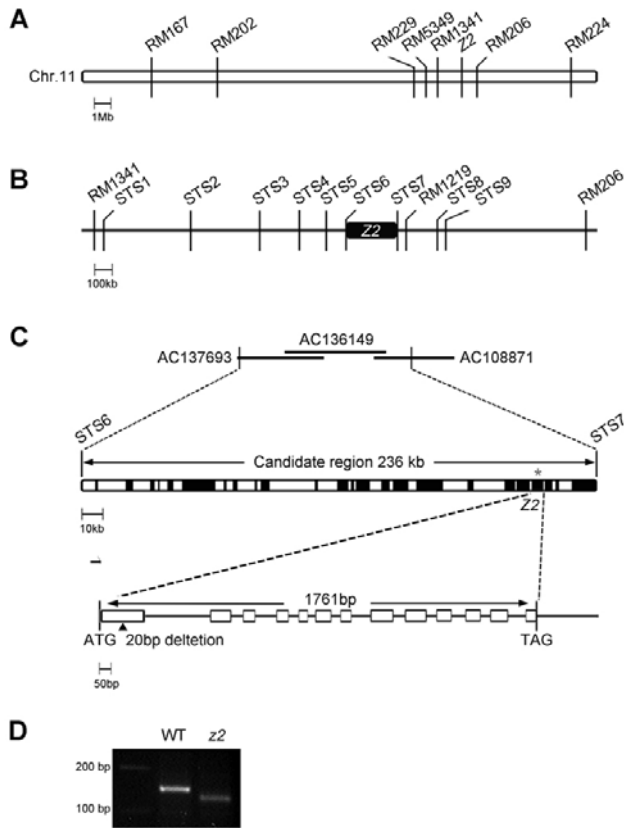


## Supplemental Data

**Table S1.** Primer information used in this study

A. The SSR and STS markers used for map-based cloning of the <i>zebra2</i> locus			
Locus	Clone	Forward primer (5' → 3')	Reverse primer(5' → 3')
RM167	AC135794	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
RM202	AC138197	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
RM229	AC150702	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT
RM5349	AC134925	AGGGCATGCTTACATCCAAC	CATTTGCTTCTATGCCCCAG
RM1341	AC134053	AACCTGGAGGTGCTGGTCTC	TTTCTCCCCCAACCAC
STS1	AC134624	ACTCGGAAACCTCAGTGTAAG	GACTTTCGCTTCTCTTCTG
STS2	AC134624	GGCAACAGTCGTAGAGCTTC	TATGCGTCGCATGCACAG
STS3	AC136905	GACTTTGACCTAGCTTTCTTG	GTGATTGATTAGCTGCAGTG
STS4	AC133291	GTCTGCATAAGCTGGAGTAC	GATAGACCAAGGTGCTCAAG
STS5	AC133291	TTCACAGGGACCACTACG	GGTCGCTCAACGAATCTG
STS6	AC109644	AGAACCATCAAACCTAGGG	TTCACAGGGACCACTACG
STS7	AC108871	ATGATGCTGTGTTCCGAG	GGTTCAGGGTAGGCATTAAG
RM1219	AC108871	GAGGAATGGAGGAGTTTGGG	CCGGCAAGGAAAAGGAAC
STS8	AC109929	GTGACAGTAGACACTGAAGC	CACAGAAGTGATAATCTCTACC
STS9	AC109929	GCCATTAAACACTGAACATC	CTTAGCTCGCAGACAACC
RM206	AC146334	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG
RM224	AC135190	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCCGGG
B. Primer sets for quantitative PCR			
Gene		Forward primer (5' → 3')	Reverse primer(5' → 3')
<i>JAmyb</i>		TTCGTTACGTCGGAGTCACAAGA	AAACTCATGGTCCACCTCCTGCAT
<i>MT2b</i>		CCTGCAACTGAATCTATCGTCGTCGT	AAGCTCTGATCGACAGTAGCAGCA
<i>OsACS6</i>		ACCTGATCGAGGAATGGAGCAAGA	TAAACTGGGCCATCGCCTTTCTGA
<i>OsLhca1</i>		GTCACCATGTCCGCCGAG	GACTCCTTGAACCGCTCGAAG
<i>OsLhcb1</i>		CCATGTTCTCCATGTTCCGGCTTCT	TAGGCCCAGGCGTTGTTGTTGA
<i>OsLhcb4</i>		TACCTGCAGTTCGAGCTGGAC	AGGCCGAACACCTCGGTGTA
<i>OsNAC4</i>		CGGAAATAGGAGTGATGGCTAGA	ACCACATTTGCAGAATCATGCT
<i>Os04g32480</i>		CTCGTGGCAATGCAATTGTAGGCA	ACCCAAGCTGGTCAACCTCTCTTT
<i>Os11g13370</i>		AAGCATCAGCAGCAGCAATGTCTC	TGCCAAGAGGATTTGGGAGACGAA



