C) or undetectable in petals (Figure 3D). During the development of stamens, *TrSEP3* transcripts were detected in sporogenous tissue (Figure 3E).

Evolutionary force analysis

Phylogenetic analyses revealed in previous studies that the *SEP* subfamily has experienced several duplication events,

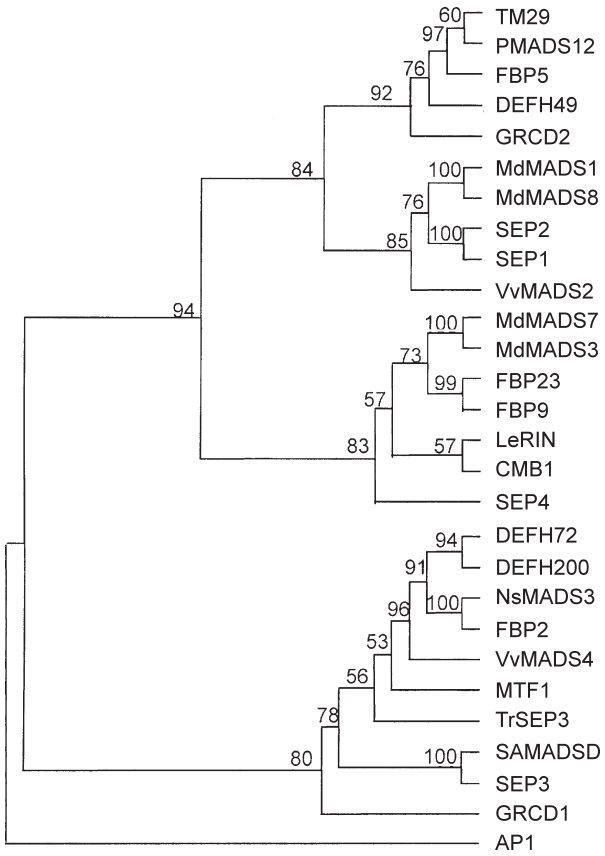

Figure 2. Phylogenetic analysis of selected MADS-box proteins.

Full-length AA sequences were used. Multiple alignment was carried out using Clustal_X (1.83) with default setting. Phylogenetic tree was constructed with ProtPars program of PHYLIP 3.63. AP1 from *Arabidopsis* was used as the out group. Bootstrap values are shown on branches. Accession no: MdMADS8 (CAA04919), CMB1 (Q39685), AP1 (Q41276). The other proteins are equivalent to those of genes in Table 2.

which might potentially change the functions of these genes (Irish and Litt 2005; Zahn et al. 2005). So it is reasonable to examine whether *TrSEP3* has evolved under relaxed or changed constraint. The nonsynonymous/synonymous substitution rate ratio ($\omega=d_N/d_S$) provides a sensitive measurement of selective pressure at the AA level. A lineage that underwent Darwinian selection may have a d_N/d_S ratio that is not equal to zero. The likelihood ratio test (LRT) can be used to distinguish the neutral evolution ($\omega=0$), purifying selection ($0<\omega<1$) and positive selection ($\omega>1$), respectively (Yang 1998). We used the CODEML program of the PAML 3.14b package (Yang 1997) to carry out a branch-specific LRT on some eudicot *SEP*-like genes. The sequences of MIK regions at AA level were used in this analysis.

The one-ratio model, which assumes the same ω parameter for the entire tree, leads to the log-likelihood value (lnL) equal to –7 026.5 with an estimated d_N/d_S (ω) 0.096 6 among the selected *SEP*-like genes. The low average ratio ($\omega<1$) suggests that purifying selection is dominating the evolution of undertaken *SEP*-like genes. The free-ratio model, which assumes a different ω value for each branch in the tree, leads to the lnL= -6 973.5. That the statistic difference (2ΔlnL = 106) between the LRTs of the two models is significant ($\chi^2_{0.01} = 76$, df = 49) suggests that the free-ratio model fits the data better than the one-ratio model, which means that the evolution forces are not equal for all the analyzed *SEP*-like genes even though these

