Supplemental Table S1. Effects of various compounds on activity of TCE purified from tulip petals.

Compound [a]	Concentration (mM)	Relative activity [b] (%)
None	-	100
NaF	0.2	20.5
PMSF	2	9.32
$AgNO_3$	0.2	< 0.1
$HgCl_2$	0.2	< 0.1
$CuSO_4$	2	25.9

Enzyme activity was measured using 4 mM 6-tuliposide A as substrate.

[a] Compounds that inhibited activity >60% are shown. The following compounds showed less or no effects on enzyme activity: 1) coenzymes (0.05 mM), FMN, FAD, riboflavin, pyridoxal-5'-phosphate, NAD(P)(H), biotin, thiamine-HCl, phenazinemethosulfate, pyrroloquinoline quinone, thiooctic acid, XTP (X; A, G, C, and T), XDP, XMP, thioglycolic acid, cysteamine, glutathione (oxidized and reduced forms), L-Cys, iodonitrotetrazolium, dehydroascorbic acid, and L-ascorbic acid; 2) metal ions (2 mM), Li^{+}, BO_3^{2+}, Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, Ca^{2+}, V^{3+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, As^{3+}, Rb^{+}, Nb^{5+}, Mo^{2+}, Cd^{2+}, Sn^{2+}, Cs^{+}, Tl^{2+}, Ba^{2+}, and Pb^{2+}; and 3) other compounds (2 mM), *p*-chloromercuribenzoic acid, EDTA, EGTA, bipyridyl, *o*-phenanthroline, disodium catechol-3,5-disulfonate, 8-hydroxyquinoline, hydrazine, diphenylhydrazine, hydroxylamine, KCN, NaN_3, iodoacetate, *N*-ethylmaleimide, 5,5'-dithiobis(2-nitrobenzoate), D-cycloserine, D-penicillamine, dithiothreitol, $K_4(Fe(CN)_6)$, and $K_3(Fe(CN)_6)$.

[b] Activity is shown as percent ratio of specific activity to that without additive (2,260 U/mg).

Supplemental Table S2. Primer sequences used in this study.

Primer name	Sequence (5' to 3')
For degenerate PCR	
TCEA-N-F1 [a]	GCIYTIGAYGAYGARATHGT
TCEA-N-F2 [a]	GAYGAYGARATHGTIYTIGA
TCEA-Int-R1 [b]	ACIGTIGTICCIARRAAICKYTC
TCEA-Int-R2 [b]	CCIARRAAICKYTCDATIGGICC
For RACE PCR	
TCEA-5RACE-R1	ACTCTTGTAAATGATCAAGAAGG
TCEA-3RACE-F1	CCTGAAGCCCTTCTTGATCATTTAC
TCEA-3RACE-F2	GCCCTTCTTGATCATTTACAAGAG
For full-length cDNA cloning, genomic DNA cloning, and RT-PCR	
TCEA-RT-F	AAGCATTTCTGTGAAATCAATTAAGTG
TCEA-RT-R	CATCATAACAATGCCTTAAAGAGG
For RT-PCR	
TCEA-RT-F1	TCAGTAGCCTCGTTCTTTAGCT
TCEA-RT-F2	CTTTAGCTCGCTGCCTGCTAGG
TCEA-RT-F3	AAGATGGGCGAGGCAGGACT
TCEA-RT-R1	CTCTCCACCAGAAAATCATTTC
TCEA-RT-R2	GTCATGAAACTCAATGTTCG
GAPDH-F	GGAATCCTTGGTTATGTTGAAG
GAPDH-R	TACTTGGTTGCGGCAATGTGG
For qRT-PCR	
TgTCEA1-qRT-F	TTGGTGAAGAGCGGGTGGC
TgTCEA1-qRT-R	GAAACCCTAACAAGAAGCAGCG
TgTCEA2-qRT-F	AAGAGCGGGTGGGGAGGT
TgTCEA2-qRT-R	CAAACCCTAACAAGAAGCAACA
For *E. coli* expression plasmids	
TCEA-F-BamHI	CGCGGATCCGCTTTAGACGACGAAATTGTG
TCEA-R-XhoI	GGGCTCGAGCTACTCGCCTTTGAGAAAAGC