**Fig. S1.** Map-based cloning of the *z2* locus.

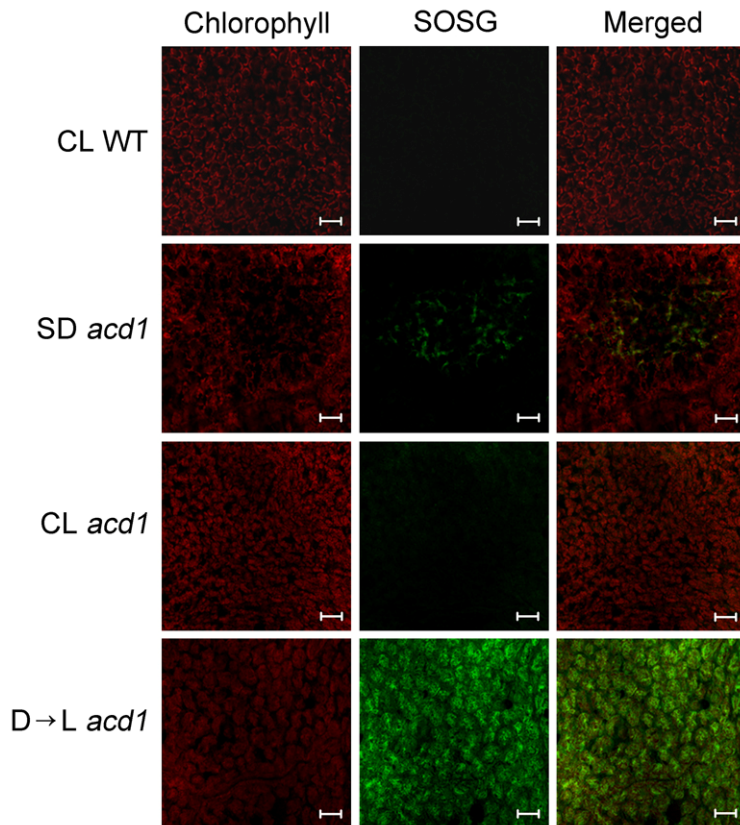
(A) Genetic mapping of the *z2* locus. The *z2* locus was initially mapped to a 2.2-Mb region between two SSR markers, RM1341 and RM206, on the long arm of chromosome 11. PCR-based SSR and STS marker primer information is listed in Table S1. (B) Physical mapping of the *z2* locus. The *z2* locus was further delimited to a 236-kb interval between STS6 and STS7. (C) Identification of *Z2* among candidate genes. The genomic structure of *OsCRTISO* encoding carotenoid isomerase (586 amino acids [aa]) is composed of 13 exons (white boxes) and 12 introns (lines). We found 20-bp deletion of exon 1 in *OsCRTISO* allele in *z2* mutant allele, leading to a frameshift mutation and premature translational termination (138 aa) containing another new open reading frame region (664-759bp, 31 aa) after the frameshift mutation. Chai et al. (2011) reported *zebra2-1* (*z2-1*) allele in which the *CRTISO* transcript is 24-bp shorter than that of the wild type due to an alteration in the splicing site. Thus, the *z2* mutants used in this study is *zebra2-2* (*z2-2*). (D) Genomic PCR analysis of the exon 1 of *Z2* allele in WT and *z2* plants, showing that the *z2* allele has a 20-bp deletion.