Table 2. Parameter estimates under two-ratio model

	lnL‡	ω_0†	ω_1††	2ΔlnL
TM29	–7 025.49	0.095 4	0.212 2	2.063 8
PMADS12	–7 025.52	0.095 4	0.191 4	1.987 4
FBP5	–7 026.52	0.096 6	0.097 9	0.001 3
DEFH49	–7 025.27	0.098 7	0.057 7	2.486 3
GRCD2	–7 025.59	0.094 7	0.150 0	1.862 6
MdMADS1	–7 026.52	0.096 6	0.807 5	0.000 0
SEP2	–7 026.37	0.097 1	0.076 5	0.298 1
SEP1	–7 024.81	0.098 2	0.031 8	3.410 3
VvMADS2	–7 026.38	0.096	0.119 4	0.280 3
MdMADS7	–7 026.23	0.096 7	0.000 1	0.580 5
MdMADS3	–7 026.52	0.096 6	0.000 1	0.000 1
FBP23	–7 025.88	0.095 3	0.144 7	1.282 9
FBP9	–7 025.52	0.095 2	0.218 0	1.997 6
LeRIN	–7 026.46	0.096 1	0.108 7	0.107 4
SEP4	–7 026.02	0.095 2	0.132 1	0.991 1
DEFH72	–7 025.8	0.097 7	0.055 1	1.438 5
DEFH200	–7 026.33	0.097	0.062 4	0.376 2
NsMADS3	–7 023.52	0.097 7	0.000 1	6.002 0*
FBP2	–7 023.03	0.098 5	0.019 1	6.973 5**
VvMADS4	–7 021.16	0.100 3	0.026 0	10.708 8**
MTF1	–7 025.73	0.098 2	0.063 1	1.579 5
TrSEP3	–7 026.39	0.096	0.117 6	0.264 7
SAMADSD	–7 024.37	0.098 1	0.028 1	4.298 5*
SEP3	–7 023.23	0.098 5	0.009 8	6.575 7*
GRCD1	–7 021.36	0.092 2	0.260 4	10.313 9**

‡log likelihood value. †background d_N/d_S when a branch specified; ††d_N/d_S for a specified branch. *, 5% significance level; **, 1% significance level.

Accession no: *TM29* (AJ302015), *PMADS12* (AY370527), *FBP5* (AF335235), *DEFH49* (X95467), *GRCD2* (AJ784156), *MdMADS1* (U78947), *SEP2* (M55552), *SEP1* (BT006224), *VvMADS2*, *MdMADS3* (U78949), *MdMADS7* (AJ000761), *FBP23* (AF335241), *FBP9* (AF335236), *LeRIN* (AF448522), *SEP4* (AY096386), *DEFH72* (X95468), *DEFH200* (X95469), *NsMADS3* (AF068722), *FBP2* (M91666), *VvMADS4* (AF373603), *MTF1* (AJ223318), *TrSEP3* (DQ372071), *SAMADSD* (Y08626), *SEP3* (AF015552), *GRCD1* (AJ400623).

