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Expression patterns of Class I *KNOX* and *YABBY* genes in *Ruscus aculeatus* (Asparagaceae) with implications for phylloclade homology

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Abstract *STM* (*RaSTM*) and *YAB2* (*RaYAB2*) homologues were isolated from *Ruscus aculeatus* (Asparagaceae, monocots) and their expressions were analyzed by real-time PCR to assess hypotheses on the evolutionary origin of the phylloclade in the Asparagaceae. In young shoot buds, *RaSTM* is expressed in the shoot apex, while *RaYAB2* is expressed in the scale leaf subtending the shoot bud. This expression pattern is shared by other angiosperms, suggesting that the expression patterns of *RaSTM* and *RaYAB2* are useful as molecular markers to identify the shoot and leaf, respectively. *RaSTM* and *RaYAB2* are expressed concomitantly in phylloclade primordia. These results suggest that the phylloclade is not homologous to either the shoot or leaf, but that it has a double organ identity.

Keywords Asparagaceae phylloclade *Ruscus aculeatus* *STM* *YABBY*

Introduction

The body plan of vascular plants is quite uniform in that they consist of three major vegetative organs: root, stem and leaf (e.g. Gifford and Forster, 1989). Contrary to this uniform body plan, some plants produce novel organs that are not strictly homologous or identical to one of the three major vegetative organs; such innovations contribute to morphological diversification of vascular plants. Phylloclades are a unique organ with a compressed, leaf-like appearance despite being located in the axillary position where a lateral shoot should arise generally (Bell, 1991). A typical phylloclade is seen in the coniferous genus *Phyllocladus* (Podocarpaceae) where it is interpreted as a laterally compressed shoot system (Tomlinson et al., 1987).

In the Asparagaceae family of basal monocots (Rudall et al., 2000; Chase, 2004), a compressed, elliptic organ with a pointed apex is formed in the axil of the scale leaf (Figs. 1a-c). It also has been designated as a phylloclade, but the organ identity and evolutionary process are not fully understood. Some studies have considered the Asparagaceae phylloclade to be a compressed stem (caulome) because of its axial position and ability to generate floral buds (e.g., Turpin, 1820 cited in Hirsch, 1977;

Zweigelt, 1913; Hirsch, 1977). Others have compared it to a leaf borne on an aborted shoot, because it grows determinately and has a venation pattern similar to that of the leaf (de Candolle, 1827 cited in Hirsch, 1977; Schlittler, 1960; Cusset and Tran, 1966). In addition to these simple interpretations, the Asparagaceae phylloclade was also considered to be a *de novo* organ with stem and leaf identities (Croizat-Chaley, 1973; Sattler, 1984; Cooney-Sovetts and Sattler, 1986). Furthermore, some authors have postulated that the phylloclade is a congenital-fusion product of an axillary branch and its prophylls (Van Tieghen, 1884 cited in Cooney-Sovetts and Sattler, 1986; Arber, 1924).

The expression patterns of transcription factor genes would be helpful in clarifying the identity of the Asparagaceae phylloclade. In some model plants with simple leaves, Class I *KNOTTED*-like homeobox (*KNOX*) genes are expressed in the shoot apical meristem (SAM), while they are down-regulated in lateral organ primordia (Vollbrecht et al., 1990; Barton and Poethig, 1993). This expression pattern is plesiomorphic for Class I *KNOX* genes (Bharathan et al., 2002; Harrison et al., 2005; Sano et al., 2005). On the other hand, some genes, such as *ASYMMETRIC LEAVES 1*, *ASYMMETRIC LEAVES 2*, Class III *HOMEODOMAIN-LEUCIN ZIPPER* genes, *KANADI* genes, and

YABBY genes, are expressed in lateral organ primordia and promote their asymmetric growth (Eshed et al., 2001; Bowman et al., 2002; Emery et al., 2003; Engstrom et al., 2004). Among them, expression of *YABBY* genes is specific to lateral organs in diverse lineages of angiosperms (Bowman, 2000; Kim et al., 2001; Yamaguchi et al., 2003; Yamada et al., 2004; Jang et al., 2004; Juarez et al., 2004; Fourquin et al., 2005). Based on these previous studies, it is probable that the expression patterns of Class I *KNOX* genes and *YABBY* genes could be markers for assessing the SAM and lateral organ identities, respectively, in most angiosperm lineages.

In this study, we isolated *SHOOTMERISTEMLESS* (*STM*) and *YABBY2* (*YAB2*) homologues from *Ruscus aculeatus* L. (Asparagaceae), which are members of Class I *KNOX* and *YABBY* genes, respectively. Their expressions were analyzed by real-time PCR to assess the proposed hypotheses on phylloclade evolution.

Materials and Methods

Plant materials and phenology of *Ruscus aculeatus*

Plants of *R. aculeatus* cultivated in the Tokyo campus of Japan Women's University were used in this study. Dormant buds enclosed by several scale leaves (bud scales) formed at the base of the current shoots became enlarged during February and March (stage 0, Figs. 1d, 2a; see also Hirsch, 1977). At stage 0, the shoot apex was round without its own scale leaves. The shoot apex formed four to six lateral shoot axes subtended by scale leaves from April to June (stage I, Fig. 2b). In stage II lasting about 5 months from July to November, phylloclade primordia emerged acropetally in the axils of scale leaf primordia on the main or lateral axes (Fig. 2c). In the subsequent 3 months (December to early February), floral buds subtended by bracts developed on the adaxial surface of the phylloclade primordia (stage III, Fig. 2d). The basal-most phylloclades on each axis were devoid of floral buds. The phylloclade primordia became flattened at stage IV (mid-February to mid-March) while the main and lateral shoot apices ceased indeterminate growth and also flattened (Fig. 2e). At this stage, the next main shoot system (stage 0) was initiated in the axil of the scale leaf remaining on the base of the current shoot. The shoot system grew above ground and the floral buds began differentiation in late March (stage V, Fig. 2f). Anthesis began in April (stage VI, Fig. 1e).

Cloning *STM* homologue and *YABBY* gene

Samples collected for cloning were frozen in liquid N_2. Total RNA was extracted from floral buds and first-strand cDNA for 3′ RACE was synthesized following Shindo et al. (1999). The partial cDNA sequence of an *STM* homologue was amplified by STM-ELK1 and UAP. Nested PCR was performed by KN4-1 and UAP (Table1). The remaining 5′ end sequence was determined by 5′ RACE following Shindo et al. (1999). Similarity between the obtained *STM* homologue and other *KNOX* genes was estimated by BLAST (http://www.ncbi.nlm.nih.gov/BLAST). A *YABBY* gene was isolated following Yamada et al. (2003). The obtained sequences were registered in DDBJ/EMBL/GenBank as AB000000 (*RaSTM*) and AB168115 (*RaYAB2*).

Phylogenetic analyses of *KNOX* and *YABBY* genes

The deduced amino acid sequences of *KNOX* genes and *BELL1* were obtained from the NCBI DNA Database. (See S1 for the accession numbers.) They were aligned with the predicted amino acid sequence of the obtained *STM* homologue of *R. aculeatus* using CLUSTAL X ver. 1.64b (Thompson et al., 1997) and the alignment was revised manually. Phylogenetic analysis was performed with CLUSTAL X ver. 1.64b based on amino acid sequences of MEIKNOX, ELK, and Homeodomains (Fig. 2, S2). Bootstrap supports with 1000 replicates were also calculated by CLUSTAL X ver. 1.64b for each cluster. The obtained tree was rooted by choosing *BELL1* as an outgroup. Alignment and phylogenetic analysis of *YABBY* genes (see S3 for their accession numbers) were conducted following Yamada et al. (2003).

Real-time PCR

Collected samples were soaked in RNAlater (Ambion Inc., Austin, TX, USA) after dissection under a binocular microscope. We extracted total RNA from: shoot apices and bud scales subtending the shoot apices at stage 0; the basal-most phylloclade primordia on each axis at stage IV; scale leaves on main and lateral axes at stage IV; floral buds at stage V; and mature basal-most vegetative phylloclades at stage VI. The sample stages and contained organ type(s) are summarized in Table 2. First-strand cDNAs were synthesized for each sample by the methods described above and were used as a template for real-time PCR. To eliminate possibly-contaminated genomic DNA, we treated total RNAs with DNase I before cDNA synthesis. TaqMan® probes and primers (Table 1) were designed by Primer Express ver. 1.5 (Applied Biosystems, Foster City, CA, USA). Mixtures for PCR were prepared using Platinum® Quantitative PCR SuperMIX-UDG (Invitrogen Co. Ltd, Carlsbad, CA, USA). As an internal control, the expression level of 18S rRNA was quantified for each sample using Pre-Developed TaqMan® Assay Reagants (Applied Biosystems). Three independent reactions were prepared for each amplification set. Threshold cycle (Ct) values were measured by PTC-200 DNA Engine Cycler (Bio-Rad Laboratories, Inc., Waltham, MA, USA). The obtained Ct values were compared with Ct values of standard templates with the known number of initial templates for estimating the initial target and control cDNA molecules in each reaction. The number of target cDNA molecules was divided by that of 18S rRNA and standard deviations among the three reactions were calculated. Experiments were replicated five times to verify the results.

