

Supplemental Data. Zeng and Cheng (2014). Plant Cell 10.1105/tpc.114.126631

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*      20      *      40      *      60      *      80
FAE1-E1 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKASRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIVTRPKPVYLVE : 82
FAE1-E2 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKASRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIVTRPKPVYLVE : 82
FAE1-E3 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKASRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIVTRPKPVYLVE : 82
CAD90159 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKAYRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIATRBPVYLVE : 82
AF490461 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKAYRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIATRBPVYLVE : 82
AF490460 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKAYRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIATRBPVYLVE : 82
ACB55612 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKAYRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIATRBPVYLVE : 82
AF274750 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKAYRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIATRBPVYLVE : 82
NP_195178 : MTSVNVKLLYHYVITNLFNLCFFPLTAIVAGKASRLTIDDLHLLYYSYLOHNLTIAELFAFTVFGSVLYIVTRBPVYLVD : 81
    
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*      100      *      120      *      140      *      160
FAE1-E1 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
FAE1-E2 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
FAE1-E3 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
CAD90159 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
AF490461 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
AF490460 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
ACB55612 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
AF274750 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
NP_195178 : YSCYLPPTHCRSSISKVMDIFYQVRKADPS-RNGTCDSSWLEFLRKIQERSGLGDETHGPEGLLOVPPPKTFAAAREBTEQ : 163
    
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*      180      *      200      *      220      *      240
FAE1-E1 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
FAE1-E2 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
FAE1-E3 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
CAD90159 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
AF490461 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
AF490460 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
ACB55612 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
AF274750 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
NP_195178 : VIIGALENLFKNTKVNPKBEIGILVVNSSMENPTPSLSAMVVNTFKLRSNVRSFNLGGMGCSAGVIAIDLAKDLLHVHKNTYA : 245
    
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*      260      *      280      *      300      *      320
FAE1-E1 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
FAE1-E2 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
FAE1-E3 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
CAD90159 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
AF490461 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
AF490460 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
ACB55612 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
AF274750 : LVVSTENITYNIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
NP_195178 : LVVSTENITQCIYAGDNRSMVSNCLFRVGGAAILLSNKPERDRRSKYELVHTVRTHTGADDKSFRVQVQGDDBSGKIGVSL : 327
    
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*      340      *      360      *      380      *      400
FAE1-E1 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFIAKKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
FAE1-E2 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFIAKKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
FAE1-E3 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFIAKKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
CAD90159 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFMGKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
AF490461 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFMGKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
AF490460 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFMGKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
ACB55612 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFMGKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
AF274750 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFMGKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
NP_195178 : SKDITDVAGRTVKKNIATLGLPLILPLSEKLLFFVTFMGKLEKDKVKHYYVPDFKLAIDHFCIHAGGRAVIDVLEKNLALAP : 409
    
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          420      *      440      *      460      *      480      *
FAE1-E1 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALSNVKASTNSPWEHCIDRYPVQID : 491
FAE1-E2 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALSNVKASTNSPWEHCIDRYPVQID : 491
FAE1-E3 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALSNVKASTNSPWEHCIDRYPVQID : 491
CAD90159 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALNNVKASTNSPWEHCIDRYPVKID : 491
AF490461 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALNNVKASTNSPWEHCIDRYPVKID : 491
AF490460 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALNNVKASTNSPWEHCIDRYPVKID : 491
ACB55612 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALNNVKASTNSPWEHCIDRYPVKID : 491
AF274750 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALNNVKASTNSPWEHCIDRYPVKID : 491
NP_195178 : IDVEASRSTLHRFGNTSSSSIWYELAYIEAKGRMKKGNKVVQIALGSGFKCNSAVWVALNNVKASTNSPWEHCIDRYPVKID : 491

