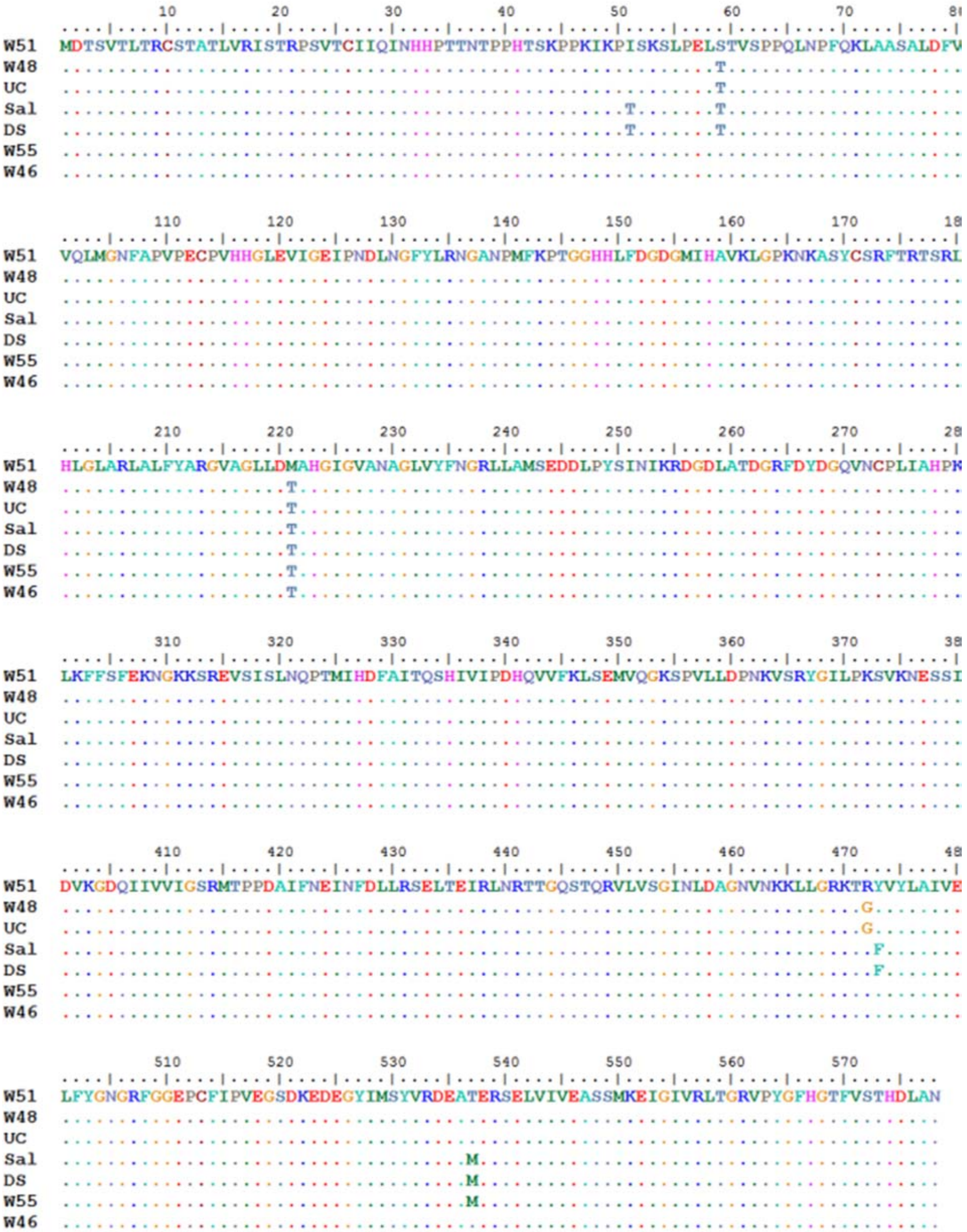
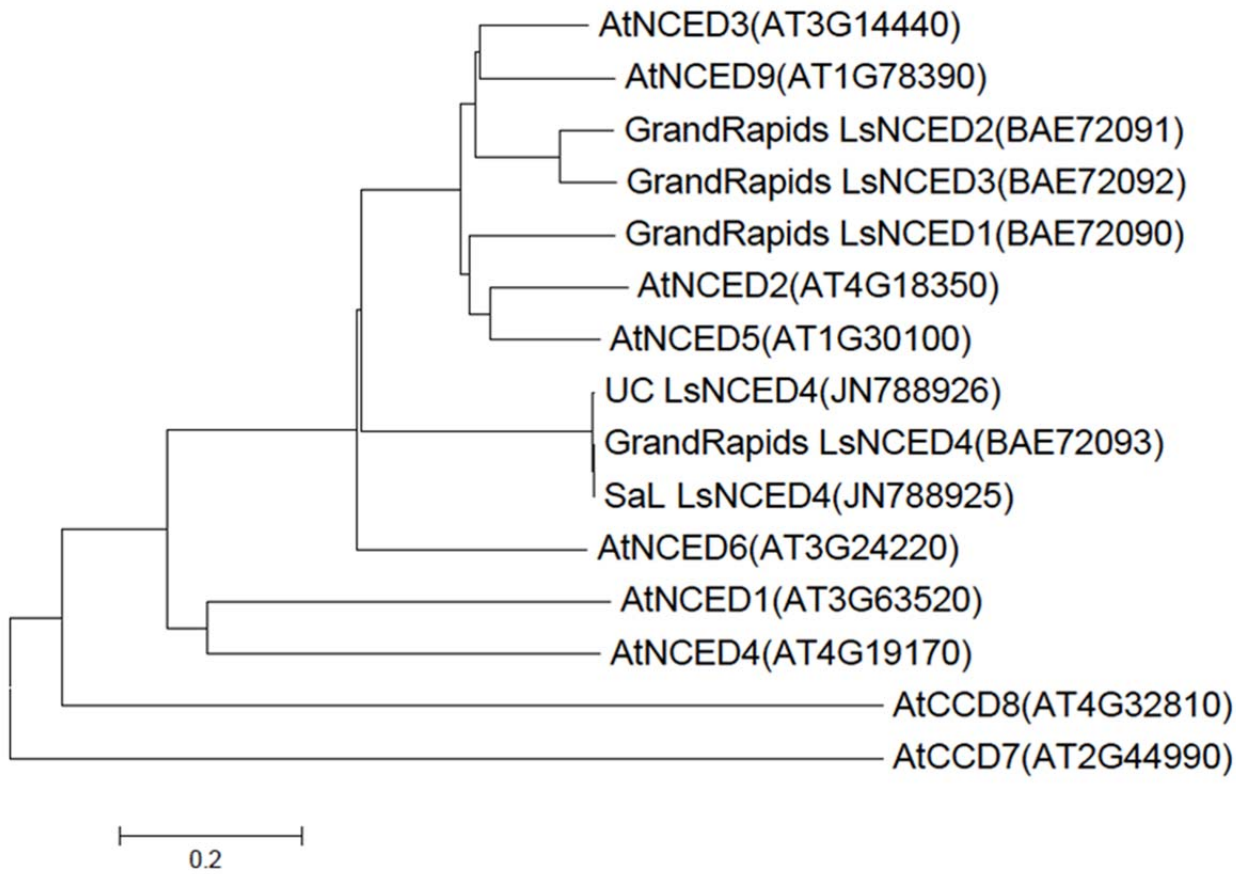


```
             10        20        30        40        50        60        70        8
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
W51  MDTSVTLTRCSTATLVRISTRPSVTCIIQINHHPTTNTPPHTSKPPKIKPISKSLPELSTVSPPQLNPFQKLAASALDFV
W48  ..........................................................T.....................
UC   ..........................................................T.....................
Sal  ..................................................T.......T.....................
DS   ..................................................T.......T.....................
W55  ................................................................................
W46  ................................................................................

            110       120       130       140       150       160       170       18
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
W51  VQLMGNFAPVPECPVHHGLEVIGEIPNDLNGFYLRNGANPMFKPTGGHHLFDGDGMIHAVKLGPKNKASYCSRFTRTSRL
W48  ................................................................................
UC   ................................................................................
Sal  ................................................................................
DS   ................................................................................
W55  ................................................................................
W46  ................................................................................

            210       220       230       240       250       260       270       28
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
W51  HLGLARLALFYARGVAGLLDMAHGIGVANAGLVYFNGRLLAMSEDDLPYSINIKRDGDLATDGRFDYDGQVNCPLIAHPK
W48  ....................T...........................................................
UC   ....................T...........................................................
Sal  ....................T...........................................................
DS   ....................T...........................................................
W55  ....................T...........................................................
W46  ....................T...........................................................

            310       320       330       340       350       360       370       38
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
W51  LKFFSFEKNGKKSREVSISLNQPTMIHDFAITQSHIVIPDHQVVFKLSEMVQGKSPVLLDPNKVSRYGILPKSVKNESSI
W48  ................................................................................
UC   ................................................................................
Sal  ................................................................................
DS   ................................................................................
W55  ................................................................................
W46  ................................................................................

            410       420       430       440       450       460       470       48
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
W51  DVKGDQIIVVIGSRMTPPDAIFNEINFDLLRSELTEIRLNRTTGQSTQRVLVSGINLDAGNVNKKLLGRKTRYVYLAIVE
W48  .......................................................................G........
UC   .......................................................................G........
Sal  ........................................................................F.......
DS   ........................................................................F.......
W55  ................................................................................
W46  ................................................................................

            510       520       530       540       550       560       570
     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
W51  LFYGNGRFGGEPCFIPVEGSDKEDEGYIMSYVRDEATERSELVIVEASSMKEIGIVRLTGRVPYGFHGTFVSTHDLAN
W48  ..............................................................................
UC   ..............................................................................
Sal  ....................................M.........................................
DS   ....................................M.........................................
W55  ....................................M.........................................
W46  ..............................................................................
```