Results

Isolation of *STM* homologue

We isolated one *STM* homologue (*RaSTM*) from *R. aculeatus*. The determined partial mRNA was 1114 bp, including a complete coding sequence. The predicted amino acid sequence consists of 321 residues and includes the MEIKNOX, ELK, and Homeodomains (Fig. 3, S2). BLAST X search clearly suggested a close similarity to Class I *KNOX* genes such as *STM* and *NTH15* (data not shown).

Phylogenetic analysis robustly supported a sister relationship of *RaSTM* to dicot *STM* homologues (100% bootstrap support), showing that *RaSTM* is distantly related to *Kn1* and *RS1* homologues, which are Class I *KNOX* genes of Poaceae (Fig. 4).

Isolation of *YAB2* homologue

The obtained putative *YABBY2* homologue (*RaYAB2*) was 793 bp long. We could not obtain a complete coding sequence, but recognized Zinc finger-like and YABBY domains in the deduced amino acid sequence (Fig. 5). *RaYAB2* shares a motif located just downstream of the Zn finger-like domain with other *YAB2* homologues (Fig. 5), suggesting homology of *RaYAB2* and *YAB2*.

Phylogenetic analysis showed that *RaYAB2* is nested in a clade consisting of *YAB2* homologues and clade monophyly is suggested by 64% bootstrap support (Fig. 6).

Expression analyses of *RaSTM* and *RaYAB2* by real-time PCR

Expression of *RaSTM* was detected in the shoot apex, phylloclade primordial, and floral buds (Fig. 7). Among them, the strongest transcription was observed in the shoot apex, and the expression level in the phylloclade primordia was higher than that in the floral buds. No significant amplification of *RaSTM* was detected in the stage-VI phylloclade and scale leaves (Fig. 7).

The *RaYAB2* expression was highest in the scale leaves, while an expression intensity of less than half the highest expression was also detected in the shoot apex, phylloclade primordia, and floral buds (Fig. 7). Expression in the stage-VI phylloclade was very weak.

Experiments were replicated five times and resulted in identical patterns (data not shown).

Discussion

STM homologue lost during monocots diversification

RaSTM is clearly identified as an *STM* homologue by the phylogenetic analysis. This is the first isolation of an *STM* homologue in the monocots despite extensive genomic research into the Poaceae, including rice and maize. In Poaceae, *Kn1*, a Class I *KNOX* gene, participates in maintenance of the shoot apical meristem instead of *STM* (Jackson et al., 1994; Bharathan et al., 1999; Reiser et al., 2000). Taking into account the phylogeny in which the Asparagaceae diverged earlier than the Poaceae (Chase, 2004), the occurrence of the *STM* homologue in *R. aculeatus* suggests that an *STM* homologue was lost during diversification of the monocots while its function was taken over by the *Kn1* homologue.

Phylloclade SAM and leaf identities

The validity of homology assessment based only on gene expression has been questioned because the same gene is co-opted for similar functions among non-homologous organs (e.g., Abouheif et al., 1997; Nielsen and Martinez, 2003; Theissen, 2005). Such functional co-option of a gene would cause expressional commonality (homocracy) among non-homologous organs (Nielsen and Martinez, 2003). Thus, a homocracy among organs does not necessarily ensure their homology, but it could be a tool to assess their organ identity (Rutishauser and Isler, 2001; Nielsen and Martinez, 2003).

In *Arabidopsis* and other eudicots, *STM* maintains proper growth of the SAM by expression in both vegetative and reproductive SAMs, while it is down-regulated in leaf primordia (Barton and Poethig, 1993; Long et al., 1996). Although we could not specify the exact function of *RaSTM*, we infer that monocot *RaSTM*, like other dicot *STM* homologues, is involved in maintenance of the SAM, because it is expressed strongly in the vegetative and reproductive shoot apices, but expression is not detected in the scale leaves as is usual in dicots. Notably, *RaSTM* is expressed in the phylloclade primordia, suggesting that young phylloclades are functionally comparable to the SAM.

Strong expression of *RaYAB2* in the scale leaves suggests that it may be involved in leaf formation. The expression detected in shoot apices might be attributed to the scale leaves (bud scales) covering them. *RaYAB2* is also transcribed in the phylloclade primordia, so the phylloclade is also partly comparable to a leaf.

The concomitant expression of *RaSTM* and *RaYAB2* in the phylloclade suggests that both SAM and leaf developmental pathways may be partly incorporated into the phylloclade developmental pathway. Similar incorporation of SAM and leaf developmental pathways confers continuous identity between SAM and leaf in a tomato compound leaf of (Sinha, 1999; Kim et al., 2003). The phylloclade twofold pattern could explain the apparently contradictory characteristics of leaf-like appearance and shoot-like axillary position.

Traditional plant morphological studies emphasize the positional criterion (homotopy) to assess organ homology and do not permit coexistence of multiple identities in a single organ (Rutishauser and Isler, 2001). Such an approach is called Classical Morphology (ClaM) (Rutishauser and Isler, 2001), and the ClaM approach has been applied to homology assessments of the phylloclade, interpreting it as either a compressed stem (Turpin, 1820 cited in Hirsch, 1977; Zweigelt, 1913; Hirsch, 1977) or a leaf borne on an aborted shoot (de Candolle, 1827 cited in Hirsch, 1977; Schlittler, 1960; Cusset and Tran, 1966).

There are many studies of organ heterotopy whereby organs with different identities are formed in an equivalent position (e.g., Rutishauser and Grubert, 1999; Rutishauser and Isler, 2001). Furthermore, developmental genetic studies clarify that amalgamation of different developmental pathways obscures the boundary between the three major vegetative organs (root, stem, leaf) (Hofer, 1998; Sinha, 1999). These findings have led to recent re-evaluation of the importance of the Fuzzy Arberian Morphology (FAM) approach named after Agnes Arber (Rutishauser and Isler, 2001), such as the Leaf –Shoot Continuum Hypothesis (Arber, 1950). The FAM approach emphasizes estimation of organ identities over homology, and accepts heterotopy and continuum identity between organs (Rutishauser and Isler, 2001). Arber (1924) explained the contradictory characteristics of the phylloclade as a fusion/coexistence of leaf and SAM and this interpretation is subsumed into later FAM approaches interpreting the phylloclade as having a double identity (Croizat-Chaley, 1973; Sattler, 1984; Cooney-Sovetts and Sattler, 1986).

The FAM interpretation of the phylloclade matches the results of our expression analyses, although it is not shown here whether the *STM* and *YABBY* genes are expressed in the same or different parts (tissues) of the phylloclade. We still need to clarify how the developmental pathways of the SAM and leaf are incorporated into phylloclade development to assess phylloclade evolution. Expression analyses of other genes involved in SAM and leaf developmental pathways, as well as *in situ* hybridization experiments, which are ongoing, will shed light on this.

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References

Abouheif E, Akam M, Dickinson WJ, Holland PWH, Meyer A, Patel NH, Raff RA, Roth VL, Wray GA (1997) Homology and developmental genes. Trends Genet 13: 432-433

APG II (2003) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Bot J Linn Soc 141: 399-436

Arber A (1924) *Danaë*, *Ruscus*, and *Semele*: a morphological study. Ann Bot 38: 229-260

Arber A (1950) The natural philosophy of plant form. Cambridge University Press, Cambridge

Barton MK, Poethig RS (1993) Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the shoot meristemless mutant. Development 119: 823-831

Bell AD (1991) Plant Form. -An illustrated guide to flowering plant morphology- Oxford University Press, Oxford

Bharathan G, Janssen BJ, Kellogg EA, Sinha N (1999) Phylogenetic relationships and evolution of the KNOTTED Class of plant homeodomain proteins. Mol Biol Evol 16: 553-563

Bharathan G, Goliber TE, Moore C, Kessler S, Pham T, Sinha NR (2002) Homologies in leaf form inferred from KNOXI gene expression during development. Science 296: 1858-1860.