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O.sativa_ZEBRA2 1 -----NLLAAARCAPLAPASRG---AVVGRP
Z.mays_1 1 -----MPLAAGHMLLWPAASPR---PARRLCAA
Z.mays_2 1 -----MPLAAGHMLLWPAASPR---PARRLCAA
S.bicolor 1 -----MPLAAGHMLLWPAASPR---PARRLCAA
P.trichocarpa 1 -----MSLQFNKSLPFGQPTVQLGSSKRSOKHLSLSEPTVSTLVDGIVLRRKIKKI
A.thaliana 1 -----NLDLCPNVKCDRLFSALMTSTYKQKTSLSLQFN-----
S.lycopersicum 1 -----MSTLSPVYVSLDRCVYVAGSKPKYVQSSCFPEFIGNCTDQOQCGSLGVEV
D.carota 1 MSTLSPVYVSLDRCVYVAGSKPKYVQSSCFPEFIGNCTDQOQCGSLGVEV
          22
O.sativa_ZEBRA2 29 SLPDPLGQTRGAGAAAAAVALKETTVAKEEHEEGCGDAIVVGGSIGGVAATOLA
Z.mays_1 30 SLPDPLGQTRGAGAAAAAVALKETTVAKEEHEEGCGDAIVVGGSIGGVAATOLA
Z.mays_2 31 SLPDPLGQTRGAGAAAAAVALKETTVAKEEHEEGCGDAIVVGGSIGGVAATOLA
S.bicolor 30 GRRGQVGFPRGALSSKAAVAVAEKAGCGEGCGDAIVVGGSIGGVAATOLA
P.trichocarpa 56 DMLGQTRGAGAAAAAVALKETTVAKEEHEEGCGDAIVVGGSIGGVAATOLA
A.thaliana 39 LENDKHKKLRPMVAVKSSVSSVASTVQTKRDKGSSLDVAIVVGGSIGGVAATOLA
S.lycopersicum 59 DKAKGRKQVTVSVAIVVDKRRKSTYGS-SDVEKSSGDAIVVGGSIGGVAATOLA
D.carota 61 CVSRKRRKPRVYVSLDRCVYVAGSKPKYVQSSCFPEFIGNCTDQOQCGSLGVEV
          22
O.sativa_ZEBRA2 89 AKGARVVLVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
Z.mays_1 90 AKGARVVLVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
Z.mays_2 91 AKGARVVLVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
S.bicolor 90 AKGARVVLVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
P.trichocarpa 116 VKGAVVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
A.thaliana 98 VKGAVVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
S.lycopersicum 118 VKGAVVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
D.carota 118 VKGAVVLEKYIIPGSSGYTRDGFYFDVSSVMPFGSDKGNLMLTQALAVGQRME
          22
O.sativa_ZEBRA2 149 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
Z.mays_1 150 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
Z.mays_2 153 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
S.bicolor 150 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
P.trichocarpa 176 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
A.thaliana 158 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
S.lycopersicum 178 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
D.carota 178 VLPDPSVTFHFLPDLVSVVHREDFPTELSKPPHEKEGIFRFYQCMKIFNLSLSL
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Z.mays_1 210 LKSLSEPIFLGQFPQKPLECLTAYLFPNAGDIARKFKDQGLLSPIDAEFCFVSTVN
Z.mays_2 213 LKSLSEPIFLGQFPQKPLECLTAYLFPNAGDIARKFKDQGLLSPIDAEFCFVSTVN
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P.trichocarpa 236 LKSLSEPIFLGQFPQKPLECLTAYLFPNAGDIARKFKDQGLLSPIDAEFCFVSTVN