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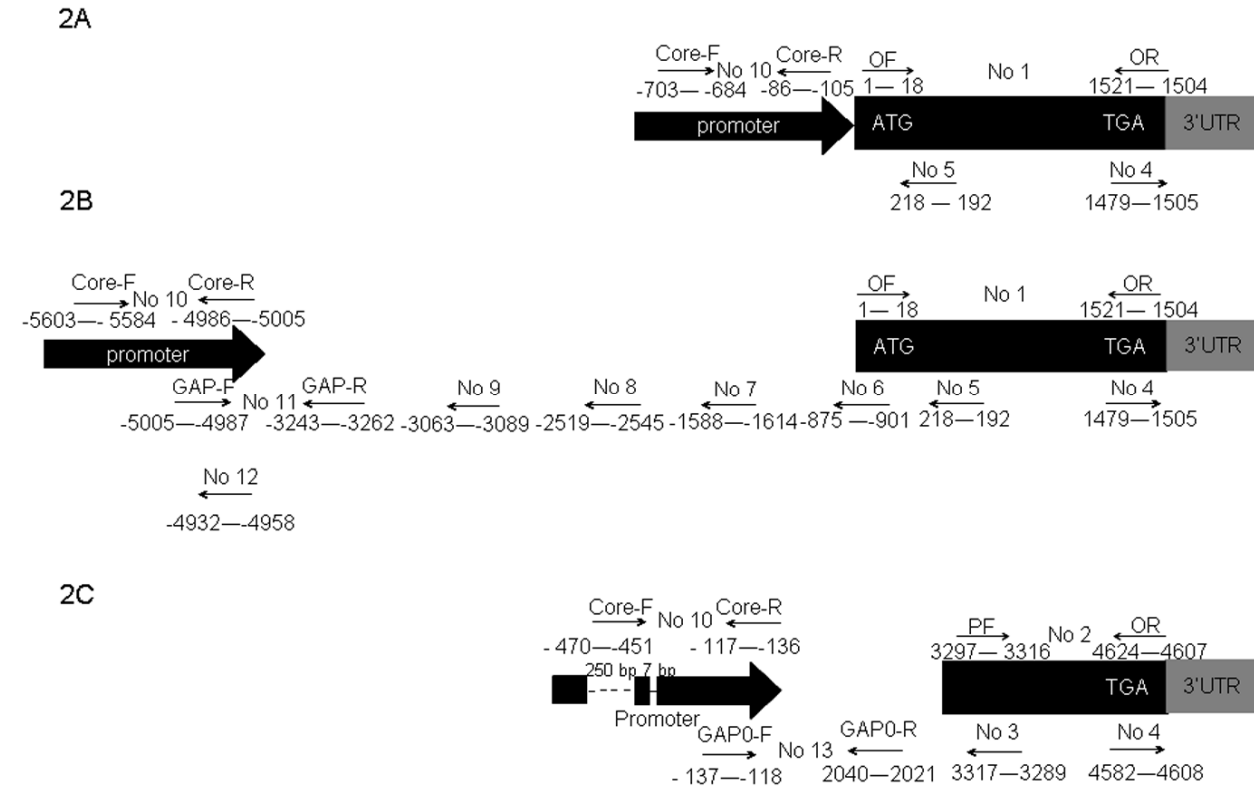
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          500
FAE1-E1 : SDSEAKSETRVQNGRS : 506
FAE1-E2 : SDSEAKSETRVQNGRS : 506
FAE1-E3 : SDSEAKSETRVQNGRS : 506
CAD90159 : SDSEKSETRVQNGRS : 506
AF490461 : SDSEKSETRVQNGRS : 506
AF490460 : SDSEKSETRVQNGRS : 506
ACB55612 : SDSEKSETRVQNGRS : 506
AF274750 : SDSEKSETRVQNGRS : 506
NP_195178 : SDSEKSETRVQNGRS : 506