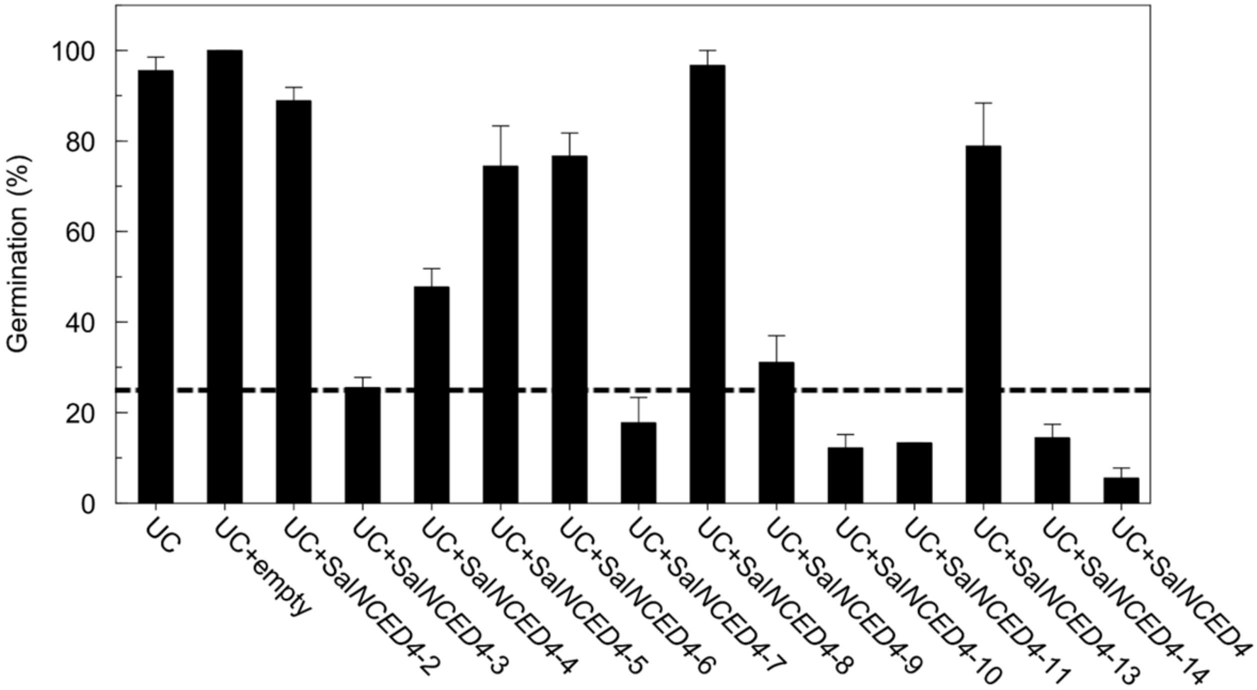
Supplemental Figure 1. Alignment of NCED4 Amino Acid Sequences from Different Lettuce Varieties.