Bowman JL (2000) The YABBY gene family and abaxial cell fate. Curr Opin Plant Biol 3: 17-22

Bowman JL, Eshed Y, Baum SF (2002) Establishment of polarity in angiosperm lateral organs. Trends Genet 18; 134-141

Chase MW (2004) Monocot relationships: an overview. Am J Bot 91: 1645-1655

Cooney-Sovetts C, Sattler R (1986) Phylloclade development in the Asparagaceae: an example of homoeosis. Bot J Linn Soc 94: 327-371

Croizat-Chaley L (1973) En torno al concepto de Hoya -ensayo de botanica analitica y sintetica. Bibl Acad Cie Fis Mat Nat 12: 1-196

Cusset G, Tran TTH (1966) Remarques sur l'éperon foliaire des *Asparagus*. Bull Soc Bot France 113: 121-151

Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL (2003) Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. Curr Biol 13: 1768-1774

Engstrom EM, Izhaki A, Bowman JL (2004) Promoter bashing, microRNAs, and *Knox* genes. New insights, regulators, and targets-of-regulation in the establishment of lateral organ polarity in *Arabidopsis*. Plant Physiol 135: 685-694

Eshed Y, Baum SF, Perea JV, Bowman JL (2001) Establishment of polarity in lateral organs of plants. Curr Biol 11: 1251-1260

Fourquin C, Vinauger-Douard M, Fogliani B, Dumas C, Scutt CP (2005) Evidence that *CRABS CLAW* and *TOUSLED* have conserved their roles in carpel development since the ancestor of the extant angiosperms. Proc Natl Acad Sci USA 102: 4649-4654

Gifford EM, Foster AS (1989) Morphology and evolution of vascular plants. 3rd ed. W.H. Freeman and Co, New York

Harrison JC, Corley SB, Moylan EC, Alexander DL, Scotland RW, Langdale JA (2005) Independent recruitment of a conserved developmental mechanism during leaf evolution. Nature 434: 509-514

Hirsch AM (1977) A developmental study of the phylloclades of *Ruscus aculeatus* L.. Bot J Linn Soc 74: 355-365

Hofer JMI, Ellis THN (1998) The genetic control of patterning in pea leaves. Trends Plant Sci 3: 439-444

Jackson D, Veit B, Hake S (1994) Expression of maize KNOTTED1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. Development 120: 405-413

Jang S, Hur J, Kim SJ, Han MJ, Kim SR, An G (2004) Ectopic expression of *OsYAB1* causes extra stamens and carpels in rice. Plant Mol Biol 56: 133-143

Juarez MT, Twigg RW, Timmermans MC (2004) Specification of adaxial cell fate during maize leaf development. Development 131: 4533-4544

Kim M, Pham T, Hamidi A, McCormick S, Kuzoff RK, Sinha N (2003) Reduced leaf complexity in tomato wiry mutants suggests a role for *PHAN* and KNOX genes in generating compound leaves. Development 130: 4405-4415

Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of Arabidopsis. Nature 379: 66-69

Nielsen C, Martinez P (2003) Patterns of gene expression: homology or homocracy? Dev Gen Evol 213: 149-154

Reiser L, Sánchez-Baracaldo P, Hake S (2000) Knots in the family tree: evolutionary relationships and functions of *Knox* homeobox genes. Plant Mol Biol 42:

151-166

Rudall PJ, Conran JG, Chase MW (2000) Systematics of Ruscaceae/Convallariaceae: a combined morphological and molecular investigation. Bot J Linn Soc 134:73-92

Rutishauser R, Grubert M (1999) The architecture of *Mourera fluviatilis* (Podostemaceae): Developmental morphology of inflorescences, flowers and seedlings. Am J Bot 86: 907-922

Rutishauser R, Isler B (2001) Developmental genetics and morphological evolution of flowering plants, especially bladderworts (Utricularia): fuzzy Arberian complements classical morphology. Ann Bot 88: 1173-1201

Sano R, Juarez CM, Hass B, Sakakibara K, Ito M, Banks JA, Hasebe M (2005) KNOX homeobox genes potentially have similar function in both diploid unicellular and multicellular meristems, but not in haploid meristems. Evol Dev 7: 69-78

Schlittler J (1960) Die Asparageenphyllokladien erweisen sich auch ontogenetisch als Blätter. Bot Fahrb Syst 79: 428-446

Shindo S, Ito M, Ueda K, Kato M, Hasebe M (1999) Characterization of MADS genes in gymnosperm *Gnetum parvifolium* and its implication for the evolution of reproductive organs in seed plants. Evol Dev 3: 180-190

Sinha N (1999) Leaf development in angiosperms. Annu Rev Plant Physiol Plant Mol Biol 50: 419-446

Theissen G (2005) Birth, life and death of developmental control genes: new challenges for the homology concept. Theor Biosci 124: 199-212

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc Acids Res 24:4876-4882

Tomlinson PB, Takaso T, Rattenbury JA (1987) Developmental shoot morphology in *Phyllocladus* (Podocarpaceae). Bot J Linn Soc 99: 223-248

Zweigelt F (1913) Was sind die Phyllokladien der Asparageen? Österreichische Botanische Zeitschrift 63: 313-335

Yamada T, Ito M, Kato M (2003) Expression pattern of *INNER NO OUTER* homologue in *Nymphaea* (water lily family, Nymphaeaceae). Dev Gen Evol 213: 510-513

Yamada T, Ito M, Kato M (2004) *YABBY2*-homologue expression in lateral organs of *Amborella trichopoda* (Amborellaceae). Int J Plant Sci 165: 917-924

Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka Y, Nagato Y, Hirano H (2003) The *YABBY* gene *DROOPING LEAF* regulates carpel specification and midrib development in *Oryza sativa*. Plant Cell 16: 500-509

Figure legends

Fig. 1. Morphology of *Ruscus aculeatus*. **a** Mature shoot system. **b** Close-up of mature phylloclade formed at main shoot apex and lateral phylloclades. **c** Phylloclade subtended by scale leaf. **d** Young bud at stage 0 covered by scale leaves (arrowhead). **e** Flower on adaxial surface of phylloclade. *p* phylloclade, *l* scale leaf. Bars: 1 cm (a, b, e), 5 mm (c, d)

Fig. 2. Phenology of *Ruscus aculeatus*. The main shoot system of the previous year is omitted in stage 0 and I. Stage VI is not shown. The dashed line in stage II illustrates the disintegrated main shoot system of the previous year.

Fig. 3. Alignment of deduced amino acid sequences of selected *KNOX* genes. Amino acid positions used for phylogenetic analysis are shaded. MEIKNOX, ELK, and Homeodomains are indicated by clumps. Asterisks indicate identical amino acids. See S1 for the full alignment.

Fig. 4. Neighbor joining tree of *KNOX* genes. Bootstrap supports (>50%) are shown above branches. Bar: 0.05 amino acid substitutions per site

Fig. 5. Alignment of deduced amino acid sequences of *YABBY* genes. Amino acid positions used for phylogenetic analysis are shaded. Zinc finger-like and YABBY domains are marked by clumps. Asterisks indicate identical amino acids. Note a motif shared by *YAB2* homologues (boxed).

Fig. 6. Neighbor joining tree of *YABBY* genes. Bootstrap supports (>50%) are shown above branches. Bar: 0.01 amino acid substitutions per site

Fig. 7. Relative expression levels of *RaSTM* (open) and *RaYAB2* (shaded) in phylloclade primordia (PP), shoot apices and bud scales subtending them (S), floral buds (F), scale leaves (L) and mature phylloclades (PM). The expression level in shoot apices is set to 100%. Double-ended bars indicate standard deviations among three independent reactions.

Table 1. Primers used in this study. I, N, R, S, W, and Y follow the IUPAC code.