A.thaliana 218 LKSLSEPIFLGQFPQKPLECLTAYLFPNAGDIARKFKDQGLLSPIDAEFCFVSTVN
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D.carota 238 LKSLSEPIFLGQFPQKPLECLTAYLFPNAGDIARKFKDQGLLSPIDAEFCFVSTVN
          22
O.sativa_ZEBRA2 269 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
Z.mays_1 270 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
Z.mays_2 271 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
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P.trichocarpa 296 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
A.thaliana 278 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
S.lycopersicum 298 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
D.carota 298 ALQPFNINSMVLCDRPFGIINYVGGVGGIAGSLADGLVKGSEIRKANVNIILENG
          22
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Z.mays_1 330 KAVGVLSHGKREFFAKTISNATRNDTFGKLLGDELPDEEFQPKNIVKAPSFSLIHMG
Z.mays_2 333 KAVGVLSHGKREFFAKTISNATRNDTFGKLLGDELPDEEFQPKNIVKAPSFSLIHMG
S.bicolor 330 KAVGVLSHGKREFFAKTISNATRNDTFGKLLGDELPDEEFQPKNIVKAPSFSLIHMG
P.trichocarpa 356 KAVGVLSHGKREFFAKTISNATRNDTFGKLLGDELPDEEFQPKNIVKAPSFSLIHMG
A.thaliana 338 KAVGVLSHGKREFFAKTISNATRNDTFGKLLGDELPDEEFQPKNIVKAPSFSLIHMG
S.lycopersicum 358 KAVGVLSHGKREFFAKTISNATRNDTFGKLLGDELPDEEFQPKNIVKAPSFSLIHMG
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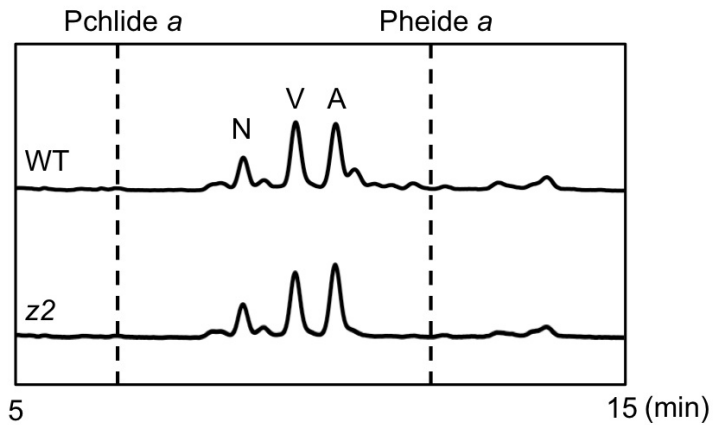
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**Fig. S2.** Protein sequence alignments of carotenoid isomerase (CRTISO) in plants. ClustalW alignment of homologs of CRTISO (<http://ch.embnet.org/software/ClustalW.html>) was performed. All protein sequences were obtained using the NCBI-BLASTP program ([http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST\\_PROGRAMS=blastp&PAGE\\_TYPE=BlastSearch&SHOW\\_DEFAULTS=on&LINK\\_LOC=blasthome](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome)). Z.mays\_1, Z.mays\_2, S.bicolor, P.trichocarpa, A.thaliana, S.lycopersicum, and D. carota have 86, 87, 89, 79, 77, 85, and 81% sequence similarity to OsZ2, respectively. The location of the mutation in the *Arabidopsis* carotenoid isomerase mutant *ccr2-1* is indicated (red arrow head). O.sativa\_ZEBRA2 (rice, *Oryza sativa* ZEBRA2, *Os11g36440*); Z.mays\_1 (*Zea mays* carotenoid isomerase 1, ACO71189); Z.mays\_2 (*Zea mays* carotenoid isomerase 2, NP\_001148055); S.bicolor (*Sorghum bicolor*, XP\_002449729); P.trichocarpa (*Populus trichocarpa*, XP\_002323362); A.thaliana (*Arabidopsis thaliana* carotenoid isomerase, At1g06820); S.lycopersicum (*Solanum lycopersicum* carotenoid isomerase, Q8S4R4); D.carota (*Daucus carota* carotenoid isomerase, Q2VEX9).