NCED4 amino acid sequences are from thermotolerant genotypes (W51, W48 and UC) or thermosensitive genotypes (Sal, DS, W55 and W46).



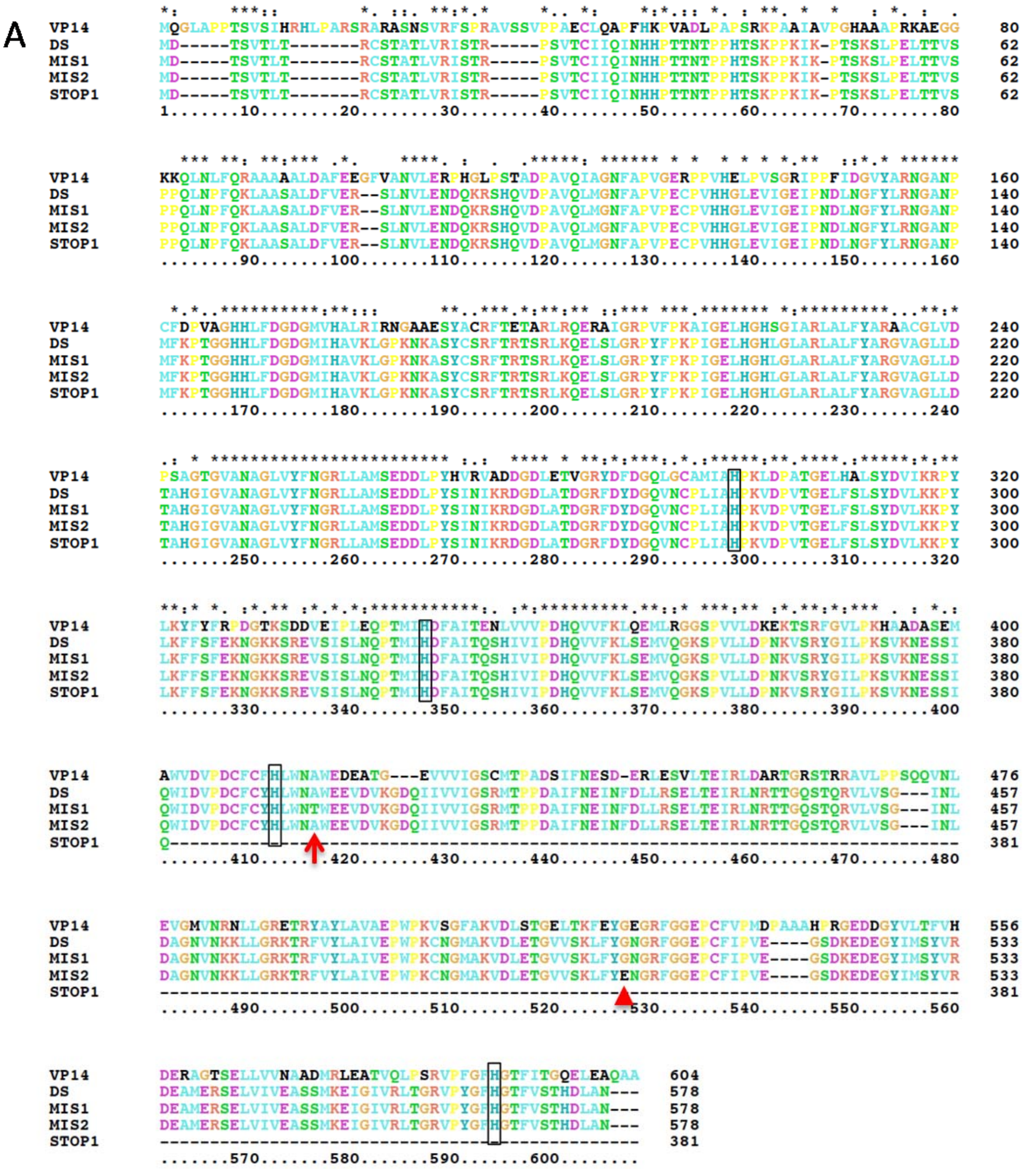
Supplemental Figure 2. Phylogenetic Analysis of NCED Proteins from Arabidopsis and Lettuce.

NCED proteins from *Arabidopsis* and from lettuce (Ls-NCED4 from Sal, UC and Grand Rapids, and Ls-NCED1, Ls-NCED2, Ls-NCED3 from Grand Rapids) were initially aligned with CLUSTAL-W and MEGA (Version 5.05, http://www.megasoftware.net) was used to conduct a phylogenetic analysis based on the Neighbor Joining method with 1000 bootstrap replications. The scale indicates the substitutions per site. The alignments used to generate this phylogeny are available in Supplemental Dataset 1.