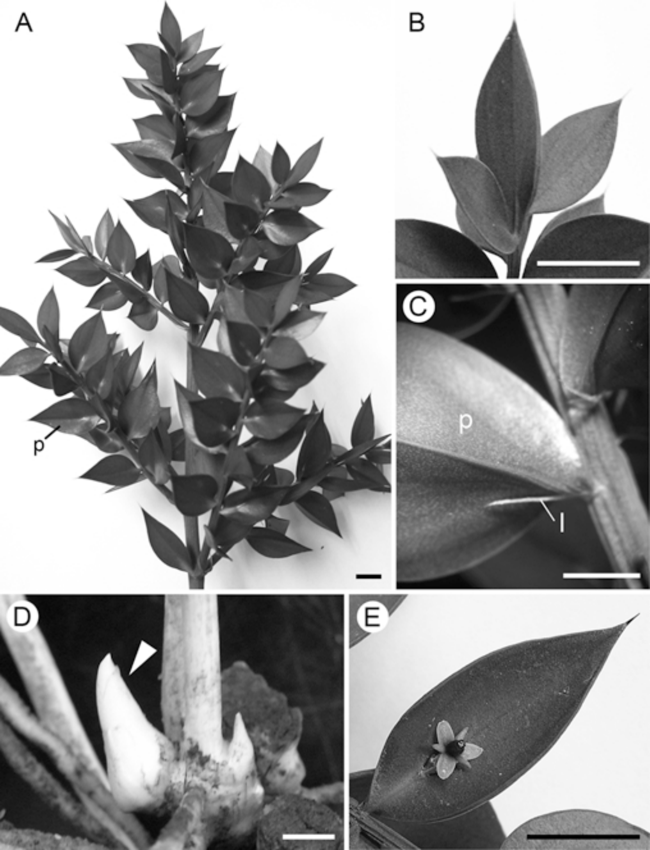
Table 2. Organs in each sample. + present, - absent

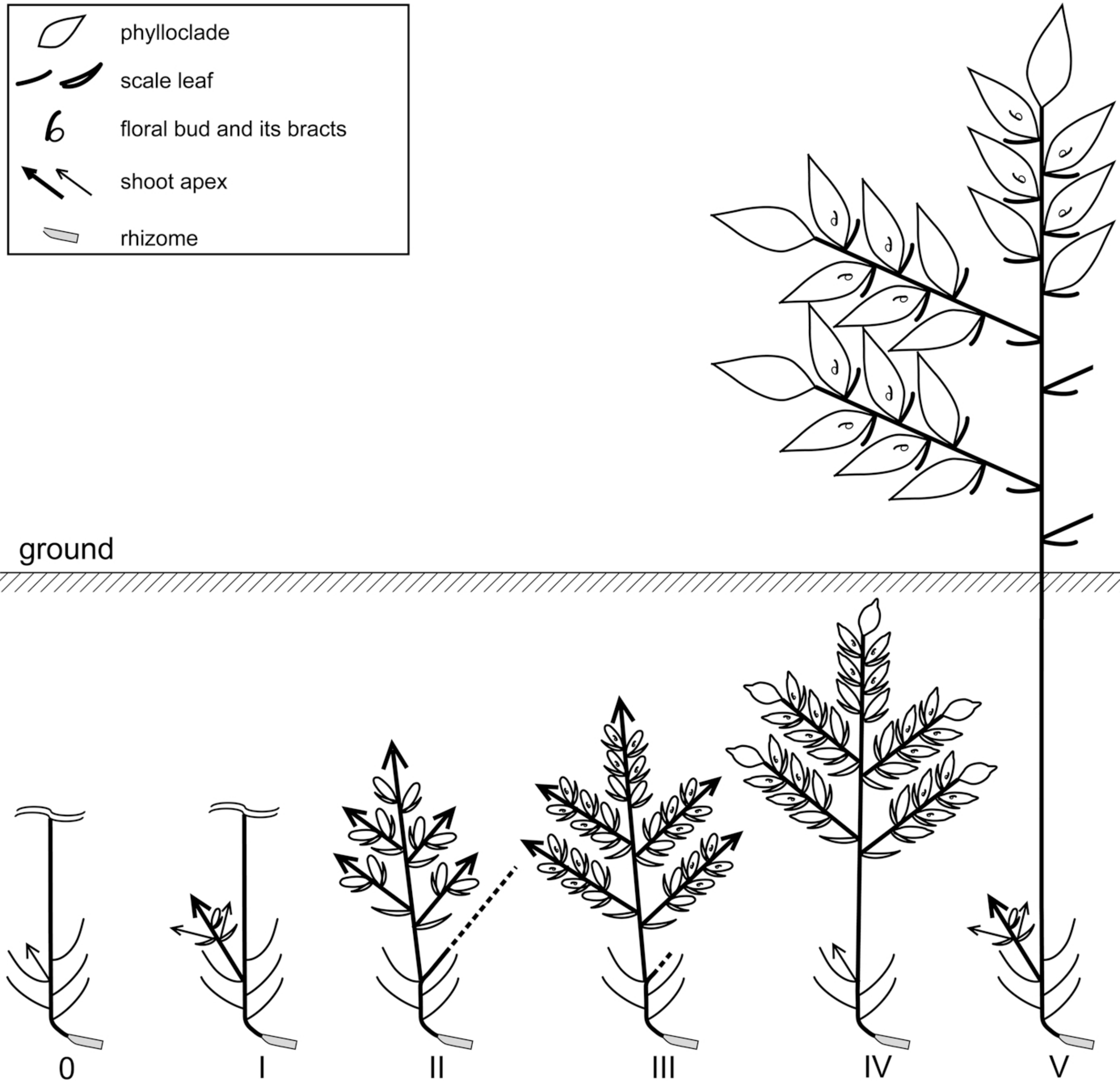
Footnote. *Abbreviations in parentheses correspond to those in Fig. 7.

S1. *KNOX* genes and *BELL1* used in phylogenetic analysis and their DDBJ/EMBL/GenBank accession numbers. Data published only in the database are indicated by asterisks.

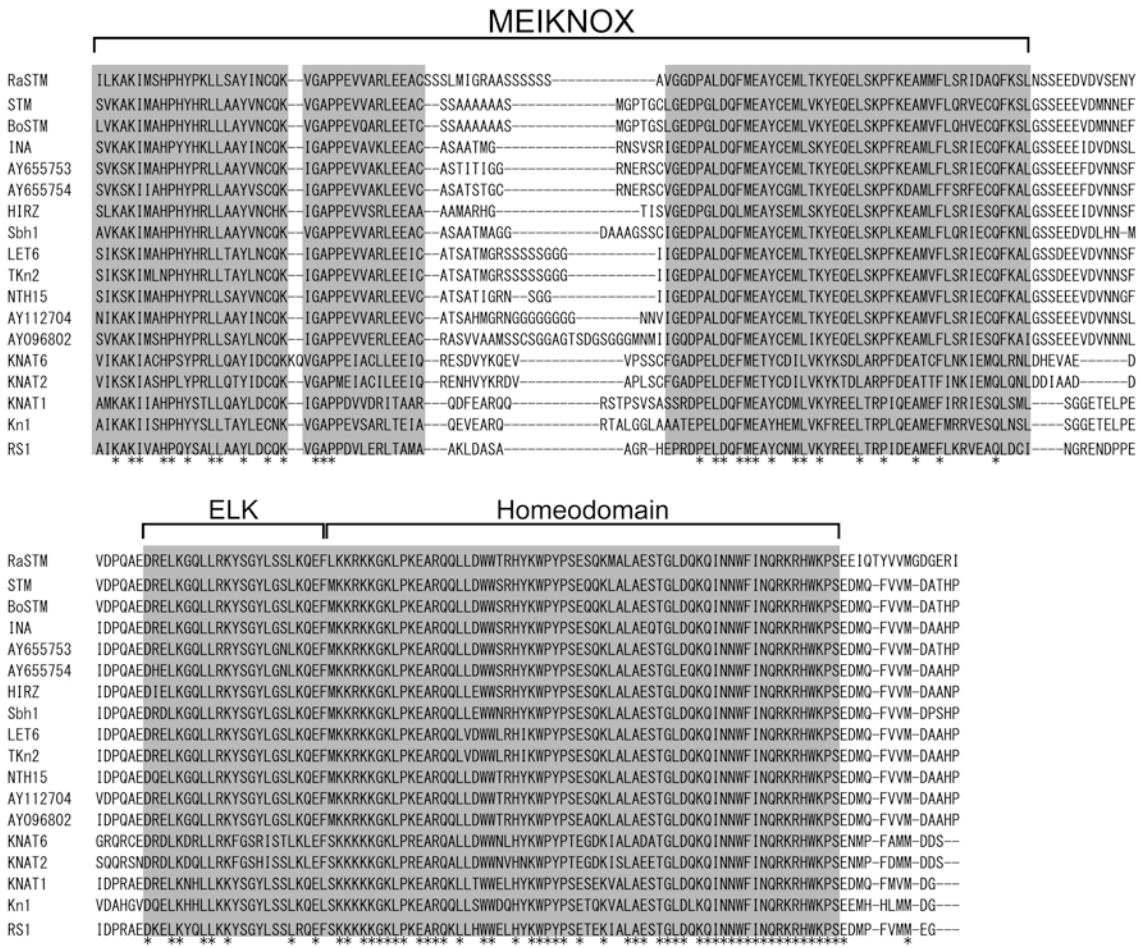
S2. Alignment of deduced amino acid sequences of *KNOX* genes and *BELL1*. Amino acid positions used for phylogenetic analysis are shaded. MEIKNOX, ELK, and Homeodomains are indicated by clumps. Asterisks indicate identical amino acids.