**Fig. S3.** Singlet oxygen ( $^1\text{O}_2$ ) accumulation in the *Arabidopsis accelerated cell death 1 (acd1)* mutant.

$^1\text{O}_2$  was examined by SOSG fluorescence in the leaves of *acd1* mutants grown under SD, CL, and D→L conditions. D→L: the SD-grown WT (Columbia) and *acd1* plants were incubated in the dark for 4 days and then exposed to light ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 3 hours. Red chlorophyll autofluorescence (left), green SOSG fluorescence (middle), and merged images (right) are shown. SOSG fluorescence was observed at 520 nm and chlorophyll autofluorescence at 680 nm using laser scanning confocal microscopy. Scale bar = 50  $\mu\text{m}$ .



**Fig. S4.** Reverse-phase HPLC analysis of chlorophyll and carotenoid intermediates in WT and z2 leaves.

Plants were grown under SD conditions. They were transferred into darkness at ZT-0, incubated for 3 days, and sampled under darkness. Chlorophyll intermediates, Pchl *a* and Pheide *a*, were not detected, indicating normal function of chlorophyll catabolism in z2 mutants. Pchl *a*, protochlorophyllide *a*; Pheide *a*, pheophorbide *a*; N, neoxanthin; V, violaxanthin; A, antheraxanthin.

ACS6 1 M V A F A T E K K Q D L N L L S K I A S G D G H G E N S S Y F D G W K A Y E E N P F H P I D R P D G V I Q M G L A E N Q  
 OsACS6 1 - - - - - M A Y Q G I D L L S T K A A G D D H G E N S S Y F D G W K A Y D T N P F D L R H N R G G V I Q M G L A E N Q

ACS6 61 L C G D L M R K W V L K H P E A S I C T S E G V N Q F S D I A I F Q D Y H G L P E F R Q A V A K F M E K T R N N K V K F  
 OsACS6 55 L S L D L I E E W S K N H P E A S I C T P E G V S Q F K R I A N F Q D Y H G L P E F R K A M A Q F M G Q V R G G K A T F

ACS6 121 D P D R I V M S G G A T G A H E T V A F C L A N P G D G F L V P T P Y Y P G F D R D L R W R T G V N L V P V T C H S S N  
 OsACS6 115 D P D R V V M S G G A T G A Q E T L A F C L A N P G E A F L V P T P Y Y P A F D R D C C W R S G I K L L P I E C H S F N

ACS6 181 G F K I T V E A L E A A Y E N A R K S N I P V K G L L V T N P S N P L G T T L D R E C L K S L V N F T N D K G I H L I A  
 OsACS6 175 D F R L T K E A L V S A Y D G A R R Q G T S V K G I L I T N P S N P L G T I T D R D T L A M L A T E A T E H R V H L V C

ACS6 241 D E I Y A A T T F G Q S E F I S V A E V I E - E I E D C N R D L I H I V Y S L S K D M G L P G L R V G I V Y S Y N D R V  
 OsACS6 235 D E I Y A G S V F A T P E Y V S I A E V I E R D V P W C N R D L I H V V Y S L S K D F G L P G F R V G I V Y S Y N D A V

ACS6 300 V Q I A R K M S S F G L V S S Q T O H L I A K M L S D E E F V D E F I R E S K L R L A A R H A E I T T G L D G L G I G W  
 OsACS6 295 V A A A R R M S S F G L V S S Q T Q Y F L A R M L S D E E F I G R F L Q E S K C R L V A R H E R F T S G L R E V G I G C

ACS6 360 L K A K A G L F L W M D L R N L L K T A T F D S E T E L W R V I V H Q V K L N V S P G G S F H C H E P G W F R V C F A N  
 OsACS6 355 L R G N A G L F S W M D L R R M L R E K T A E A E L E L W R V I V H Q V K L N V S P G T S F H C R E P G W F R V C H A N

ACS6 420 M D H K T M E T A L E R I R V F T S Q L E E E T K P M A A T T M M A K K K K K C W Q S N L R L S F S D T R R F D D G F F  
 OsACS6 415 M D D E T M E V A L G R I H D F V R Q H Q O R - - - - - R V K A E R W A A N R Q L R L S L P H H H H L S P A H L S S

ACS6 480 S P H S P V P P S P L V R A Q T  
 OsACS6 468 P L A L L S P Q S P M V R A T S

**Fig. S5.** Comparison of amino acid sequences of the *Arabidopsis* and rice ACS6 homologs. Identical amino acids are shaded in black, and the amino acids that have similar characteristics are shaded in gray. The *Arabidopsis* ACS6 (At4g11280) and its rice homolog (OsACS6; LOC\_Os4g48850) are aligned.