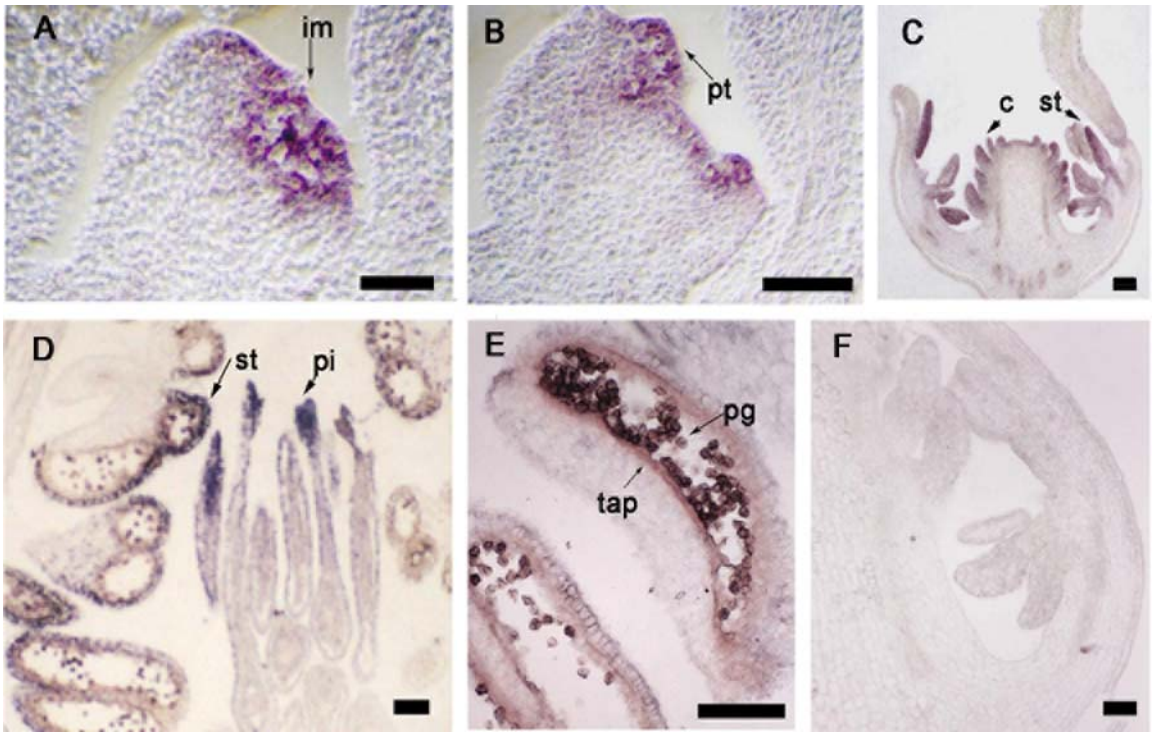

Figure 3. *In situ* hybridization analysis of *TrSEP3* in wild-type *Taihangia rupestris*.

(A) *TrSEP3* is expressed at the tips of inflorescence meristems (im).

(B) Floral primordial with the emergency of perianth tube primordial (pt).

(C) Flower bud with petals and two inner floral organ primordia. *TrSEP3* expression is reduced in the petals (p) and is localized to primordial of stamens (st) and carpels (c).

(D) Developed stamens (st) and pistils (pi). *TrSEP3* transcripts are concentrated on anthers and tips of pistils.

(E) Developed anthers. *TrSEP3* expressed in the tapetal region (tap) and pollen grains (pg).

(F) Tissue section with sense RNA as negative control. Bars =100 μm .

genes have spent a majority of time under negative selection during the evolution.

To test whether the *TrSEP3* has evolved under-changed constraint, the two-ratio model was used and the result was compared with that of the one-ratio model. The two-ratio model assigns two ω ratios for the designated branch (ω_1) and for all the other branches (ω_0). When *TrSEP3* is designated, the resulted lnL was −7 026.4 with estimated parameter ω_0 =0.096 for the background branches and ω_1 = 0.117 6 for the *TrSEP3* branch (Table 2). The statistic difference of lnLs was not significant ($2\Delta\text{lnL} = 0.26$, $\chi^2_{0.05} = 3.84$, df = 1), indicating that *TrSEP3* has not undergone intensified purifying selection.

Discussion

We isolated a MADS-box gene from *Taihangia*, designated as *TrSEP3* because of the high similarity of its deduced AA sequence to the other *SEP3*-like genes.

TrSEP3 contains conserved motifs like other *SEP3*-like proteins. The M-domains of the selected MADS-box proteins are nearly completely conserved, and the K1, K2, and K3 regions are highly conserved (Figure 1). The C-termini of these proteins are less conservative compared with the other domains (Figure 1). Previous studies have shown that K- and C-domains of SEP1, 2, 3 and 4 proteins are necessary for the interaction with AG protein, while M- and I-domains are not (Fan et al. 1997), and the K-domain may be critical for the interaction strength of all MADS-box proteins (Yang et al. 2003b). The interaction between PI and SEP3 requires the interhelical region between K1 and K2, and part of K3 (Yang and Jack 2004). The C-terminus of MADS-box proteins may mediate the ternary complex formation, and specify the regulation of MADS-box genes (Egea-Cortines et al. 1999). So it is the

less conserved C-domain of TrSEP3 that has the potential, if any, to contribute to its special function.