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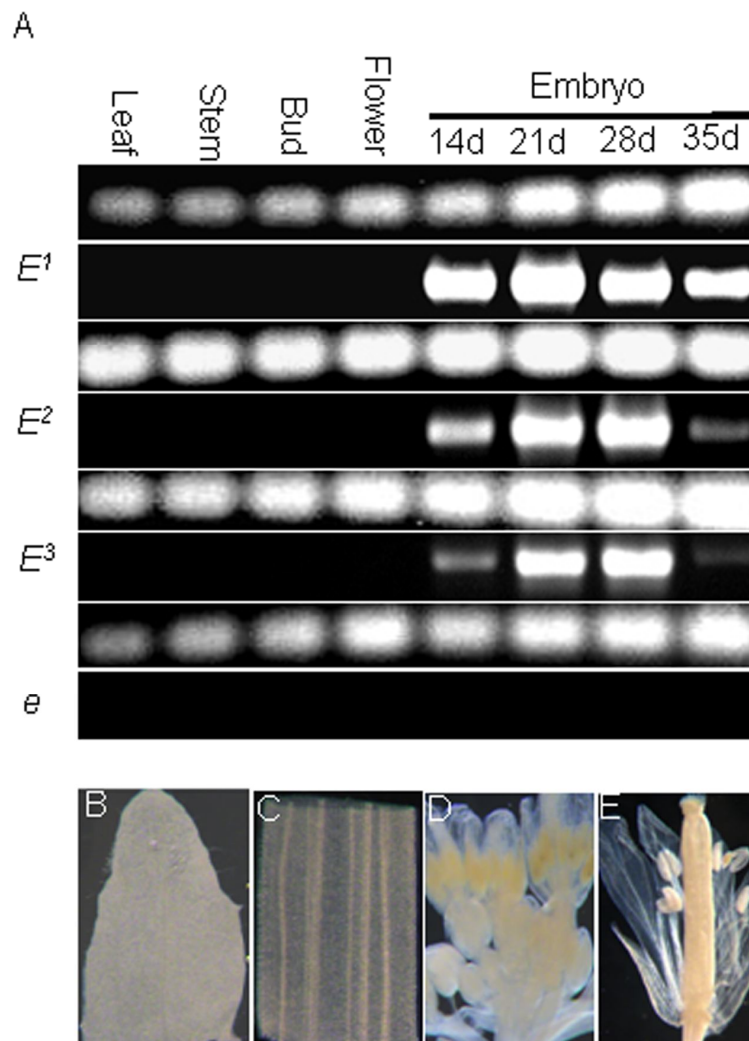
Supplemental Figure 1. Alignment of amino acid sequences encoded by the *FAE1* alleles E^1 , E^2 and E^3 of yellow mustard, CAD90159 of *B. juncea*, AF490461 of *B. rapa*, AF490460 of *B. oleracea*, ACB55612 and AF274750 of *B. napus* and NP_195178 of *Arabidopsis*.

The amino acid sequence alignment was carried out using the ClustalW program (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Conserved cysteine and histidine residues are indicated by a diamond and a triangle, respectively.



Supplemental Figure 2. Schematic diagram showing the position of each primer used for cloning of the *FAE1* alleles E^1 , E^2 , E^3 and e .

(A) Primers used for cloning the *FAE1* allele E^1 . **(B)** Primers used for cloning the *FAE1* allele E^2 and E^3 . **(C)** Primers used for cloning the *FAE1* allele e . Arrows indicated the direction of the primers. The number under each arrow indicates its nucleotide position. The “A” of translation start codon ATG represents the first nucleotide.



Supplemental Figure 3. Expression pattern of the *FAE1* alleles E^1 , E^2 and E^3 .

(A) The *FAE1* alleles E^1 , E^2 and E^3 were expressed in 14-35d old embryos, but not in the vegetative tissues of leaf, stem, bud and flower as revealed by RT-PCR. The *FAE1* allele e was not expressed in the embryo or vegetative tissues. Actin (FG576123) was used as an internal control. (B-E) GUS activity was not detected in leaf (B), stem (C), bud (D) or flower (E) of transgenic *Arabidopsis* plants containing the construct pBI101 Pro E^1 -GUS with the promoter of the *FAE1* allele E^1 .