Supplemental Table S2 (continued)

TCEA-G152A-F	CTACTTCCACGCTGGCGGCTTCGT
TCEA-G152A-R	ACGAAGCCGCCAGCGTGGAAGTAG
TCEA-G153A-F	CTTCCACGGAGCCGGCTTCGTCA
TCEA-G153A-R	TGACGAAGCCGGCTCCGTGGAAG
TCEA-G154A-F	CCACGGAGGCGCCTTCGTCATCG
TCEA-G154A-R	CGATGACGAAGGCGCCTCCGTGG
TCEA-S235A-F	CTTTCAGGTGACGCCGCCGGCGGCAA
TCEA-S235A-R	TTGCCGCCGGCGGCGTCACCTGAAAG
TCEA-D327N-F	GTGGCGGGAAATAATTTTCTGGTG
TCEA-D327N-R	CACCAGAAAATTATTTCCCGCCAC
TCEA-H359A-F	GAGGGGGTCGGTGCTGTGTTTCATCT
TCEA-H359A-R	AGATGAAACACAGCACCGACCCCCTC
TCEA-H362A-F	GGTCATGTGTTTGCTCTGTCTGAT
TCEA-H362A-R	ATCAGACAGAGCAAACACATGACC
For GFP reporter plasmids	
TCEA-rep-F1	TCTAGAGGATCCATGTCAGTAGCCTCGTTCTTTAGC
TCEA-rep-F2	TCTAGAGGATCCATGGCTTTAGACGACGAAATTGTGTTG
TCEA-rep-R1	CCCTTGCTCACCATGGACTCGCCTTTGAGAAAAGCAAT C
TCEA-rep77-R	CCCTTGCTCACCATGGATGTGGGCGTCGGAGAGAGAGA TG
TCEA-rep60-R	CCCTTGCTCACCATGGAAAATACAGAAGTAACAGAACC
TCEA-rep55-R	CCCTTGCTCACCATGGAAGAACCATGGTATGTATTGC
TCEA-rep50-R	CCCTTGCTCACCATGGAATTGCTTTCTATAGGACGAC
TCEA-rep45-R	CCCTTGCTCACCATGGAACGACAGGCTAGAACCGGCGG CT
TCEA-rep44-R	CCCTTGCTCACCATGGAACAGGCTAGAACCGGCGGCTT AAC

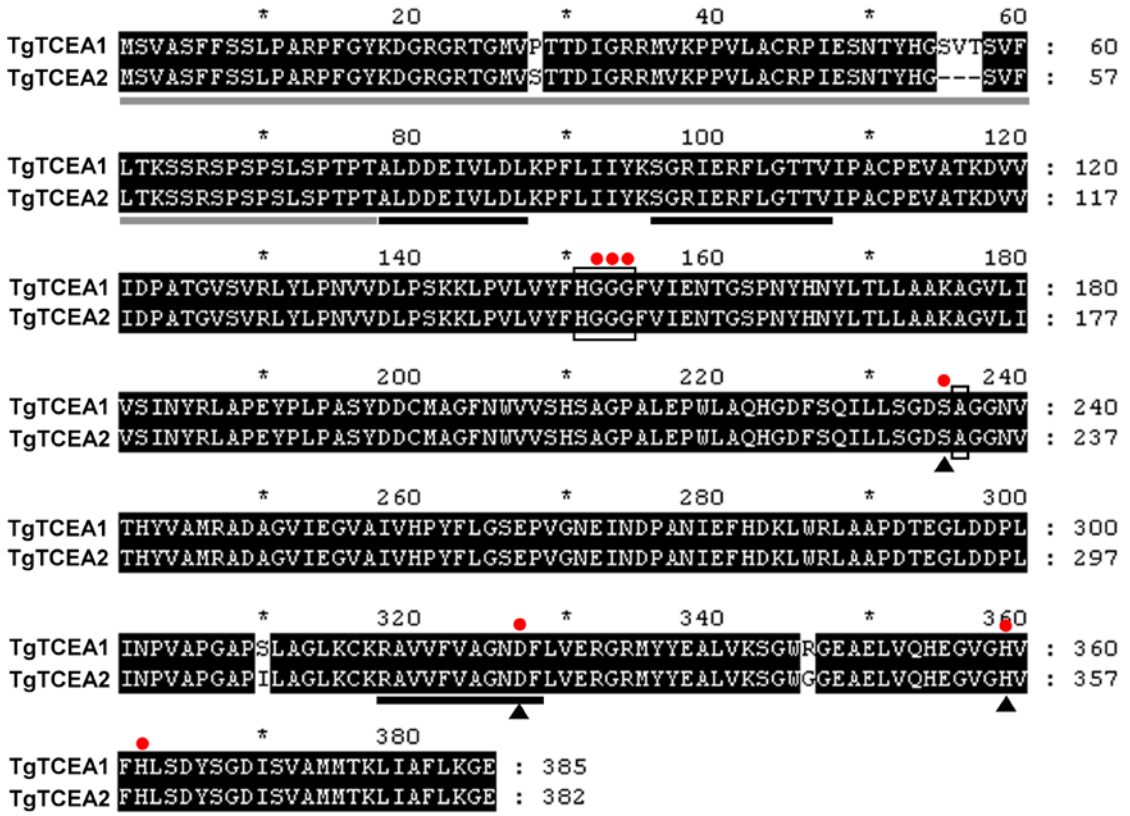
Supplemental Table S2 (continued)