Like other *SEP3*-like genes, the *TrSEP3* expression was strongly detected in floral meristems and in the organ primordia of the inner three whorls. *TrSEP3* was strongly expressed in the central domain of floral meristems before the emergence of floral organ primordia, then in the three inner whorls of floral organ primordia. At the late stage of flower development, *TrSEP3* expression was confined in the three inner whorls of floral organs. This expression pattern is similar to that of *DEFH72* and *DEFH200* from *Antirrhinum* (Davies et al. 1996) and *SEP3* from *Arabidopsis* (Mandel and Yanofsky 1998), suggesting that this gene may be involved in determining the floral meristems and specifying the organ identities of the innermost three flower whorls. In contrast, *TrSEP3* was strongly expressed at the tips of pistils and weakly expressed in anther walls, similar to that of *TM5* of tomato (Pnueli et al. 1994) and different from that of *SEP3* of *Arabidopsis* (Mandel and Yanofsky 1998), indicating that *TrSEP3* may have gained novel functions in matured reproductive organs.

Functionally characterized *SEP* genes playing various roles suggests there has been extensive redeployment of different *SEP* genes in different plant species (Pnueli et al. 1994; Kotilainen et al. 2000; Pelaz et al. 2000; Ampomah-Dwamena et al. 2002; Immink et al. 2002; Vrebalov et al. 2002; Ferrario et al. 2003; Ditta et al. 2004). Duplication is one of the two main molecular mechanisms having altered body forms in evolution (Weatherbee and Carroll 1999; Ng and Yanofsky 2001) and may have provided the raw materials for the diversification of MADS-box genes (Irish 2003; Kramer et al. 2003; Zahn et al. 2005). There are also other *TrSEP3* paralogues identified from *Taihangia* (Lü et al. 2007, in press). Experiments using RNAi to downregulate the expression of this gene will help elucidate whether *TrSEP3* has evolved new functions.

SEP proteins are involved in the formation of higher order complexes according to the floral quartets model (Theissen 2001), so the functional variation of *SEP* genes might reflect the varied combination specificity within such complexes (Irish and Litt 2005), and it will help us examine the *SEP* function by further study on *TrSEP3* and its paralogues in *Taihangia*.

Methods and Materials

Isolation of *TrSEP3* nucleotide acid sequence

Total RNA was isolated from floral buds of *Taihangia rupestris* Yü et Li using Trizol Reagent (Invitrogen, Cat.No.15596). Polyadenylated RNA was purified with the Oligotex mRNA Mini Kit (QIAGEN, Cat.No.70022) and converted into double-stranded cDNA with the use of SuperScriptII (Invitrogen, Cat.No.18064) and an oligo $(dT)_{17}$ primer PTA (5'-CCGGATCCTCTAGAGCGGCCGC$(T)_{17}$-3'). A 5'-degeneration primer SL (5'-GTTCT(G/C)TGTGATGCTGAGGTTGC-3') and a 3'-adapter primer AP (5'-CCGGATCCTCTAGAGCGGCCGC-3') were used to amplify the synthesized cDNA by PCR. The PCR products were cloned into pGEM-T vector (Promega, Cat.No.A3600) and sequenced. According to the sequence, two nested reversed primers XD1 (5'-CTTCAATGACATATCCAGCTGCC-3') and XD2 (5'-CCAGCATGCACTGTGTCC-3') were designed to amplify the 5'-end sequence in 5'-RACE with PTA primer and AP primer. After sequencing, the sequences were assembled. Sequencing of cDNAs was performed with the ABI PRISM dye terminator kit (PE Applied Biosystems, Foster City, CA).

Sequence analysis of *TrSEP3* and construction of phylogenic tree

The sequence characters of *TrSEP3* were analyzed primarily with BioEdit (Yang et al. 2003a) and the longest open reading frame was translated into the peptide sequence which was used to do BLAST search (Wheeler et al. 2003) against Swisspot and NCBI databases. Multiple sequence alignment at amino acid level was performed using the Clustal_X (1.83) with manual adjustments. The phylogenetic tree of amino acid sequences was constructed with the Protpars program of the PHYLIP 3.63 package (Felsenstein 2004). Bootstrap analysis was performed with Seqboot program.

Evolutionary force analysis

The codon region alignment was obtained with DAMBE 4.2 package (Xia and Xie 2001) according to the amino acid multiple alignment. Only the sequences of MADS, I, and K domains were used in the alignment. The branch-specific model of PAML 3.14b package (Yang 1997) was used to analyze selection forces.

In situ hybridization analysis of *TrSEP3*

The fixation and *in situ* hybridization methods used here have been reported before (Li et al. 2005). The less-conserved sequences (nucleotides 170–766 counted from the start codon ATG) were used as the template to synthesize the sense and antisense probes initiated.

