Isolation and characterization of a TERMINAL FLOWER 1 homolog, RsTFL1, from radish (Raphanus sativus)

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Abstract  The gene TERMINAL FLOWER1 (TFL1) regulates the floral phases and the inflorescence architecture of Arabidopsis. A TFL1 homolog designated as RsTFL1 was isolated from radish (Raphanus sativus). The deduced amino acid sequence had 96% identity with TFL1—higher than the values obtained for other reported TFL1-like proteins. The genomic organization of RsTFL1 is very similar to that of TFL1 and consists of 4 exons and 3 introns. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed that RsTFL1, like TFL1, is continuously expressed in both vegetative and reproductive tissues. RsTFL1 was expressed throughout seed development until seed desiccation. RsTFL1 mRNA was detected after seed imbibition, suggesting that the gene expression is initiated during germination. In situ hybridization analysis revealed that RsTFL1 was expressed in the inflorescence meristem but not in the floral meristems. The expression was not limited to the inner cells of the inflorescence meristem, unlike the expression of TFL1 in Arabidopsis. This expression pattern of RsTFL1 may make it possible that radish maintains its indeterminate inflorescence.

Key words:  Indeterminate inflorescence, radish, Raphanus sativus, shoot apical meristem, TERMINAL FLOWER1.

Arabidopsis and radish (Raphanus sativus) are both cruciferous plants that have indeterminate inflorescences with typical racemes. Radish has long been grown as a food crop worldwide and consists of extremely variable cultivars. In particular, in Southeast Asia (Japan, Korea, and China), the large-rooted form is one of the most important vegetables. In addition, some have importance in Chinese medicine, the seeds of some plants are used as oil sources, and the young siliques (pods) or leaves of some plants are consumed (Banga 1976). Radish shows considerable variations in temperature and photoperiod some plants are consumed (Banga 1976). Radish shows as oil sources, and the young siliques (pods) or leaves of in Chinese medicine, the seeds of some plants are used important vegetables. In addition, some have importance and China), the large-rooted form is one of the most cultivars. In particular, in Southeast Asia (Japan, Korea, and Nomura 2008). Moreover, since TFL1 is known to play a key role in plant architecture in Arabidopsis, we isolated and characterized a TFL1 homolog from radish that was designated as RsTFL1 (Raphanus sativus TFL1) in order to understand the basic molecular mechanism of flowering in this plant. The cDNA sequence data have been deposited in the GenBank database (accession number: AB435524). In this study, the rat’s tail radish cultivar “Pakki-hood” was used (Nomura et al. 1996). This particular variety does not require a chilling step to induce flowering. Total RNA extracted from radish shoot apices was used to isolate TFL1-like genes by reverse transcriptase-polymerase chain reaction (RT-PCR). The longest cDNA clone had a 531-bp open reading frame that was preceded by a 67-bp 5’-untranslated region. Two polyadenylation sites were found at positions 700 and 736 in the 3’-untranslated region. The cDNA showed extensive similarity to TFL1. PCR amplification of the corresponding genomic sequences revealed that RsTFL1 contains 4 exons of 277, 66, 37, and 356 bp and 3 introns of 195, 227, and 90 bp (Figure 1A), and these were located at the same positions as in TFL1 of Arabidopsis (Ohshima et al. 1997). The 531-bp open reading frame is predicted to encode 177 amino acid residues, and the deduced amino acid sequence has 96% identity with that of TFL1 (Figure 1B). Phylogenetic analysis revealed that RsTFL1 and TFL1 were positioned on the same branch.
of a phylogenetic tree, indicating that they have the closest relationship among the TFL1-like genes (Figure 1C). In Arabidopsis, TFL1 and FT belong to the PEBP family (Bradley et al. 1997; Ohshima et al. 1997; Yeung et al. 1999; Kardailsky et al. 1999; Kobayashi et al. 1999). Both these genes regulate meristem identity and flowering time; however, despite the similarities in their sequences, they play antagonistic roles: FT induces floral