Supplemental Figure 3. Germination at High Temperature of Seeds of UC lines Transformed with ProSal:Sal-*NCED4.*

Germination of 12 independent heterozygous lines (UC+Sal*NCED4*-2 to -11, 13-14), UC wild type (UC), one control with empty vector (UC+empty), and one homozygous line derived from the heterozygous UC+Sal*NCED4*-3 line (UC+Sal*NCED4*) were examined at 35°C in the light for 3 d. If heterozygous seeds are segregating for a single dominant transgene, only 25% (horizontal dashed line) of UC+Sal*NCED4* seeds would be expected to germinate. Error bars denote SE (n≥ 3).



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          *:     ***::         *. ::. **:*.*     *.. *:    *:*.:: *. : **. *  *   : *. :  .
VP14      MQGLAPPTSVSIHRHLPARSRARASNSVRFSPRAVSSVPPAECLQAPFHKPVADLPAPSRKPAAIAVPGHAAAPRKAEGG   80
DS        MD-----TSVTLT-------RCSTATLVRISTR-----PSVTCIIQINHHPTTNTPPHTSKPPKIK-PTSKSLPELTTVS   62
MIS1      MD-----TSVTLT-------RCSTATLVRISTR-----PSVTCIIQINHHPTTNTPPHTSKPPKIK-PTSKSLPELTTVS   62
MIS2      MD-----TSVTLT-------RCSTATLVRISTR-----PSVTCIIQINHHPTTNTPPHTSKPPKIK-PTSKSLPELTTVS   62
STOP1     MD-----TSVTLT-------RCSTATLVRISTR-----PSVTCIIQINHHPTTNTPPHTSKPPKIK-PTSKSLPELTTVS   62
          1.......10........20........30........40........50........60........70........80

           *** **: **:*** .*.    ****. :  .  .*****: ****** * *  * * * *.**  ::*.* ******
VP14      KKQLNLFQRAAAAALDAFEEGFVANVLERPHGLPSTADPAVQIAGNFAPVGERPPVHELPVSGRIPPFIDGVYARNGANP   160
DS        PPQLNPFQKLAASALDFVER--SLNVLENDQKRSHQVDPAVQLMGNFAPVPECPVHHGLEVIGEIPNDLNGFYLRNGANP   140
MIS1      PPQLNPFQKLAASALDFVER--SLNVLENDQKRSHQVDPAVQLMGNFAPVPECPVHHGLEVIGEIPNDLNGFYLRNGANP   140
MIS2      PPQLNPFQKLAASALDFVER--SLNVLENDQKRSHQVDPAVQLMGNFAPVPECPVHHGLEVIGEIPNDLNGFYLRNGANP   140
STOP1     PPQLNPFQKLAASALDFVER--SLNVLENDQKRSHQVDPAVQLMGNFAPVPECPVHHGLEVIGEIPNDLNGFYLRNGANP   140
          ........90.......100.......110.......120.......130.......140.......150.......160

           *.*..**********:**:::      **..***.*:**:** ::*** ***.******* *:*********...**:*
VP14      CFDPVAGHHLFDGDGMVHALRIRNGAAESYACRFTETARLRQERAIGRPVFPKAIGELHGHSGIARLALFYARAACGLVD   240
DS        MFKPTGGHHLFDGDGMIHAVKLGPKNKASYCSRFTRTSRLKQELSLGRPYFPKPIGELHGHLGLARLALFYARGVAGLLD   220
MIS1      MFKPTGGHHLFDGDGMIHAVKLGPKNKASYCSRFTRTSRLKQELSLGRPYFPKPIGELHGHLGLARLALFYARGVAGLLD   220
MIS2      MFKPTGGHHLFDGDGMIHAVKLGPKNKASYCSRFTRTSRLKQELSLGRPYFPKPIGELHGHLGLARLALFYARGVAGLLD   220
STOP1     MFKPTGGHHLFDGDGMIHAVKLGPKNKASYCSRFTRTSRLKQELSLGRPYFPKPIGELHGHLGLARLALFYARGVAGLLD   220
          .......170.......180.......190.......200.......210.......220.......230.......240

          .: * ************************ :.:  **** * **:*:***:.*.:*****:**.****.:*****:*:**
VP14      PSAGTGVANAGLVYFNGRLLAMSEDDLPYHVRVADDGDLETVGRYDFDGQLGCAMIAHPKLDPATGELHALSYDVIKRPY   320
DS        TAHGIGVANAGLVYFNGRLLAMSEDDLPYSINIKRDGDLATDGRFDYDGQVNCPLIAHPKVDPVTGELFSLSYDVLKKPY   300
MIS1      TAHGIGVANAGLVYFNGRLLAMSEDDLPYSINIKRDGDLATDGRFDYDGQVNCPLIAHPKVDPVTGELFSLSYDVLKKPY   300
MIS2      TAHGIGVANAGLVYFNGRLLAMSEDDLPYSINIKRDGDLATDGRFDYDGQVNCPLIAHPKVDPVTGELFSLSYDVLKKPY   300
STOP1     TAHGIGVANAGLVYFNGRLLAMSEDDLPYSINIKRDGDLATDGRFDYDGQVNCPLIAHPKVDPVTGELFSLSYDVLKKPY   300
          .......250.......260.......270.......280.......290.......300.......310.......320