TCEA-rep43-R	CCCTTGCTCACCATGGAGGCTAGAACCGGCGGCTTAAC C
TCEA-rep42-R	CCCTTGCTCACCATGGATAGAACCGGCGGCTTAACCATC
TCEA-rep41-R	CCCTTGCTCACCATGGAAACCGGCGGCTTAACCATCC
TCEA-rep40-R	CCCTTGCTCACCATGGACGGCGGCTTAACCATCCGTC
TCEA-rep20-R	CCCTTGCTCACCATGGACCCATCTTTGTATCCAAAAGG

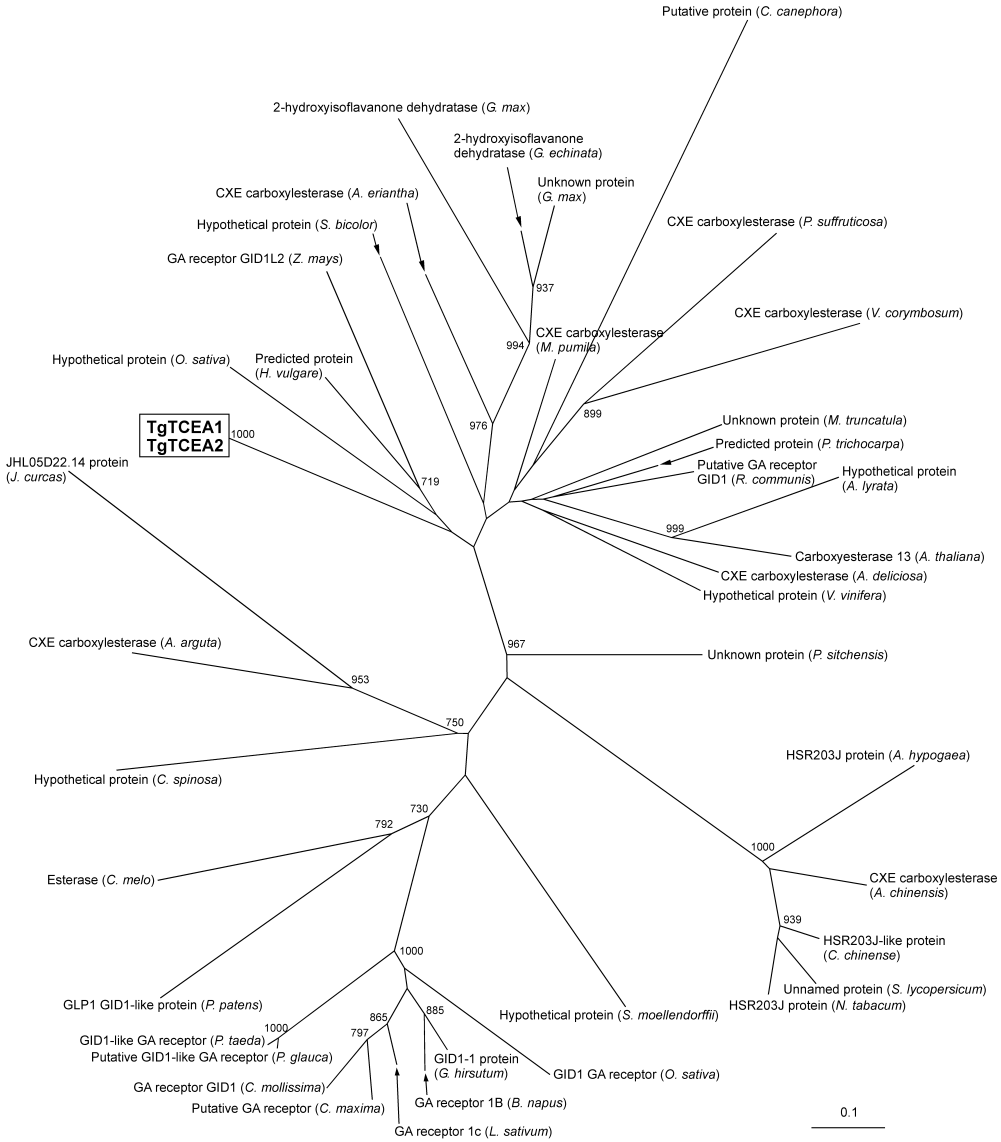
'F' and 'R' in the primer name indicate 'forward' and 'reverse' primers, respectively.