S3. *YABBY* genes used in phylogenetic analysis and their DDBJ/EMBL/GenBank accession numbers


A
p
B
C
p
l
D
E



phylloclade
scale leaf
floral bud and its bracts
shoot apex
rhizome
ground
0
I
II
III
IV
V


MEIKNOX
RaSTM ILKAKIMSHPHYPKLLSAYINCQK--VGAPPEVVARLEEACSSSLMIGRAASSSSSS-------------AVGGDPALDQFMEAYCEMLTKYEQELSKPFKEAMMFLSRIDAQFKSLNSSEEDVDVSENY
STM SVKAKIMAHPHYHRLLAAYVNCQK--VGAPPEVVARLEEAC--SSAAAAAAS--------------MGPTGCLGEDPGLDQFMEAYCEMLVKYEQELSKPFKEAMVFLQRVECQFKSLGSSEEEVDMNNEF
BoSTM LVKAKIMAHPHYHRLLLAYVNCQK--VGAPPEVQARLEETC--SSAAAAAAS--------------MGPTGSLGEDPGLDQFMEAYCEMLVKYEQELSKPFKEAMVFLQHVECQFKSLGSSEEEVDMNNEF
INA SVKAKIMAHPYYHKLLAAYINCQK--IGAPPEVAVKLEEAC--ASAATMG-----------------RNSVSRIGEDPALDQFMEAYCEMLSKYEQELSKPFREAMLFLSRIECQFKALGSSEEEIDVDNSL
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AY655754 SVKSKIIAHPHYPRLLAAYVSCQK--IGAPPEVVAKLEEVC--ASATSTGC----------------RNERSCVGEDPALDQFMEAYCGMLTKYEQELSKPFKDAMLFFSRFECQFKALGSSEEEFDVNNSF
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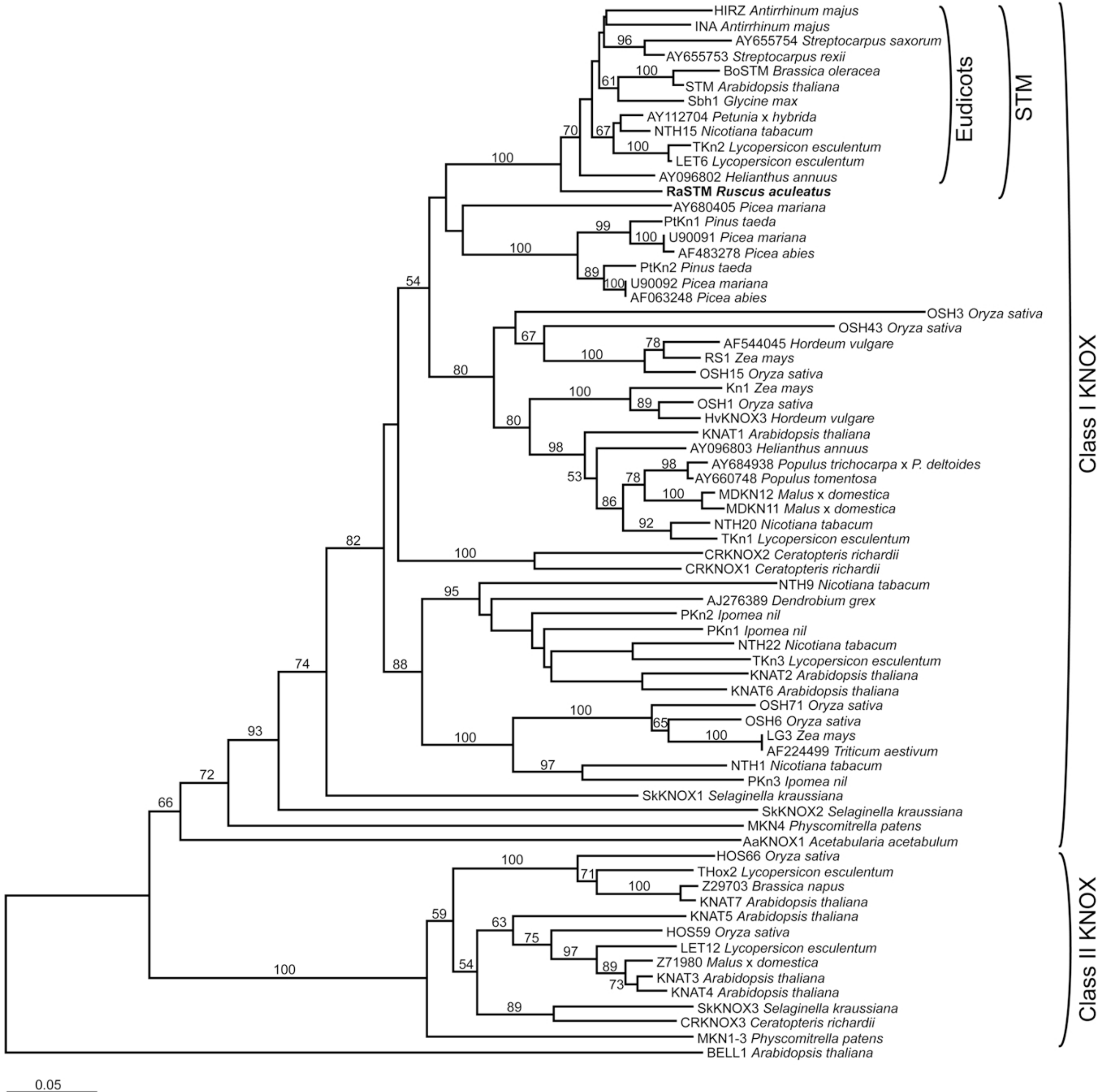
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INA Antirrhinum majus
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BoSTM Brassica oleracea
STM Arabidopsis thaliana
Sbh1 Glycine max
AY112704 Petunia x hybrida
NTH15 Nicotiana tabacum
TKn2 Lycopersicon esculentum
LET6 Lycopersicon esculentum
AY096802 Helianthus annuus
RaSTM Ruscus aculeatus
AY680405 Picea mariana
PtKn1 Pinus taeda
U90091 Picea mariana
AF483278 Picea abies
PtKn2 Pinus taeda
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AF063248 Picea abies
OSH3 Oryza sativa
OSH43 Oryza sativa
AF544045 Hordeum vulgare
RS1 Zea mays
OSH15 Oryza sativa
Kn1 Zea mays
OSH1 Oryza sativa
HvKNOX3 Hordeum vulgare
KNAT1 Arabidopsis thaliana
AY096803 Helianthus annuus
AY684938 Populus trichocarpa x P. deltoides
AY660748 Populus tomentosa
MDKN12 Malus x domestica
MDKN11 Malus x domestica
NTH20 Nicotiana tabacum
TKn1 Lycopersicon esculentum
CRKNOX2 Ceratopteris richardii
CRKNOX1 Ceratopteris richardii
NTH9 Nicotiana tabacum