          **:* *. :*.** :*.*.*:***********:. :*:*********.**::* ***:** :*.**:*:*** . : *.:
VP14      LKYFYFRPDGTKSDDVEIPLEQPTMIHDFAITENLVVVPDHQVVFKLQEMLRGGSPVVLDKEKTSRFGVLPKHAADASEM   400
DS        LKFFSFEKNGKKSREVSISLNQPTMIHDFAITQSHIVIPDHQVVFKLSEMVQGKSPVLLDPNKVSRYGILPKSVKNESSI   380
MIS1      LKFFSFEKNGKKSREVSISLNQPTMIHDFAITQSHIVIPDHQVVFKLSEMVQGKSPVLLDPNKVSRYGILPKSVKNESSI   380
MIS2      LKFFSFEKNGKKSREVSISLNQPTMIHDFAITQSHIVIPDHQVVFKLSEMVQGKSPVLLDPNKVSRYGILPKSVKNESSI   380
STOP1     LKFFSFEKNGKKSREVSISLNQPTMIHDFAITQSHIVIPDHQVVFKLSEMVQGKSPVLLDPNKVSRYGILPKSVKNESSI   380
          .......330.......340.......350.......360.......370.......380.......390.......400

VP14      AWVDVPDCFCFHLWNAWEDEATG---EVVVIGSCMTPADSIFNESD-ERLESVLTEIRLDARTGRSTRRAVLPPSQQVNL   476
DS        QWIDVPDCFCYHLWNAWEEVDVKGDQIIVVIGSRMTPPDAIFNEINFDLLRSELTEIRLNRTTGQSTQRVLVSG---INL   457
MIS1      QWIDVPDCFCYHLWNTWEEVDVKGDQIIVVIGSRMTPPDAIFNEINFDLLRSELTEIRLNRTTGQSTQRVLVSG---INL   457
MIS2      QWIDVPDCFCYHLWNAWEEVDVKGDQIIVVIGSRMTPPDAIFNEINFDLLRSELTEIRLNRTTGQSTQRVLVSG---INL   457
STOP1     Q-------------------------------------------------------------------------------   381
          .......410.......420.......430.......440.......450.......460.......470.......480

VP14      EVGMVNRNLLGRETRYAYLAVAEPWPKVSGFAKVDLSTGELTKFEYGEGRFGGEPCFVPMDPAAAHPRGEDDGYVLTFVH   556
DS        DAGNVNKKLLGRKTRFVYLAIVEPWPKCNGMAKVDLETGVVSKLFYGNGRFGGEPCFIPVE----GSDKEDEGYIMSYVR   533
MIS1      DAGNVNKKLLGRKTRFVYLAIVEPWPKCNGMAKVDLETGVVSKLFYGNGRFGGEPCFIPVE----GSDKEDEGYIMSYVR   533
MIS2      DAGNVNKKLLGRKTRFVYLAIVEPWPKCNGMAKVDLETGVVSKLFYENGRFGGEPCFIPVE----GSDKEDEGYIMSYVR   533
STOP1     --------------------------------------------------------------------------------   381
          .......490.......500.......510.......520.......530.......540.......550.......560

VP14      DERAGTSELLVVNAADMRLEATVQLPSRVPFGFHGTFITGQELEAQAA   604
DS        DEAMERSELVIVEASSMKEIGIVRLTGRVPYGFHGTFVSTHDLAN---   578
MIS1      DEAMERSELVIVEASSMKEIGIVRLTGRVPYGFHGTFVSTHDLAN---   578
MIS2      DEAMERSELVIVEASSMKEIGIVRLTGRVPYGFHGTFVSTHDLAN---   578
STOP1     ------------------------------------------------   381
          .......570.......580.......590.......600........
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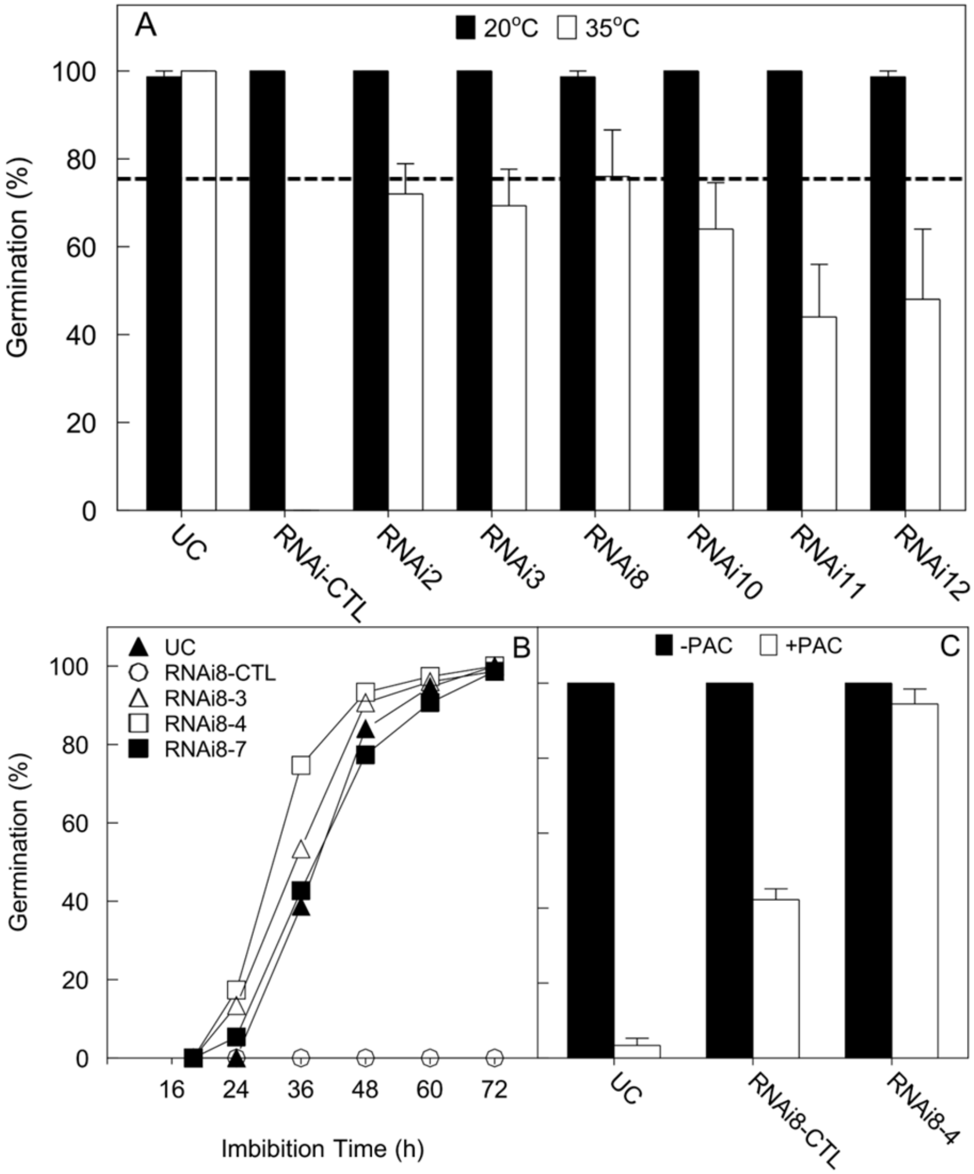
Supplemental Figure 4. Comparison of NCED4 Amino Acid Sequence and Structure to VP14 from Maize.