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TATTACGATATATGTTATATCGTATATTCTATGCACTCGATAATATTAACCTTCCTATT
GTTTAGCTAGAGATAAGTATAGTTTCCTATTTATCGACAGAAAGGTTTATACTAAAC
CGTCATAGAAGCTTGTATATAATGCAAAGTTAGCATAACTTAATAAACAAGTCAGTTT
GATATCTTATTCTACA**TGGTATCAGAGCTGA**GATCTCTTTGATTCTCTTGCTTAAAAT

PBS

CGAGTTCTTGTTCAAAGTTTGAACAAATTGTCTATCGTCGGTGACG**ATG**TCTGTCTGA
AAGTTCTGAAGTTAAGAACACACCCGAGAAGAGTACGAGAGATGTATCGTCTCTTC
CATCCCCGTACATCTTGTATGGTCCGATAATCCCGGAGCCATGATTACTCCAGTC
ATGTTGAACGGAGAGAAGTACAATCAATGGGCTAACGAGATGCTTAATGCGCTTCA
AGCCAAGCGCAAGGTCTGCTTCATCAACGAACTTTGAAGAAACCTGCAACAGAC
GATCCAGACTATGATAATTGGGTAGCAGTGAATTCTATGATTATCGGTTGGATACG
AGCATCTATTGATCCAAAGATAAAGGCGTCTGTGACGTTTGTAAAGCGAGGCGAGTT
TGTTGTGGACGGATTGAAGCAGCGGTTCTCGGTTGGGAACAAAGTTCGTATTCAT
CAAATCAAAGCACAGCTGGCAGCCTGTCGACAGGAAGGTCAAAGTGTGCTTGAAT
ACTATGGgAAAGTTGTGCACTCATTGGGAAGAACTCGACGTCTATCGACCTCTGCC
AATGTGTACGTGgTgGGGGCTGCTAAAGATATCAGAAAGGAAAGAGACGATGACAA
GGTCCACCAGTTCATAaTGGGTCTTGATGACTCACGTTTGGGAGTCTTTGTACGT
CTCTAATCGGCATGGATCCCCTGCCATCGATCGGAGAAGTCTACTCCAAGGTAGTT
CAAGAAGAACAAGGTTGAGTAcTTCACGAAACAGAGAACAGCAGCAAGAGGTG
ATAGGTTTTCGTTGCTCGCAGTGACAATCAAATCAAGAAACATCAATTCTCCGAAAC
AGAGATCGTGTGACAATTTGTTCACTGTGGACGTCGAGGTCATGATAAGAAGGA
TTGCTGGCAGATCATTGGCTTTCCGGAGGGGTGGAATGAACGTACGGACCGTGGC
GGTTCTGTTCTGTTGGCAGAGGTCGCGGTGGACGCAACACAGGCACAGTTGGACGC
GGCAGAGGCCAAGTAGCAGCAGCACACGCCACCAGCTCCAACCTTTCTGCCTTTC
CAGATTTTACACCGGACCAGTGGAAGTTCTGACTCAACTGATACAAGAGAAATCA
GGTTCAGACAAGCTATCTGGTAAGGACAAATATGGCGACGTTATTTTCGATACTGG
GGCTTCTCACCACATGACCGGGAAGCTTTCTCTTTGAAAAATCTTGTCTCCATACC
ACCTTGTTCCGTGGGTTTCGCAGATGGGAGTCGAACATTTGCCTTGAGTATGGGC
GATCTTCCACTGTCAGGAAGAGTGTGCTTAACATAATGTTCTATATGTGCCATCCTTG
AACTGCACTTTGATATCGGTTGCGAAGTTTTTAAACAACAAGCTGTCTTGCAACC
TTTACTGATGCTGTTTGTATTTTACAGGACCGTTTCTCGAGGACTCTGATTGGAACC
GGTGAAGAGCGTGGTGGGTTTACTACTTAACAGATGTGGCTACAGCAAAGATAC
ACACAGTTGACGCATCTTCTGACCAATCACTGTGGCATCAGCGTTTAGGGCATCCT
AGTTTTTCTGTGCTTTCGTCTTACCTTTGAGTTTTTCTATCAATAAAAGCTCGCGTT
CTTGACGTATGTTTTCGAGCTAAACAAGCTCGTGAGATTTTTCCAGACAGTATTA