AJ276389 Dendrobium grex
PKn2 Ipomea nil
PKn1 Ipomea nil
NTH22 Nicotiana tabacum
TKn3 Lycopersicon esculentum
KNAT2 Arabidopsis thaliana
KNAT6 Arabidopsis thaliana
OSH71 Oryza sativa
OSH6 Oryza sativa
LG3 Zea mays
AF224499 Triticum aestivum
NTH1 Nicotiana tabacum
PKn3 Ipomea nil
SkKNOX1 Selaginella kraussiana
SkKNOX2 Selaginella kraussiana
MKN4 Physcomitrella patens
AaKNOX1 Acetabularia acetabulum
HOS66 Oryza sativa
THox2 Lycopersicon esculentum
Z29703 Brassica napus
KNAT7 Arabidopsis thaliana
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HOS59 Oryza sativa
LET12 Lycopersicon esculentum
Z71980 Malus x domestica
KNAT3 Arabidopsis thaliana
KNAT4 Arabidopsis thaliana
SkKNOX3 Selaginella kraussiana
CRKNOX3 Ceratopteris richardii
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BELL1 Arabidopsis thaliana
Eudicots
STM
Class I KNOX
Class II KNOX
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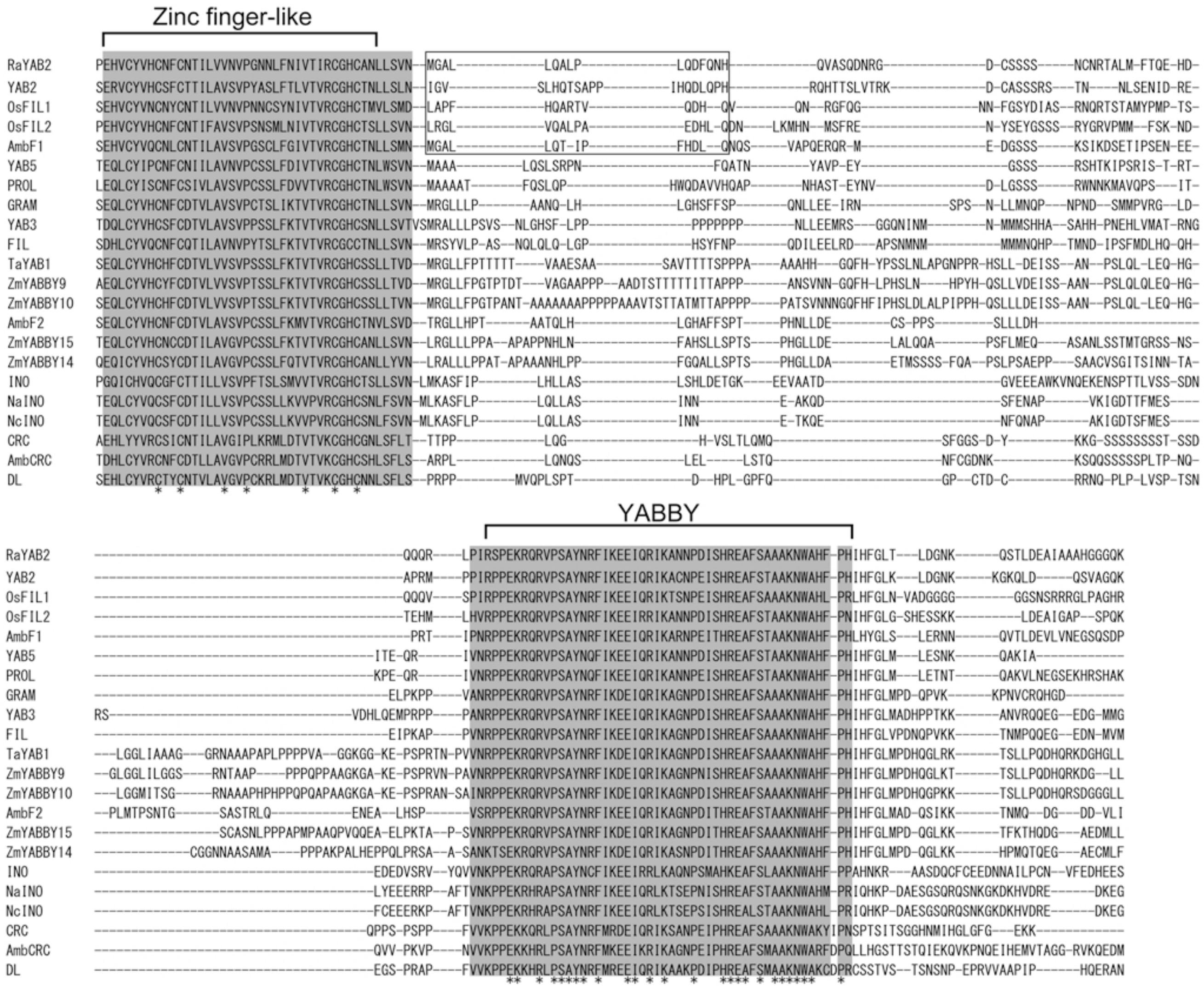
Zinc finger-like
YABBY
RaYAB2
YAB2
OsFIL1
OsFIL2
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PROL
GRAM
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TaYAB1
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AmbCRC
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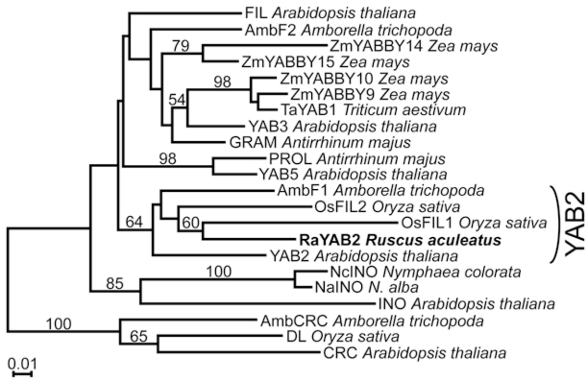
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AmbF2 Amborella trichopoda
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54
TaYAB1 Triticum aestivum
YAB3 Arabidopsis thaliana
GRAM Antirrhinum majus
98
PROL Antirrhinum majus
YAB5 Arabidopsis thaliana
AmbF1 Amborella trichopoda
OsFIL2 Oryza sativa
60
OsFIL1 Oryza sativa
64
RaYAB2 Ruscus aculeatus
YAB2 Arabidopsis thaliana
YAB2
100
NcINO Nymphaea colorata
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INO Arabidopsis thaliana
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AmbCRC Amborella trichopoda
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DL Oryza sativa
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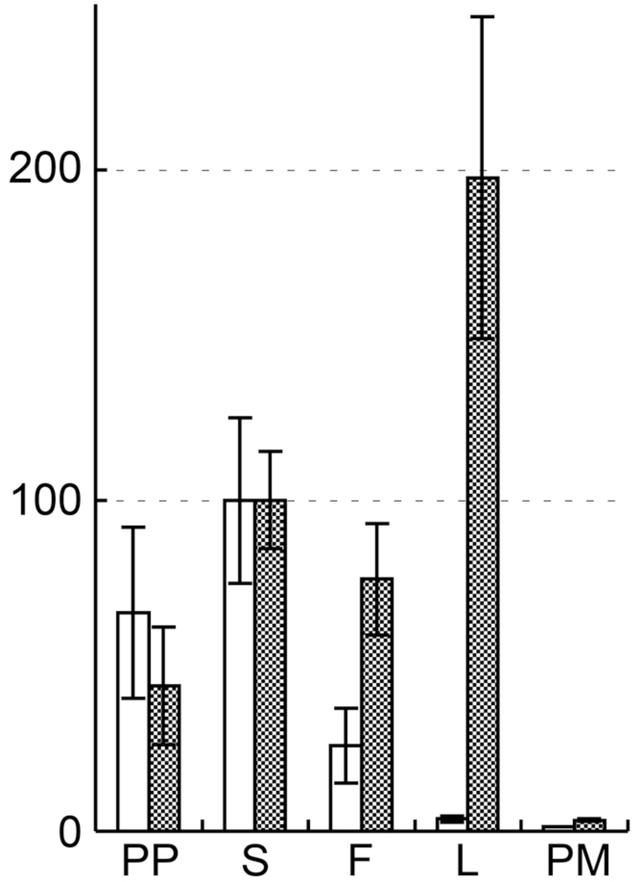
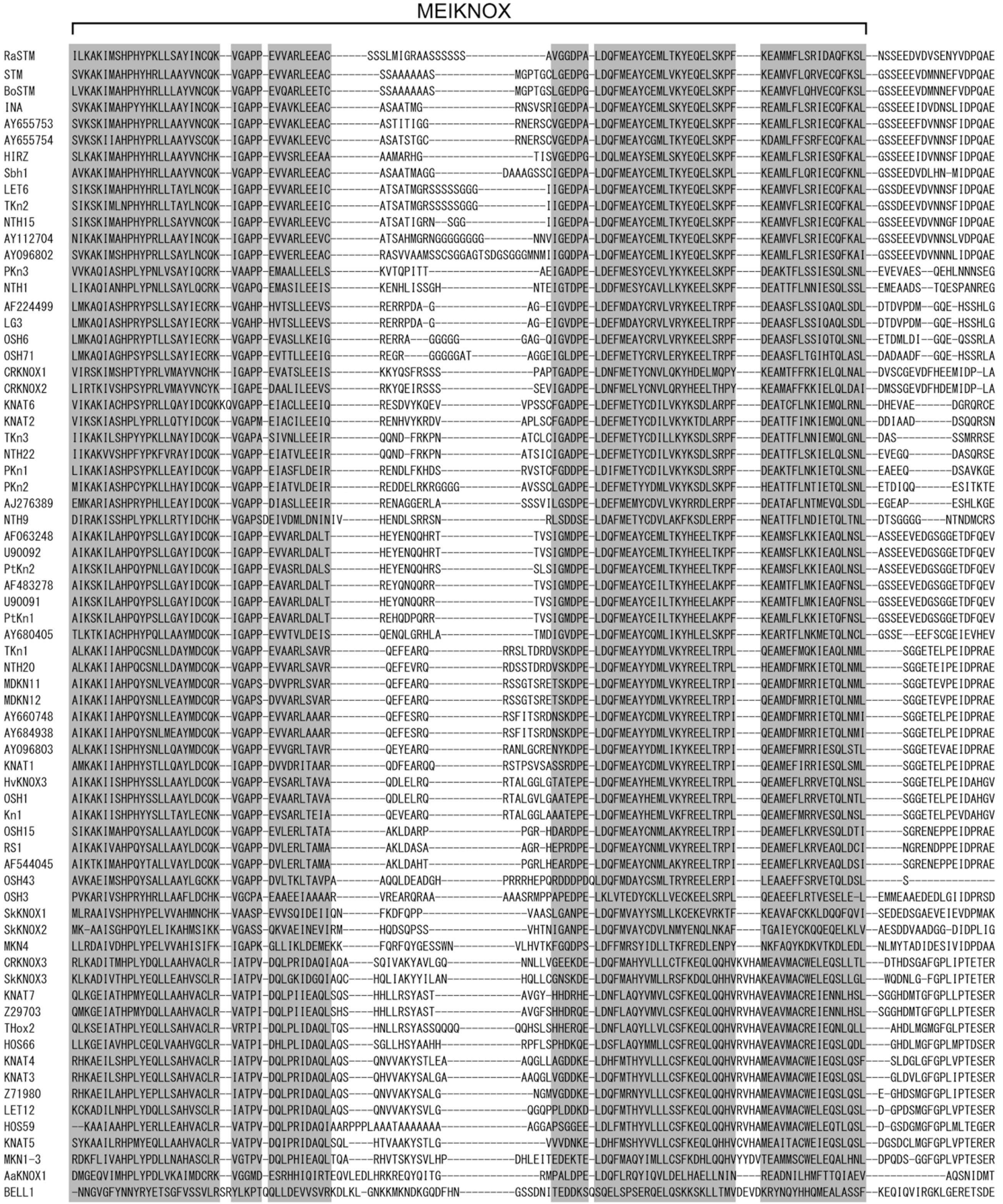