[a] Designed from N-terminal amino acid sequence, ALDDEIVLDL.

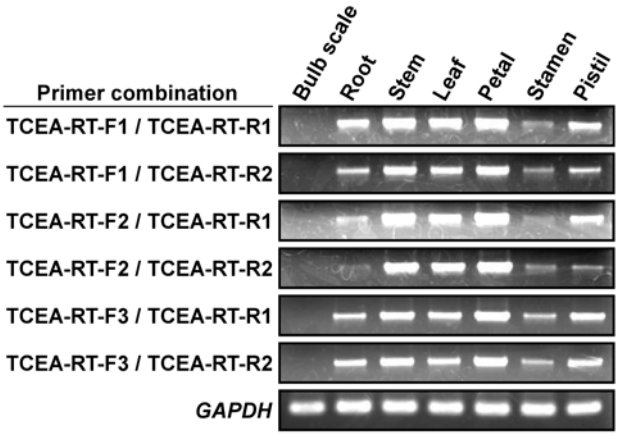
[b] Designed from internal amino acid sequence, SG(R/P)IERFLGTTV.



Supplemental Figure S1. Alignment of amino acid sequences of TgTCEA1 and TgTCEA2. Sequences found by protein sequencing of enzyme purified from tulip tissues are indicated with *black* underlines. N-terminus of mature enzyme is based on protein sequencing of anther enzyme, as petal enzyme did not yield exclusively single amino acid signals in its N-terminal sequencing. Internal peptide sequences are from petal enzyme. Signal peptides are indicated with *gray* underline. Residues constituting putative catalytic triad (S, D, and H) and oxyanion hole (HGGG-motif and Ala next to catalytic Ser) are indicated with *black* triangles and boxes, respectively, and those subjected to the site-directed mutagenesis are marked with *red* circles.