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References

A**mpomah-Dwamena C, Morris BA, Sutherland P, Veit B, Yao JL** (2002). Down-regulation of *TM29*, a tomato *SEPALLATA* homolog, causes parthenocarpic fruit development and floral reversion. *Plant Physiol*. **130**, 605–617.

Angenent GC, Franken J, Busscher M, van Dijken A, van Went JL, Dons HJM et al. (1995). A novel class of MADS box genes is involved in ovule development in petunia. *Plant Cell* **7**, 1569–1582.

Angenent GC, Franken J, Busscher M, Weiss D, van Tunen AJ (1994). Co-suppression of the petunia homeotic gene *FBP2* affects the identity of the generative meristem. *Plant J*. **5**, 33–44.

Becker A, Theissen G (2003). The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Mol*. *Phylogenet*. *Evol*. **29**, 464–489.

Coen Es, Meyerowitz EM (1991). The war of the whorls: Genetic interactions controlling flower development. *Natrue* **353**, 31–37.

Colombo L, Franken J, Koetje E, Vanwent J, Dons HJM, Angenent GC et al. (1995). The petunia MADS box gene *FBP11* determines ovule identity. *Plant Cell* **7**, 1859–1868.

Davies B, Egea-Cortines M, de Andrade Silva E, Saedler H, Sommer H (1996). Multiple interactions amongst floral homeotic MADS box proteins. *EMBO J*. **15**, 4330–4343.

Ditta G, Pinyopich A, Robles P, Pelaz S, Yanofsky MF (2004). The *SEP4* gene of *Arabidopsis thaliana* functions in floral organ and meristem identity. *Curr*. *Biol*. **14**, 1935–1940.

Egea-Cortines M, Saedler H, Sommer H (1999). Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus*. *EMBO J*. **18**, 5370–5379.

Fan HY, Hu Y, Tudor M, Ma H (1997). Specific interactions between the K domains of AG and AGLs, members of the MADS domain family of DNA binding proteins. *Plant J*. **12**, 999–1010.

Felsenstein J (2004). Phylip (phylogeny inference package) version 3.6. Genome Sciences, University of Washington, Seattle.

Ferrario S, Immink RGH, Shchennikova A, Busscher-Lange J, Angenent GC (2003). The MADS box gene *FBP2* is required for *SEPALLATA* function in petunia. *Plant Cell* **15**, 914–925.

Honma T, Goto K (2001). Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. *Nature* **409**, 525–529.

Immink RGH, Gadella TWJ Jr, Ferrario S, Busscher M, Angenent GC (2002). Analysis of MADS box protein-protein interactions in living plant cells. *Proc*. *Natl*. *Acad*. *Sci*. *USA* **99**, 2416–2421.

Irish VF (2003). The evolution of floral homeotic gene function. *Bioessays* **25**, 637–646.

Irish VF, Litt A (2005). Flower development and evolution: Gene duplication, diversification and redeployment. *Curr*. *Opin*. *Genet*. *Dev*. **15**, 454–460.

Johansen B, Pedersen LB, Skipper M, Frederiksen S (2002). MADS-box gene evolution—structure and transcription patterns. *Mol*. *Phylogenet*. *Evol*. **23**, 458–480.

Kotilainen M, Elomaa P, Uimari A, Albert VA, Yu D, Teeri TH (2000). *GRCD1*, an *AGL2*-like MADS box gene, participates in the C function during stamen development in *Gerbera hybrida*. *Plant Cell* **12**, 1893–1902.

Kramer EM, Di Stilio VS, Schluter PM (2003). Complex patterns of gene duplication in the *APETALA3* and *pistillata* lineages of the Ranunculaceae. *Int*. *J*. *Plant Sci*. **164**, 1–11.

Li GS, Meng Z, Kong HZ, Chen ZD, Theissen G, Lu AM (2005). Characterization of candidate class a, B and E floral homeotic genes from the perianthless basal angiosperm chloranthus spicatus (chloranthaceae). *Dev*. *Genes Evol*. **215**, 437–449.

Lü S, Du X, Lu W, Chong K, Meng Z (2007). Two *AGAMOUS*-like MADS-box genes from *Taihangia rupestris* (Rosaceae) reveal independent trajectories in the evolution of class C and class D floral homeotic functions. *Evol. Dev.* **9**, 92–104.