Figure 1. Genomic organization of RsTFL1 and similarity of deduced protein with other plant PEBPs. (A) The structure of RsTFL1 could be determined by comparison of cDNA clones with genomic DNA. Filled boxes, untranslated regions; open boxes, protein-coding regions; lines, introns. (B) Alignment of the predicted amino acid sequence of RsTFL1 with TFL1, CEN, and FT. Identical residues are in white text on black background. Dashed lines indicate gaps introduced to maximize alignment. Asterisks indicate amino acids that are likely to be most critical to the function of TFL1/CEN and FT (Hanzawa et al. 2005; Ahn et al. 2006). (C) Phylogenetic tree based on the predicted TFL1-like proteins and drawn by CLUSTALW. The lengths of horizontal lines are proportional to the similarity between predicted protein sequences. Accession numbers: CET1 (AF142559), FT (AB027504), BNTFL1-1 (AB017525), TFL1 (D87130), IdTFL1A (AJ888758), CsTFL (AB027456), Ljcen1 (AY423715), PsTFL1a (AY340579), VvTFL1A (DQ871591), CEN (S81193), SP (U84140), CET2 (AF145260), CET4 (AF145261), LpTFL1 (AF316419), RCN1/FDR2 (AF159882).
in the inflorescence meristem but not in the vegetative meristem (Figure 3A). This suggests that it may control floral transition in radish. RsTFL1 transcripts were not detected in leaves or young floral buds, but they were detected in inflorescence stems and floral buds immediately before flowering; moreover, low levels of expression were also detected in pedicels (Figure 3B). RsTFL1 was expressed in carpels but not in sepals, petals, and stamens. To gain an understanding of the expression pattern of RsTFL1 during seed maturation, we extracted RNA from developing seeds. RsTFL1 was expressed 20 d after flowering and continued to be expressed up to 60 d (Figure 4).

In situ hybridization analysis revealed that RsTFL1 is expressed in the inflorescence meristem but not in the floral meristem (Figure 5A), like in the case of TFL1 expression in Arabidopsis. In vitro transcribed, digoxigenin-labeled antisense and sense RNA probes

Figure 2. Southern blot analysis of the RsTFL1 gene. Raphanus sativus genomic DNA was digested with BglII (Bg) and PstI (Ps). The genomic sequence corresponding to RsTFL1 included a site for PstI. Full-length RsTFL1 cDNA was labeled with digoxigenin and used as a probe. Hybridization and washing were carried out under high stringent conditions as described in the manufacturer’s protocol (Roche).

Figure 3. RT-PCR analysis of RsTFL1 in radish. Total RNA was isolated from various tissues and germinating seeds with the RNeasy Mini Kit (Qiagen). Total RNA (1 μg) was treated with RNase-free DNase I (Invitrogen) to eliminate genomic DNA contamination. For RT-PCR, 1 μg of total RNA was used to generate first-strand cDNA in the SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen). Primers for RsTFL1 fragment amplification were F325 (5′-AGAGCACCTGCATTGGATCGTAAC-3′) and R3E2 (5′-CAG-AATTCAGACGATAGCAACATGGC-3′). The thermocycler program was 2 min/94°C; 30 cycles of 30 s/94°C, 30 s/60°C, 45 s/72°C; and a final extension of 5 min/72°C. A fragment of β-TUBULIN (TUB) gene was amplified from the same cDNA as a standard control to normalize the cDNA amount used in the RT-PCR. (Marks et al. 1987).

A) Expression of RsTFL1 in imbibed seeds (0, 24, and 48 h) and shoot apices. (B) Expression of RsTFL1 in various tissues: VM, vegetative meristem; RM, reproductive meristem; Lf, leaves; Yb, young floral buds; Fb, floral buds just before flowering; Se, sepals; Pe, petals; St, stamens; Cp, carpels; Pd, pedicels; Is, inflorescence stems.

Figure 4. Expression of RsTFL1 in developing seeds. Flowers were tagged on the day of flowering. Every 20 days during seed development (20, 40, and 60 d), siliques were harvested and seeds were picked out to extract total RNA. RT-PCR was performed using gene-specific primers. A fragment of TUB was amplified from the same cDNA as a standard control to normalize the cDNA amount used in the RT-PCR. (Marks et al. 1987).