Supplemental Figure S2. Unrooted phylogenetic tree of TgTCEA enzymes with selected members of plant α/β hydrolase fold superfamily proteins. Full-length amino acid sequences were aligned using ClustalW (version 1.83; http://clustalw.ddbj.nig.ac.jp/top-j.html). Phylogenetic tree was built based on calculation by neighbor-joining method with bootstrap analysis of 1,000 replicates and visualized with Treeview (version 1.66). Numbers at each node are bootstrap values per 1,000 trials (values >70% are shown). Scale bar indicates substitutions per site. Accession numbers for proteins listed are BAE45340 (*Oryza sativa*, GID1 GA receptor), NP_001063395 (*Oryza sativa*, hypothetical protein), XP_002285067 (*Vitis vinifera*, hypothetical protein), XP_002336023 (*Populus trichocarpa*, predicted protein), XP_002462498 (*Sorghum bicolor*, hypothetical protein), XP_002518790 (*Ricinus communis*, putative GA receptor GID1), BAJ94344 (*Hordeum vulgare*, predicted protein), XP_002875213 (*Arabidopsis lyrata*, hypothetical protein), NP_001152298 (*Zea mays*, GA receptor GID1L2), ABB89006 (*Malus pumila*, CXE carboxylesterase), NP_190439 (*Arabidopsis thaliana*, carboxyesterase 13), ACJ84504 (*Medicago truncatula*, unknown protein), BAD80839 (*Glycyrrhiza echinata*, 2-hydroxyisoflavanone dehydratase), BAD80840 (*Glycine max*, 2-hydroxyisoflavanone dehydratase), ABW74473 (*Paeonia suffruticosa*, CXE carboxylesterase), ACU19943 (*Glycine max*, unknown protein), ABK23572 (*Picea sitchensis*, unknown protein), ABB89023 (*Actinidia eriantha*, CXE carboxylesterase), ABB89018 (*Actinidia deliciosa*, CXE carboxylesterase), ABB89000 (*Vaccinium corymbosum*, CXE carboxylesterase), XP002970604 (*Salaginella moellendorffii*, hypothetical protein), ADF18551 (*Arachis hypogaea*, HSR203J protein), BAA74434 (*Solanum lycopersicum*, unnamed protein), BAC15624 (*Nicotiana tabacum*, HSR203J protein), ABB89024 (*Actinidia chinensis*, CXE carboxylesterase), BAD11070 (*Capsicum chinense*, HSR203J-like protein), XP_001757066 (*Physcomitrella patens*, GLP1 GID1-like protein), ABB89014 (*Actinidia arguta*, CXE carboxylesterase), ACN86356 (*Gossypium hirsutum*, GID1-1), CAP64321 (*Picea glauca*, putative GID1-like GA receptor), CAN87127 (*Cucurbita maxima*, putative GA receptor), ABZ89192 (*Coffea canephora*, putative protein), ADN93297 (*Lepidium sativum*, GA receptor 1c), ABD96915 (*Cleome spinosa*, hypothetical protein), BAJ53143 (*Jatropha curcas*, JHL05D22.14), ABQ53633 (*Cucumis melo*, esterase), CAP64323 (*Pinus taeda*, GID1-like GA receptor), ADV36285 (*Castanea mollissima*, GA receptor GID1), and ADT78692 (*Brassica napus*, GA receptor 1B).