ATAAATCAGATGATTGTTTTCTCTAATTCATGTCGATGTCTGGGGTCCATACCGTG
TACCTTCTTCTTGCGGTGCAGTATATTTCTCACGATTGTAGATGATTACTCCAGAT
CAGTTTGACTTACCTAATGTTGGAGAAATCAGAAGTTAARACCATTCTTAAAACT
TCATTGCATACGCAGAAAAACAGTTTGGCAAAGAAGTTAAGATGGTCCGAAGCGAC
AATGGCACAGAggcATTTATGTGtTCTCTCTCAGTATTTTCGTGAAATCGGTGTCATT
CAiTCAGACCTCATGTGTTGGCACTCCGCAGCAGAATGGTCGTGTCGAGCGCcAAG
CACAGGCATATTCTTAACGTGGCACGTgGCGTTACTGTTTCAGGCAAGCTTACCGA
TAAGCTTTTGGGGTGAAGCTATTTTAACAGCAGCGTATCTTATAAATCGGACACCTA
CATCACTTCACAACGGTCGTTACCCTATGAAGTTCTTCACGGGTCTAAACCAGA
TTACACACAGCTACGTGTCTTTGGgTTCGGCTTGCTATACTCATCGAGTCACAAGAA
GCAAAGACAAGTTTGGTGAGAGAAGTCGCCTTTGTGTGTTTGTGGATATCCTTTT
GGGAAAAGGGATAaCAAAGTTTATGATATGGAACGAGGGGAGTTCATTACTTCTC
GTGATGTTGTGTTTCGAGAGGATGgTGTTCATATGCTACAAAATCTGTACCGGCT
ACCACAACAATCCCGGTGGTAGCAAGCGATGgATGAGGACTgGGAGTTTACTGTT
CCTCCAGTTCTGGTGGACTTGATCGATAGGGGGAGCTTGGCTGGTGTACCCCTA
CAACTGATTCACCCGTTAACACAGCCAGTGGCAGTGAGAATTTATCACCTTTAGCC
GACGAGAGTGATGATATTGATGAAGAAGTYACAACAGGAGCAGCTGATTTAACAGA
TGATACGAGTGGTTCAGAAACAGTAAACGAAGCTCTTGGTCGTGGTAGAAGACAC
GCGGTTCCCTCTGTAAAGCTCAAGGACTATGTCACCTACAATGCAGAAGCACACGC
CTTAAGTACCCACCACGCTCACACCATCTCTGTTCCCTCAGTCCTCGTCTTCGGTCC
AAGGTAACACACTCTACCCTTTGACAAAATTCGTGTGTGACTCTAATTTTTCTCCAC
AGCAGCAAGCTTTCTTAGCTGCAATTACAGCCGGCGTTGAACCCAAGCATTTTAAG
GAAGCTGTTGGGATTGATGTGTGGGATAATTCTATGGTCGACGAGATTGTTGCTCT
TGAAGGACAACATACGTGGGATATTTGTGATCTTCCTCTCAACAAAATGCGCTTG
GAAGCCAATGGGTCTATAAAATCAAATATAACTGACGGTACTATTAGACGTCATA
AGTCTCGCGTTGTTGTCATGGGAAATAACAAGTAGAAGGAGAAGATTATAACGAA
ACCTTTGCTCCAGTGGTTAAGATGACGACCGTCCGTATGTTTCTGCGTCTAGTCGC
AGCCAATCAATGGGAAGTGTTTCAGATGGATGTCAACAACGCATTTCTCCATGGTG
ATCTCGACGAGGAAGTATATATGAAACTCCCACCTGGGTTTCGCCACTCTCATCCA
AATAAAGTTTGTGACTCCGGAAAAGTTTGTATGGGCTCAAGCAAGCTCCACGATG
TTGGTTTAAAAGCTCTCTGACGCCCTGCTCAAGTTCGGTTTCTGTGAGTCCTACG
ATGACTACTCATTATTCTCATACACTCGTAAAGGCATTGAGCTCCATGTGCTGATTT
ATGTTGATGATCTGCTCATCAGTGGTAATGATAGACACATGGTACAAAGGTTCAA
GACTACTTGGGAAAATGTTTTCTATGAAGGATCTTGGCAAACCTCAAGTATTTTCTA
GGCATTGAGGTCAGTCGAGGGCCAGAAGGAATATTTCTCTCTCAAAGAAAGTATGC
CTTAGACATTATAGCTGAAACAGGAAATCTCGGTTGCAGACCGGCTGCTACTCCGC
TCGAACAAAACCATCAACTTGGCAAAGTCGAGAGTCCAGTGCTTGGAGACCCCA
CAAGTATCGGAGGCTCGTAGGTCGTCTGCTCTATCTTGTTACACACCAGACCTGAGT
TAAGTTATTCGGgTTCATGTTTTGTACAGTATATGCAGACTCCGAAAGAAGCTCAC
TGGGATGCAGCTCAACGAGTTGTGCgGGTTTTTAAAAGGCTCACCTGGACAAGGC