(A) Predicted protein amino acid sequences of Ls-NCED4 from three TILLING mutants (MIS1, MIS2 and STOP1) and their wild type DS were compared to those of VIVIPAROUS 14 (VP14, AAB62181.2) from maize (*Zea mays*). Arrow, the position at which there is a missense mutation in MIS1 (A to T); arrowhead, the position at which there is a missense mutation in MIS2 (G to E). The STOP1 mutation truncated the transcript just prior to the third of four conserved His residues (boxed) that are essential for coordinating an iron ion in the catalytic site of the enzyme.

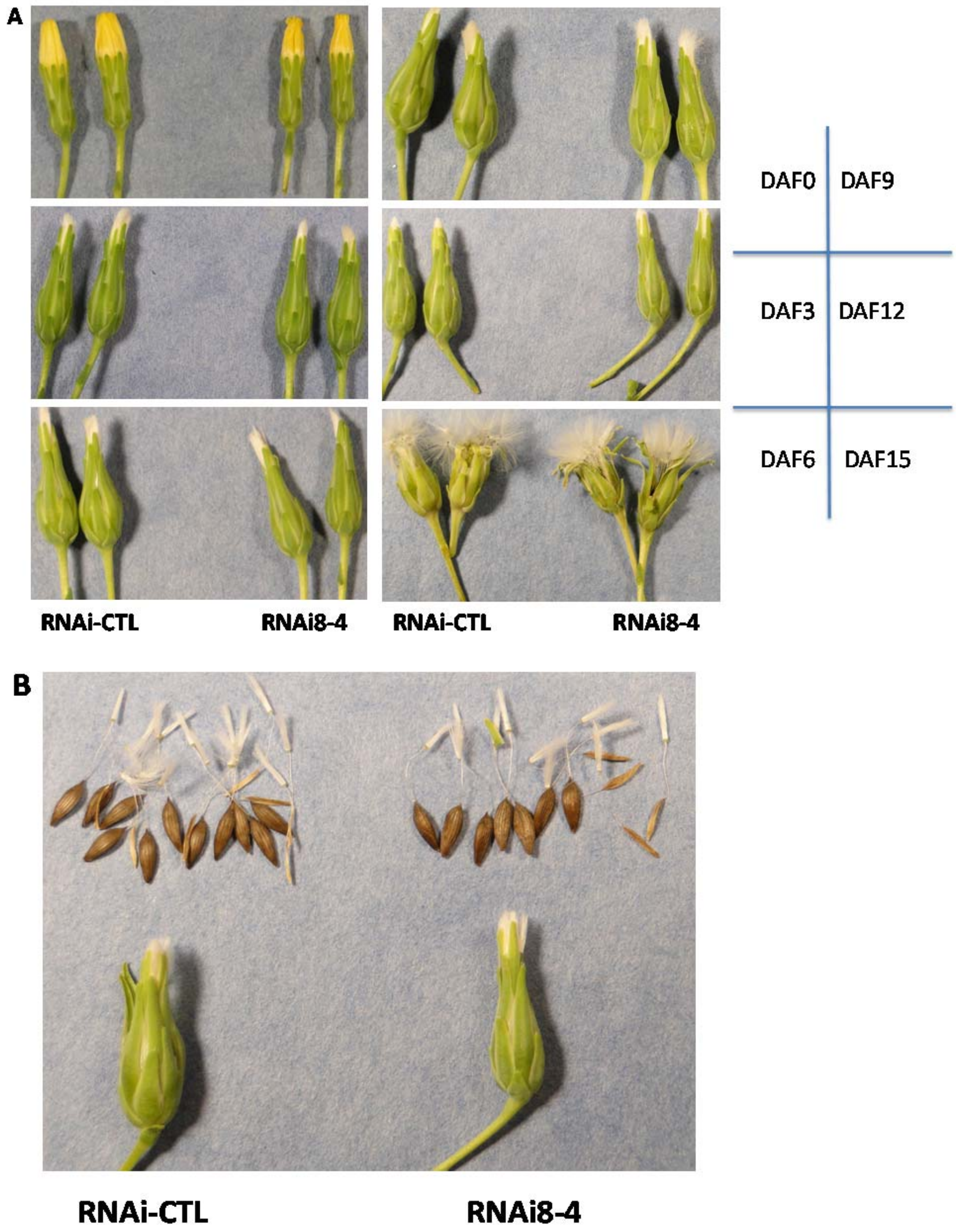
(B) His-412 (white arrow), Ala-416 (red arrow) and Gly-523 (red arrowhead) are highlighted in bright yellow in the tertiary structure of VP14. Ala-416 was replaced with Thr in the MIS1 mutant and the STOP1 mutation truncated the coding region just prior to His-412, while the Gly-523 was replaced with Glu in MIS2. In maize, mutations near the conserved His residues reduced enzymatic activity (Messing et al., 2010).