200
100
0
PP
S
F
L
PM

Primer name	Reaction used	Oligonucleotide sequence
KN4-1	Isolation of *STM* homologue	5′-CAUCAUCAUCAUAARAARGGIAARYTNCC-3′
STM-ELK1	Isolation of *STM* homologue	5′-GGNWSYYTNAARCARGARTTYAT-3′
RaSTM-RTF	Real-time PCR	5′-GCGCATCACCAGCATTATTTC-3′
RaSTM-RTR	Real-time PCR	5′-CAGATAAGGGCTGGAGTGACATC-3′
RaSTM-TaqMan® Probe	Real-time PCR	5′-GGCGTAGGGATTGCCGAAGCCATTT-3′
RaYAB2-RTF	Isolation of *YAB2* homologue	5′-TGGGCACATTTTCCACACAT-3′
RaYAB2-RTR	Isolation of *YAB2*homologue	5′-CGTCCAGCGTTGATTGCTTA-3′
RaYAB2-TaqMan® Probe	Real-time PCR	5′-CCCGTCAAGAGTGAGCCCGAAATG-3′
UAP	Isolation of *STM* homologue	5′-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3′

Sample*	Stage	Shoot apex	Phylloclade	Scale leaf	Floral organs
Phylloclade primordium (PP)	IV	-	+	-	-
Shoot apex (S)	0	+	-	+	-
Floral bud (F)	V	-	-	-	+
Scale leaf (L)	IV	-	-	+	-
Mature phylloclade (PM)	VI	-	+	-	-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RaSTM
STM
BoSTM
INA
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AY655754
HIRZ
Sbh1
LET6
TKn2
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PKn3
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LG3
OSH6
OSH71
CRKNOX1
CRKNOX2
KNAT6
KNAT2
TKn3
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PKn1
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OSH1
Kn1
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AF544045
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KNAT5
MKN1-3
AaKNOX1
BELL1