We determined the expression pattern of RsTFL1 in various radish tissues (Figure 3). A single 376-bp product was amplified from total RNA by using gene-specific primers. RT-PCR analysis revealed that RsTFL1 was not expressed in dry seeds or imbibed seeds before germination, but was expressed when the cotyledons emerged, 48 h after the initiation of imbibition. RsTFL1 expression was detected at the shoot apex in both the vegetative and reproductive phases. TFL1 is expressed in both vegetative and reproductive meristems and regulates floral transition in Arabidopsis (Bradley et al. 1997). In contrast, CENTRORADIALIS (CEN), a TFL1 ortholog found in Antirrhinum, is expressed in reproductive meristems but not in vegetative meristems, and it does not influence floral transition in Antirrhinum (Bradley et al. 1996). RsTFL1 was expressed in both vegetative and reproductive meristems (Figure 3A). This suggests that it may control floral transition in radish. RsTFL1 transcripts were not detected in leaves or young floral buds, but they were detected in inflorescence stems and transition whereas TFL1 suppresses it. Some conserved amino acids are responsible for the difference in the activities of TFL1 and FT (Hanzawa et al. 2005; Ahn et al. 2006). Moreover, amino acids that are essential to the function of TFL1 are conserved in RsTFL1 (Figure 1B). These results therefore indicate that the deduced amino acid sequence of the RsTFL1 protein is quite similar to TFL1, suggesting that RsTFL1 functions in the same manner as TFL1. Genomic DNA gel blot hybridization analysis was performed with a RsTFL1 cDNA probe, and a single hybridizing restriction fragment was detected (Figure 2), indicating that the radish genome contains only a single copy of RsTFL1.
were synthesized for RsTFL1. The apical meristem consists of epidermal (L1), subepidermal (L2), and inner (L3) layers. Although the activity of TFL1 throughout the apical meristem is necessary to control inflorescence meristem identity, the expression is restricted to the inner cells (part of L3) of apical meristems (Simon et al. 1996; Bradley et al. 1997; Ratcliffe et al. 1999); however, TFL1 protein is present in a wide region of the apical meristem because of its movement to outer cells (L1 and L2). TFL1 functions as a mobile signal across the meristem tissues in order to control the inflorescence meristem (Conti and Bradley 2007). On the other hand, RsTFL1 was found to be expressed in L1, L2, and a part of L3—within the central region of the inflorescence meristem—unlike the expression pattern of TFL1 in Arabidopsis (Figure 5A). This region is almost coincident with the region in which the TFL1 protein is localized in Arabidopsis to control inflorescence identity (Conti and Bradley 2007). This indicates that the RsTFL1 protein is localized solely in the central region and may control indeterminate inflorescence in the absence of a mobile signal. RsTFL1 expression was restricted to the sub-apical region of the young axillary meristem (Figure 5C). After the development of the floral meristem from the axillary shoot meristem, RsTFL1 was widely expressed throughout the axillary inflorescence meristem as well as the primary inflorescence meristem (Figure 5A). FT, LFY, and TFL1 have antagonistic functions in Arabidopsis. FD, a bZIP transcription factor of Arabidopsis, was identified as a partner of FT (Abe et al. 2005). Ahn et al. (2006) suggested that FT and TFL1 compete for a common interacting partner (FD) in the shoot apical meristem (SAM). The ratio of LFY/TFL1 activity in the SAM controls the developmental fate of the meristem (Ratcliffe et al. 1999; Ferrandiz et al. 2000). In the Arabidopsis SAM, TFL1 suppresses the floral signals induced by genes that promote flowering, namely, LFY and FT. Similarly, in radish, RsTFL1 must also suppress flowering signals in the SAM. However, the mechanism of floral suppression in radish and Arabidopsis may differ. Because RsTFL1 mRNA is present in not only the inner layers but also the outer layers of the inflorescence meristem, it may not always be necessary for the RsTFL1 protein to function as a mobile signal by moving across cells in order to control the inflorescence meristem, like the Arabidopsis TFL1 protein. On the basis of the RsTFL1 expression pattern, we can assume that the RsTFL1 protein localizes quickly throughout the inflorescence meristem, as compared to TFL1. As a result, the radish inflorescence meristem may be able to resist flowering signals, irrespective of the strength of the flowering signals or the intensity of the break-out signal induced by certain environmental factors. Further experiments are needed to elucidate the biological function of RsTFL1 in radish.

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References

Conti L, Bradley D (2007) TERMINAL FLOWER1 is a mobile