Supplemental Figure 5. Germination of Heterozygous and Homozygous RNAi-*NCED4* lines.

(A) Germination percentages of heterozygous RNAi seeds at 35°C in the light for 3 d. UC wild type, a control line transformed with empty vector (RNAi-CTL), and six independent transformed lines (RNAi2, 3, 8, 10, 11 and 12) are shown. As heterozygous RNAi seeds were segregating for the silencing insert, only 75% would be expected to germinate at 35°C (indicated by horizontal dashed line). Error bars denote SE (n≥ 3).

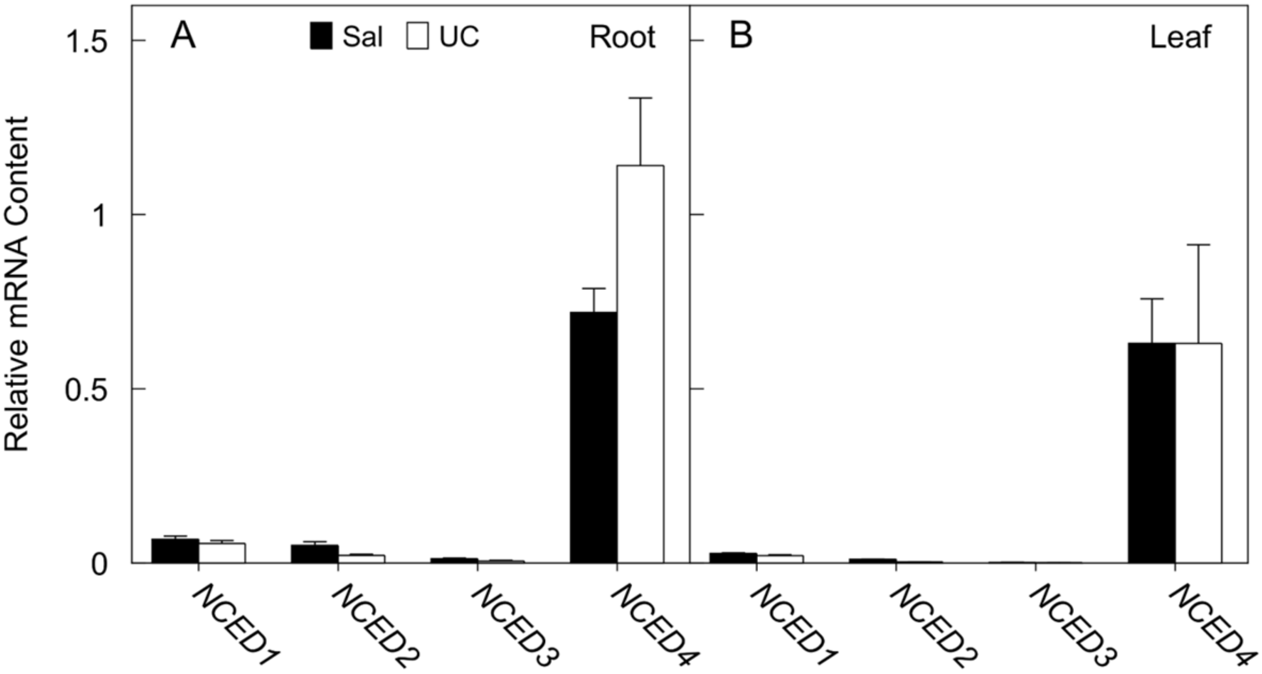
(B) Time courses of seed germination of UC, a control line (RNAi8-CTL) and three homozygous RNAi lines (RNAi8-3, RNAi8-4, RNAi8-7) at 35°C in the light. Error bars denote SE (n≥ 3).
(C) Germination of homozygous UC, RNAi control (RNAi8-CTL) and RNAi-*NCED4* (RNAi8-4) seeds in the presence of 200 μM paclobutrazol (PAC). Seeds were treated at 4°C for 3 d and then incubated at 31°C for 48 h in the light. Error bars denote SE (n≥ 3).



Supplemental Figure 6. Effect of *NCED4* Silencing on Inflorescence and Seed Development.

(A) Representative flower buds at six different developmental stages from RNAi8-4 (right in each panel) and RNAi8-CTL (left in each panel) plants are shown.

(B) Representative 9-days after flowering (DAF9) seeds and buds of RNAi control (left) and homozygous RNAi8-4 (right). Seeds from one bud are shown.



Supplemental Figure 7. Transcript Abundance of *NCED1-4* in Roots and Leaves of Sal and UC Plants.

Normalized mRNA contents of *NCED4* in roots **(A)** and leaves **(B)** of Sal and UC plants. Samples were collected from 5-week-old plants. Error bars denote SE (n= 3).

Supplemental Figure 8. Drought Tolerance Assay.

Water was withheld from five-week-old seedlings of DS, MIS1, RNAi-8, RNAi-2, RNAi-CTL (control line) for 10 days (upper panel) and the seedlings were then rewatered for 10 additional days (bottom panel). For each line, two rows of six seedlings were alternately planted in a 60-well tray. Three replicate trays were utilized for each treatment and a representative tray is shown. Watering and fertilization frequency and amount were automatically controlled. RNAi8 and RNAi2 seedlings were either heterozygous or homozygous for RNAi constructs based on germination following imbibition at 35°C for 24 h. No differences among genotypes were observed for either drought symptoms or recovery.

Supplemental Table 1. Primer Combinations Used for Isolation of Ls-*NCED4* and Its Promoters.