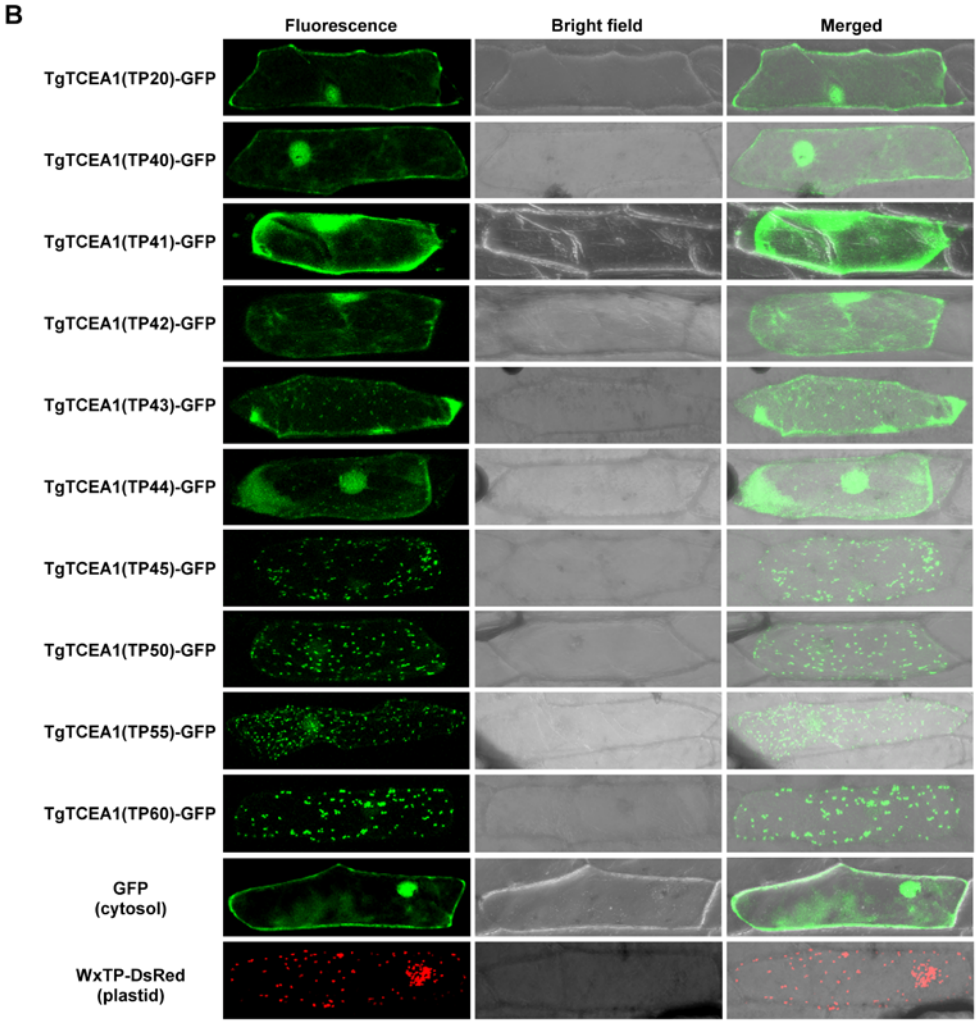


Supplemental Figure S3. Transcript analysis of *TgTCEA* genes in tulip tissues by RT-PCR with various primer sets. Three forward- and two reverse primers were designed from common sequences to the *TgTCEA1* and *TgTCEA2* genes (see Supplemental Table S2 for primer sequences). PCR products were not detected in bulb scales with any primer combinations. *GAPDH* gene was amplified to show the validity of each cDNA template.

A

TP20:MSVASFFSSLPARPFGYKDG
TP40:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPP
TP41:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPV
TP42:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVL
TP43:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLA
TP44:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLAC
TP45:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLACR
TP50:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLACRPIESN
TP55:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLACRPIESNTYHGS
TP60:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLACRPIESNTYHGSVTSVF
TP77:MSVASFFSSLPARPFGYKDGRGRTGMVPTTDIGRRMVKPPVLACRPIESNTYHGSVTSVFLTKSSRSPSPSLSPTPT

B



Supplemental Figure S4. Subcellular localization of GFPs fused with deletion series of the plastid-targeted signal of TgTCEA1 in onion epidermal cells. Different length of N-terminal plastid-targeted signal of TgTCEA1 was fused to GFP and transiently expressed in onion epidermal cells to determine minimal length of N-terminal peptide that functions as plastid-targeted signal. (**A**) Amino acid sequences of the deletion series of N-terminal transit peptide (TP). The number indicates peptide length in amino acid. Seventy-seven amino-acid peptide (TP77) that was demonstrated to function as plastid-targeted signal (Fig. 6) was indicated with *red*. (**B**) Subcellular localization of the GFPs fused with deletion series of N-terminal TPs shown in (**A**). Non-targeted GFP is the control for cytosolic localization. WxTP-DsRed that expresses transit peptide of Waxy protein fused to DsRed is the control for plastidial localization (Kitajima et al., 2009). Fluorescence signals in the nucleus are attributable to free diffusion of the protein (Dingwall and Laskey, 1986).