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                ELK                    Homeodomain
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STM       ------DRELKGQLLRKYSGYLGSLKQEFMKKRKKGKLPKEARQQLLDWWSRHYKWPYPSEQQKLALAESTGLDQKQINNWFINQRKRHWKPS---------EDMQ-FVVM-DATHP
BoSTM     ------DRELKGQLLRKYSGYLGSLKQEFMKKRKKGKLPKEARQQLLDWWSRHYKWPYPSEQQKLALAESTGLDQKQINNWFINQRKRHWKPS---------EDMQ-FVVM-DATHP
INA       ------DRELKGQLLRKYSGYLGSLKQEFMKKRKKGKLPKEARQQLLDWWSRHYKWPYPSESQKLALAEQTGLDQKQINNWFINQRKRHWKPS---------EDMQ-FVVM-DAAHP
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LET6      ------DRELKGQLLRKYSGYLGSLKQEFMKKRKKGKLPKEARQQLVDWWLRHIKWPYPSESQKLALAESTGLDQKQINNWFINQRKRHWKPS---------EDMQ-FVVM-DAAHP
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LG3       ------DHELKEMLLKKYSGCLSRLRSEFLKKRKKGKLPKDARTVLLEWWNTHYRWPYPTEEDKVRLAAMTGLDPKQINNWFINQRKRHWKPS---------EDMR-FALM-EGVAG
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RS1       ------DKELKYQLLKKYSGYLSSLRQEFSKKKKKGKLPKEARQKLLHWWELHYKWPYPSETEKIALAESTGLDQKQINNWFINQRKRHWKPS---------EDMP-FVMM-EG---
AF544045  ------DKDLKYQLLKKYSGYLSSLRQEFSKKKKKGKLPKEARQKLLHWWELHYKWPYPSETEKIALAESTGLDQKQINNWFINQRKRHWKPS---------EDMP-FVMM-EG---
OSH43     ------DKQLKHQLLRKYGGSLGDLRQVFSKRTKKGKLPKEARQKLLHWWELHYKWPYPSEMEKMTLAQTTGLDQKQINNWFINQRKRHWKPTPVAGTA---------FPTM-EAAGG
OSH3      ------DKALKRHLLRKYSGYLGGLRKELSKKKKKGKLPKEARQKLLTWWELHYRWPNPSEMEKIALAESTGLEQKQINNCFINQRKRHWKPT---------EEME-FAVM-EAYHH
SkKNOX1   ------DKELKEQLMRKYSGYISSLKHEFLKKKKKGKLPKDSRQILLNWWSVHYKWPYPSESEKASLAESTGLDQKQINNWFINQRKRHWKPS---------DELTALSGQ-PSQST
SkKNOX2   ------DKEIKRALMKKYGGYLGGLTQEYLKKKKKSKLPSAATKTLRDWWFQHLEHPYPSEAQKATLAATTKLDPKQINNWFINQRKRHWDPS---------AAAASARGE-SLQQQ
MKN4      ------DEELKKMLRLKYGKHIAGLKAEFNRVRKKGKLPTNARQILKDWFSRHSYWPYPSEMEKAYLQRLCGLNLKQINNWFINERKRHWSCK---------GKCMYPNTK-FYPRD
CRKNOX3   TLMERVRQELKNELKNGYKDRIVDVREEILRKRRAGKLPGDTTSVLKAWWHAHSKWPYPTEDEKARLVQETGLQLKQINNWFINQRKRNWHSNPSSTAA---------MKTK-RKR--
SkKNOX3   TLMERVRQELKHELKHGYRARIVDVREEILRKRRAGKLPGDTTSVLKAWWHAHSKWPYPTEDEKARLVQETGLELKQINNWFINQRKRNWHHHPSSSAS---------TTSK-LKCKS
KNAT7     SLMERVRQELKLELKQGFKSRIEDVREEIMRKRRAGKLPGDTTTVLKNWWQQHCKWPYPTEDDKAKLVEETGLQLKQINNWFINQRKRNWHNNSHSLTS---------LKSK-RKH--
Z29703    SLMERVRQELKLELKQGFKSRIEDVREEIMRKRRAGKLPGDTTTVLKNWWQQHCKWPYPTEDDKAKLVEETGLQLKQINNWFINQRKRNWHNNSHSLTS---------LKSK-RKH--
THox2     SLMERVRQELKIELKQGFRSRIEDVREEILRKRRAGKLPGDTTTVLKNWWQQHSKWPYPTEDDKAKLVEETGLQLKQINNWFINQRKRNWHSNSQSVTS---------LKSK-RKR--
HOS66     SLMERVRQELKIELKQGFKSRIEDVREEILRKRRAGKLPGDTTTILKQWWQQHSKWPYPTEDDKAKLVEETGLQLKQINNWFINQRKRNWHNNSQ-TST---------LKSK-RKR--
KNAT4     SLMERVRQELKHELKQGYKEKIVDIREEILRKRRAGKLPGDTTSVLKSWWQSHSKWPYPTEEDKARLVQETGLQLKQINNWFINQRKRNWHSNPSSSTV---------SKNK-RRSNA
KNAT3     SLMERVRQELKHELKQGYKEKIVDIREEILRKRRAGKLPGDTTSVLKAWWQSHSKWPYPTEEDKARLVQETGLQLKQINNWFINQRKRNWHSNPSSSTV---------LKNK-RKSNA
Z71980    SLMERVRQELKHELKQGYKEKIVDIREEILRKRRAGKLPGDTTSVLKAWWQSHSKWPYPTEEDKARLVQETGLQLKQINNWFINQRKRNWHSNPSTSTV---------LKSK-RKR--
LET12     SLMERVRQELKHELKQGYKEKIVDIREEILRKRRAGKLPGDTTSVLKAWWQSHSKWPYPTEEDKARLVQETGLQLKQINNWFINQRKRNWHSNPSTSSS---------QKSQ-TQECR
HOS59     SLVERVRQELKHELKQGYREKLVDIREEILRKRRAGKLPGDTASTLKAWWQAHSKWPYPTEEDKARLVQETGLQLKQINNWFINQRKRNWHSNPASSSS---------DKSK-RKRYR
KNAT5     SLMERVKKELKHELKQGFKEKIVDIREEIMRKRRAGKLPGDTTSVLKEWWRTHSKWPYPTEEDKAKLVQETGLQLKQINNWFINQRKRNWNSNSSTSST---------LTKN-KRKRT
MKN1-3    TLMERVRQELKYELKQGYRARIVDVREEILRKRRAGKLPEGTTTVLKAWWQAHSKWPYPTEDEKERRIQETGLELKQVNNWFINQRKRNWHSNPLSSSSE--------LKSK-RKK--
AaKNOX1   WFEIRNEQEQRVLLKQKYRQELLALKEEFSKRKKRGKLPTHSIEVLKSWWKEHIAWPYPTDSAKRSLASQTNLTSIQINNWFINQRKRHWHKLFPEGVPNRSLKARGMLGM-DSSGP
BELL1     QGERIPRLRYLDQRLRQQRALHQQLGMVRPAWRPQRGLPENSVSILRAWLFEHFLHPYPKESEKIMLSKQTGLSKNQVANWFINARVRLWKPMIEEMYKE--ESAELLSNS-NQDTK
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Gene	Accession No.	Species	Family	Reference
AaKNOX1	AF170172	*Acetabularia acetabulum*	Dasycladaceae	Serikawa and Mandoli 1999
AF063248	AF063248	*Picea abies*	Pinaceae	*
AF224499	AF224499	*Triticum aestivum*	Poaceae	Takumi 2000
AF483278	AF483278	*Picea abies*	Pinaceae	Hjortswang et al. 2002
AF544045	AF544045	*Hordeum vulgare*	Poaceae	Lin and Muller 2002
AJ276389	AJ276389	*Dendrobium grex*	Orchidaceae	*
AY096802	AY096802	*Helianthus annuus*	Asteraceae	*
AY096803	AY096803	*Helianthus annuus*	Asteraceae	*
AY112704	AY112704	*Petunia x hybrida*	Solanaceae	*
AY655753	AY655753	*Streptocarpus rexii*	Gesneriaceae	Harrison et al. 2005
AY655754	AY655754	*Streptocarpus saxorum*	Gesneriaceae	Harrison et al. 2005
AY660748	AY660748	*Populus tomentosa*	Salicaceae	*
AY680405	AY680405	*Picea mariana*	Pinaceae	Guillet-Claude et al. 2004
AY684938	AY684938	*Populus trichocarpa x P. deltoides*	Salicaceae	Guillet-Claude et al. 2004
BELL1	AY085278	*Arabidopsis thaliana*	Brassicaceae	Haas et al. 2002
BoSTM	AF193813	*Brassica oleracea*	Brassicaceae	Zheng et al. 2002
CRKNOX1	AB043954	*Ceratopteris richardii*	Adiantaceae	Sano et al. 2005
CRKNOX2	AB043956	*Ceratopteris richardii*	Adiantaceae	Sano et al. 2005
CRKNOX3	AB043957	*Ceratopteris richardii*	Adiantaceae	Sano et al. 2005
HIRZ	AY072736	*Antirrhinum majus*	Scrophulariaceae	Golz et al. 2002
HvKNOX3	X83518	*Hordeum vulgare*	Poaceae	Mueller et al. 1995
HOS59	AB061818	*Oryza sativa*	Poaceae	Ito et al. 2002
HOS66	AB061819	*Oryza sativa*	Poaceae	Ito et al. 2002
INA	AY072735	*Antirrhinum majus*	Scrophulariaceae	Golz et al. 2002
Kn1	X61308	*Zea mays*	Poaceae	Vollbrecht et al. 1991
KNAT1	AF482995	*Arabidopsis thaliana*	Brassicaceae	Venglat et al. 2004
KNAT2	NM_105719	*Arabidopsis thaliana*	Brassicaceae	*
KNAT3	NM_122431	*Arabidopsis thaliana*	Brassicaceae	*
KNAT4	NM_121144	*Arabidopsis thaliana*	Brassicaceae	*
KNAT5	NM_119356	*Arabidopsis thaliana*	Brassicaceae	*
KNAT6	NM_102187	*Arabidopsis thaliana*	Brassicaceae	*
KNAT7	NM_104977	*Arabidopsis thaliana*	Brassicaceae	*
LET6	AF000141	*Lycopersicon esculentum*	Solanaceae	Janssen et al. 1998
LET12	AF000142	*Lycopersicon esculentum*	Solanaceae	Janssen et al. 1998
LG3	AF100455	*Zea mays*	Poaceae	Muehlbauer et al. 1999
MDKN11	Z71978	*Malus x domestica*	Rosaceae	Watillon et al. 1996
MDKN12	Z71979	*Malus x domestica*	Rosaceae	Watillon et al. 1996
MKN4	AF284817	*Physcomitrella patens*	Funariaceae	Champagne et al. 2001
MKN1-3	AF285148	*Physcomitrella patens*	Funariaceae	Champagne et al. 2001
NTH1	AB025573	*Nicotiana tabacum*	Solanaceae	*
NTH9	AB025713	*Nicotiana tabacum*	Solanaceae	Nishimura et al. 1999
NTH15	AB004785	*Nicotiana tabacum*	Solanaceae	Tamaoki et al. 1997
NTH20	AB025714	*Nicotiana tabacum*	Solanaceae	Nishimura et al. 1999

Gene	Accession No.	Species	Family	Reference
NTH22	AB025715	*Nicotiana tabacum*	Solanaceae	Nishimura et al. 1999
OSH1	D16507	*Oryza sativa*	Poaceae	Matsuoka, 1993
OSH3	AB028882	*Oryza sativa*	Poaceae	Sentoku et al. 1999
OSH6	AB028883	*Oryza sativa*	Poaceae	Sentoku et al. 1999
OSH15	AB016071	*Oryza sativa*	Poaceae	Sato et al. 1998
OSH43	AB028884	*Oryza sativa*	Poaceae	Sentoku et al. 1999
OSH71	AB028885	*Oryza sativa*	Poaceae	Sentoku et al. 1999
PKn1	AB015999	*Ipomoea nil*	Convolvulaceae	*
PKn2	AB016000	*Ipomoea nil*	Convolvulaceae	*
PKn3	AB016002	*Ipomoea nil*	Convolvulaceae	*
PtKn1	AY680402	*Pinus taeda*	Pinaceae	Guillet-Claude et al. 2004
PtKn2	AY680403	*Pinus taeda*	Pinaceae	Guillet-Claude et al. 2004
RaSTM	AB000000	*Ruscus aculeatus*	Asparagaceae	This study
RS1	L44133	*Zea mays*	Poaceae	Schneeberger et al. 1995
Sbh1	L13663	*Glycine max*	Fabaceae	Ma et al. 1994
SkKNOX1	AY667449	*Selaginella kraussiana*	Selaginellaceae	Harrison et al. 2005
SkKNOX2	AY667450	*Selaginella kraussiana*	Selaginellaceae	Harrison et al. 2005
SkKNOX3	AY667451	*Selaginella kraussiana*	Selaginellaceae	Harrison et al. 2005
STM	NM_104916	*Arabidopsis thaliana*	Brassicaceae	Long et al. 1996
THox2	U76410	*Lycopersicon esculentum*	Solanaceae	*
TKn1	U32247	*Lycopersicon esculentum*	Solanaceae	Hareven et al. 1996
TKn2	U76407	*Lycopersicon esculentum*	Solanaceae	*
TKn3	U76408	*Lycopersicon esculentum*	Solanaceae	*
U90091	U90091	*Picea mariana*	Pinaceae	Rustledge et al. 1997
U90092	U90092	*Picea mariana*	Pinaceae	Rustledge et al. 1997
Z29073	Z29073	*Brassica napus*	Brassicaceae	Boivin et al. 1994
Z71980	Z71980	*Malus x domestica*	Rosaceae	Watillon et al. 1996