Primer ID	Forward primer	Reverse primer	Purpose in this study
328	AACCTCCCACCACTATCTTCAA	ATTTTCGTCATTTCACACCACA	Isolation of *NCED4* promoter
391	ATCGTATTTGTGTGGAGGAA		Isolation of *NCED4* promoter
331	TGCCAAATGTCAGGTTTTCA	TGATAATGGTTTACATCCAACAA	Isolation of *NCED4* promoter
332	TCGAATATAACATTACAACGTCAAC	TAGACGAATGTCCGCGTGT	Isolation of *NCED4* promoter
333	TTCAAACGATTGAGATTATGCAG	AAAGCCTTAAATCTTGTTCCAA	Isolation of *NCED4* promoter
334	AAGGCAACATGCATACTTTCG	CATGTAACTGATGGGCGTGT	Isolation of *NCED4* promoter
410	CACAAGGACAATCTATGAGG		Isolation of *NCED4* promoter
421	CCATGGACCACCATTAAAGCTATCAC		Isolation of *NCED4*
422		AGCTTAATTAGCCAAGTCATGGG	Isolation of *NCED4*
305	GCCGGCCGTATTTCCCTAAG	GAAACCATACGGCACTCTACCAG	Isolation of *NCED4*
241	TGATCCAGCGGTTCAGCTAA	TTCACCAATTACCTCCAGACCAT	Isolation of *NCED4* from BAC
449	ATGACTGGGCACAACAGACAAT	GATACCGTAAAGCACGAGGAAG	*npt*II-specific
450	ATGAAAAAGCCTGAACTCACCG	CTGCTCCATACAAGCCAACCAC	hygromycin-specific

Supplemental Table 2. Lettuce Genes and Primer Combinations Used for qRT-PCR.

Gene Name	Accession ID	Forward primer	Reverse primer	Arabidopsis homologue locus
18S1	DW138800	GCATGAGTGGTGTTTGGTTTGT	GAGGTAAGAACGCATGGAAGTTG	AT3G41768
UBQ	DW144476	TCTTAGATCACCGTCCCATCGT	TCTGAGATTGTCCGAGGATATGAG	AT5G25760
PP2A2	DW137232	TTGACGGAATCGGAGGTAAAA	CCGGCTGCACATTCCATT	AT1G10430
NCED4	AB120110	TGATCCAGCGGTTCAGCTAA	TTCACCAATTACCTCCAGACCAT	AT3G24220
NCED1	AB120107	CGCAATCACCGAGAACTTTGT	GGCGATCCTCCTTTTATCATTTC	AT1G30100
NCED2	AB120108	TCGACTCTTTGTGCACTTCATACTC	CAACGGGACAAGTTGAGGTTTTA	AT3G14440
NCED3	AB120109	AGCTCAGCTTGGTTCCCTGTTATA	CTTCACAAACTGGCTGAAAACGTAT	AT3G14440
ZEP1	AB120106	GGGCTACACTTGTTACACTGGGATA	GCCCCAAGAACACCCGATA	AT5G67030
ABA8ox4	AB235920	CCCTCAACCTCACAAATTCGA	CCATTCCCGAACGGCATATA	AT2G29090
ABI3	DY964887	ACGCAATGCTTGGTGATGAG	AAGGCGGAGATGGCCAGTA	AT3G24650
ABI5	BU007567	CGGACGGAAGCGGATAAT	TTCTAGCTCGAGACCTTGCAG	AT2G36270
SNF4	DY978381	GGATTGTATGGAGGTGTTTAGCAAA	CCGGATACGTTTTCCATGGA	AT1G15330
GA3ox1	AB012205	GGTGACCTCCTCCACATATTATCC	TGTTGGGTTCGGTTCACCAT	AT1G80340
GA20ox1	AB012203	GAAAGAGACGCTATCTTTCCGATT	GCCCATTGTGTTCTCGAAGTAGT	AT4G25420
GA2ox1	AB031206	TCGTTAACCATGGAGTTCCC	CTCAAGAATTTCACACGCCA	AT1G30040
RGL2	DY960761	CCAATCAAGCCATCCTCGAA	ATCCCTTGGTTCAAGCTGAAATC	AT3G03450
ACS1	AF380836	GACTGTGGCGTTTTGCTTAGCTA	AAGTCACGGTCAAATCCTGGATAAT	AT4G11280
ACO2	AB158346	AGCGGTCTTCAGCTTCTCAAAG	CGAGATTGATGACGATGGAATG	AT1G05010
LEA	DW129442	ACGTTAAAGGAGACGGAGGAA	CCTCACCACATCATCACGCT	AT2G36640
SPT	DW124777	GCGGATCTATACGGAACCAA	TGGAACCAAGGTCTGCAAAG	AT4G36930
PKL	DW131866	CAGCAGCAGCTTGTGATGAT	TCAACTGATTCAACCGCATT	AT2G25170
DOG1	DW045365	CCAAAAAAATCGTCTCCCACTT	CAAAAAGGAAGGCCCATCGT	AT5G45830

Supplemental Table 3. Accession Numbers for Sequences Used in This Study

Gene	**Accession ID**
Zm-*VP14*	AAB62181
At-*NCED1*	At3g63520
At-*NCED2*	At4g18350
At-*NCED3*	At3g14440
At-*NCED4*	At4g19170
At-*NCED5*	At1g30100
At-*NCED6*	At3g24220
At-*NCED9*	At1g78390
At-*CCD7*	At2g44990
At-*CCD8*	At4g32810
cv. Grand Rapids *NCED1*	BAE72090
cv. Grand Rapids *NCED2*	BAE72091
cv. Grand Rapids *NCED3*	BAE72092
cv. Grand Rapids *NCED4*	BAE72093
cvs. Sal and DS *NCED4*	JN788925
UC and W48 *NCED4*	JN788926
W46 *NCED4*	JN788927
W55 *NCED4*	JN788928
W51 *NCED4*	JN788929
cv. Diana BAC containing *NCED4*	KC676791