Ma H, Yanofsky M, Meyerowitz E (1991). *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. *Genes Dev*. **5**, 484–495.

Mandel MA, Yanofsky MF (1998). The *Arabidopsis AGL9* MADS box gene is expressed in young flower primordia. *Sex Plant Reprod*. **11**, 22–28.

Ng M, Yanofsky M (2001). Function and evolution of the plant MADS-box gene family. *Nat*. *Rev*. *Genet*. **2**, 186–195.

Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF (2000). B and C floral organ identity functions require *SEPALLATA* MADS-box genes. *Nature* **405**, 200–203.

Pelaz S, Tapia-Lopez R, Alvarez-Buylla ER, Yanofsky MF (2001). Conversion of leaves into petals in *Arabidopsis*. *Curr*. *Biol*. **11**, 182–184.

Pnueli L, Hareven D, Broday L, Hurwitz C, Lifschitz E (1994). The *TM5* MADS box gene mediates organ differentiation in the three inner whorls of tomato flowers. *Plant Cell* 6, 175–186.

Soltis PS, Soltis DE, Chase MW (1999). Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* **402**, 402–404.

Sung SK, Yu GH, Nam J, Jeong DH, An G (2000). Developmentally regulated expression of two MADS-box genes, *MdMADS3* and *MdMADS4*, in the morphogenesis of flower buds and fruits in apple. *Planta* **210**, 519–528.

Theissen G (2001). Development of floral organ identity: Stories from the MADS house. *Curr*. *Opin*. *Plant Biol*. **4**, 78–85.

Thompson J, Gibson T, Plewniak F, Jeanmougin F, Higgins D (1997). The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids*. *Res*. **25**, 4876–4882.

Uimari A, Kotilainen M, Elomaa P, Yu D, Albert VA, Teeri TH (2004).

Integration of reproductive meristem fates by a *SEPALLATA*-like MADS-box gene. *Proc*. *Natl*. *Acad*. *Sci*. *USA* **101**, 15817–15822.

Vandenbussche M, Theissen G, Van de Peer Y, Gerats T (2003). Structural diversification and neo-functionalization during floral MADS-box gene evolution by C-terminal frameshift mutations. *Nucleic Acids Res*. **31**, 4401–4409.

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R et al. (2002). A MADS-box gene necessary for fruit ripening at the tomato Ripening-inhibitor (Rin) locus. *Science* **296**, 343–346.

Weatherbee SD, Carroll SB (1999). Selector genes and limb identity in arthropods and vertebrates. *Cell* **97**, 283–286.

Wheeler DL, Church DM, Federhen S, Lash AE, Madden TL, Pontius JU et al. (2003). Database resources of the national center for biotechnology. *Nucleic Acids Res*. **31**, 28–33.

Xia X, Xie Z (2001). Dambe: Software package for data analysis in molecular biology and evolution. *J*. *Hered*. **92**, 371–373.

Yang P, Craig PA, Goodsell D, Bourne PE (2003a). Bioeditor—simplifying macromolecular structure annotation. *Bioinformatics* **19**, 897–898.

Yang Y, Fanning L, Jack T (2003b). The K domain mediates heterodimerization of the *Arabidopsis* floral organ identity proteins, APETALA3 and PISTILLATA. *Plant J*. **33**, 47–59.

Yang Y, Jack T (2004). Defining subdomains of the K domain important for protein-protein interactions of plant MADS proteins. *Plant Mol*. *Biol*. **55**, 45–59.

Yang Z (1997). Paml: A program package for phylogenetic analysis by maximum likelihood. *Comput*. *Appl*. *Biosci*. **13**, 555.

Yang Z (1998). Likelihood ratio tests for detecting positive selection and application to primate lysozyme evolution. *Mol*. *Biol*. *Evol*. **15**, 568–573.

Yu T, Li C (1983). The systematic position of genus *taihangia* in *rosaceae*. *Acta Phytotax*. *Sin*. **21**, 229–235 (in Chinese with an English abstract).

Zahn LM, Kong H, Leebens-Mack JH, Kim S, Soltis PS, Landherr LL et al. (2005). The evolution of the *SEPALLATA* subfamily of MADS-box genes: A preangiosperm origin with multiple duplications throughout angiosperm history. *Genetics* **169**, 2209–2223.

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