ATTATGTTAAGTTCGTCTACTGACTTGTCTTTAACAATTTACTGCGATTCTGATTGGT
 CTTCTGTCCCTCCACTCGCCGGTCACTCAGTGCGTTTGTAGCCATGCTTGGAGAC
 TCGCCTATCAGTTGGAAAACGAAGAAGCAGGACACAGTATCTCACTCTTCCGCAGA
 AGCCGAGTATCGAGCTATGTCTGATGCTCTTAAAGAGGTTAAATGGTTGCGTAAGT
 TGTTACATGGATTGATATCAAACAAGTCTCTACCCGCTTCTTCTGTGACAGCAAAG
 CAGCAATTTACATCGCCACTAACCCTGTCTTCCATGAACGTAATAACATGTTGAAA
 ATGACTGTCATGCGGTTTCGTGATGCTGTTTCGAGATGGTCTCATTATTCTTCATCACA
 TACGAACAAATGAGCAGATTGCGGACATTCTGACAAAGGCATTGGGACGAGCTCA
 GTTCACTACTCTACTGTCCAAGTTGGGCGTTTGTGATCTTCACGCTCCAACGTGAG
GGGGAGTATTACGATATATGTTATATCGTATATTCTATGCACTCGATAATATTAACTT
 PPT
 TCCTATTGTTTAGCTAGAGATAAGTATAGTTTCCTATTTATCGACAGAAAGGTTTATA
 CTAACCCTCATAGAACTTGTATATAATGCAAAGTTAGCATAACTTAATAACAAGT
 CAGTTGATATCTTATTCTACA

Supplemental Figure 4. DNA sequence of the retrotransposable element Sal-*T1* inserted in the 5' UTR of the *FAE1* alleles *E*² and *E*³.

The long terminal repeats (LTR) was highlighted in red. The motifs representing the primer binding site (PBS) and polypurine tract (PPT) are underlined, and highlighted in green and pink, respectively. Sequence annotation of the transposable element Sal-*T1* was predicted using GenScan at GeniusNet (<http://genome.dkfz-heidelberg.de/cgi-in/GENSCAN/genscan.cgi>). The start codon (ATG) and stop codon (TGA) are in red type. The open reading frame is highlighted in grey.

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MSVESSEVKNTPEKSTRDVSSLPS **PYILYGSDNPGAMITPVMLNGENYNQWANEMLN**
ALQAKRKVCFINETLKKPATDDPDYDNWVAVNSMIIGWIRASIDPKIKASVTFVSEASLL
WTDLKQRFSVGNKVRHQIKAQLAACRQEGQSVLEYYGKVVHSLGRTRRLSTSANVY
VVGAAKDIRKERDDDKVHQFIMGLDDSRFGSLCTSLIGMDPLPSIGEVYSKVVQEEQR
LSTFTKQRTAARDFTPQWKVLTQLIQEKSGSDKLSGPFLESDWNRLHTATCLWVR
LAILIESQEAKTSLVREVAFVCLLDILLGKRDNKVYDMERGEFITSRDVVFREDGVSICY
KICTGYHNNPGGSKRWMRTGSFTVPPVLVDLIDRGSAGVTPTTDSPVNTASGSENLS
PLADESDDIDEVTTGAADLTDDTSGSETVNEALGRGRRHAVPSVKLKDYVTYNAEAH
ALSTHHAHTISVPQSSSSVQGNTLYPLTKFVCDNFSPQQQAFLAAITAGVEPKHFKEA
VGIDVWDNSMVDEIVALEGQHTWDIC **DLPLNKTALGSQWVYKIKYNTDGTIRRHKSRV**
VVMGNKQVEGEDYNETFAPVVKMTTVMFLRLVAANQWEVFQMDVNNAFLHGDLDE
EYMKLPPGFRHSHPNKVCRLRKSLYGLKQAPRCWFKKLSDALLKFGFCQSYDDYSL
FSYTRKGIQLHVLIYVDDLLISGNDRHMVQRFKDYLGKCFSMKDLGKLYFLGIEVSRG
PEGIFLSQRNICRLRKKLTGMQLNELCGFLKGSPGQGIMLSSSTDL **SLTIYCDSDWSSC**
PSTRRSLSAFVAMLGDSPISWTKKQDTVSHSSAEAEYRAMSDALKEVKWLRKLLHG
FDIKQVSTRFFCDSKAAIYIATNPVHERTKHVENDCHAVRDAVRDGLIILHHIRTNEQIA
DILTKALGRAQFTTLLSKLGVCDLHAPT

Supplemental Figure 5. The 959 amino acids of the deduced protein of the retrotransposable element Sal-71 predicted using GenScan at GeniusNet (<http://genome.dkfz-heidelberg.de/cgi-in/GENSCAN/genscan.cgi>).

The putative locations of gag integrase, reverse transcriptase and RNase H are highlighted in green, yellow, and red, respectively.

GAGCACCTCATCGCTGGTGCTTAGGGGATTGCTTAACCTAATTTTGTGTTTAAAT
TAAATAAAAAGCCAATTAACCTTAAGCTTCGTTGCCTAATTAAGCGCATGAAGCAA
CCGTGTTTTTGGACACGTGTCATGACGAGAGACGAGAGAGGAGGGTGAAGTCGAC
TCGTCTCcATTCTGctTTCgtTctTTCTCTCTCTCTCCCGTCTTCTCTCCCACGAAGG
CGATGTGTCTCTC**TCACCGTGGCTCGTCGACGgAGACGGCGACCGGTCA**tTCTGTC
TCGGTGTGGTTCGTCGACGACATGGTCGATGA**tGTCTT**CCTCACTGTGGGTCTCAT
CGTGGGCGGTGGACCGGTAAATCGTTGTTGCCTTTCCGCGAATCTGAAGGTAAAG
ATGATTTTGTTCGGTTTTATCATGCATGTCTTAGATTAGTGTGGTCTTGGTCTA
ATCGATGTTTTTTTTCTTTCTCTGTTTCGATGATGTCTCTCACAACGGCGAAGGA
GGAGCTCGATGATGTCTCTCACAACGGCGAAGGAGGAGCTCGATGATGGTGGGT
CTCCGGTGTCTCGTATCGGTCTGGTAAAGCTTCGTCTTTACTTCTTTCACTTTG
ATTCTTCTTTCAATTATTTGTTAAATCGATGTATATTCTTTTATGTTGATTCTTTG
ATTTGTTTCTGACTTTTTGTGGTGAGCTGGAGTGATCAAATCGCTGTCTCTTGTTC
GGTGTTAAGAGACGTCAGGAAGGTTCA**TCGAACAGAGGAGTTCAATAAGTTCACA**
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GTTTGAATTGAATTAGCTTCTGGTAAGAATAGAATTAGCTTCTGGTTATGTATGAA
ATTAGCTTCTGCTTGTGTATGAAATTAATAGAACTTGTGTTGGTTGTGTGATGAAT
GCATTGGTTAAATGGATGTCATGTCGTTAGGTTGTTGGTTGTGTGATGAATGCATA
GGTAGTTGTGTGATGAATGTTTCATCTTAAATGGATG**wCAAGTCCTTCATCTCTTC**
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TCTTTACTTCTTCTATCTTTTCTTCTTCTCTCCCTTCCCATTCTCCTCAAATGGATT
CTACTCcCcATTGCGTACAATGCAAATTTGTTGAGCTTCTTAATAGCCAACAAGATA
GTGATTTTCGTTAGTAGAAGCTACTGTCGAACCAAGTTCATGAGCAGTGTCTGG
CACCTTTTGAAGGTGATTTTGTTCCTTTGTGTCAAGGT**CAT**GTCATGTATTTTGT
GAGTCACTTGGAGTCACTTGGAGTCAGTTGGAGTCATGTGTTTTGATCAATTGTGT
CACGGACTGTCCTTGGAGTCCCTTCTCCTTATGTAATTTGYAATCTAATTTGTTTGT
TAATATTGTGTCACGGATTTAGTTGTTATGTATGTTGATGTACTATAAATTATTATC
AACTTCTTCTCTTTTCTCATCAATAGCAAAGCCAAATCATATCCTCTCTTCTCTCA
CCATTACCAAACCGAAATCATCTCTTCTCTTTTATCCATCTCCAAGATTCAAATAAAA
AAAAGT**ATGTCTTCTTCTTCTGAAAATACTTTGAAGATTTATTTGATGATGCATTTG**
ATCAATATTTGATCAAACCTTTGAAAAATTTACCATGGGTCATCAAGAAGAACCAA
GGAAACwAAAAAAAAAAGAGAGCTTATATTGAAAGAAATCGTGAAGAAGGCCATATT
CGATTATGGATCAGTCTCTCTCCCTTCAAAGGTGTACTG**CAGCCATCCGTGTCTT**
GGCATATGGTTGTGCGGGTGATATGGTCGACGAATACCTTCGGCTCGGTGAAACA
ACAACTCGTTTATGTGT**CGAAAATTTGTGGAAGGCGTAATTAATTTGTT**CGGCGAT****

GAGTACTTAAGAAGACCAACCCCGACTGATCTTCAACGtTTACTTGATGTTGGGGA
GTATCGTGGATTTCCCGGGATGATAGGAaGCATCGATTGTATGCATTGGGAGTGGA
AGAATTGTCCAACcCGCTTGAAAGGACAATATTCTCGTGGTTCGGGCAAACCTACT
ATCGTTTTAGAGGCGGTTGCTTCGTATGATCTCTGGATATGGCATGCGTTTTTTGG
ACCTCCAGGTACCTTAAATGATATCAACGTTCTTGATCGTTCACCTGTTTTTGATGA
CATAACATGGTCAAGCTCCGCCAGTCACTTTCTCTGTCAATGGAAGACAATATC
ATATGGCTTACTATCTGACTGATGGTATTTATCCGAAATGGGCAACTTTTATCCAAT
CTATTCGTTTACCACAGGTGCCGAAAGCAGTTTTATTTGCTCAACGTCAAGAGGCT
GTCCGAAAAGATGTCGAGCGTGCTTTTGGAGTCTACAAGCTCGTTTTTGCAATTGT
TAAAATCCAGCTCTGTTCTGGGATAAAGTCAAATTGGGAAAATTATGAGAGCAT
GTATCATACTCCATAATATGGTAGTAGAAGACGAACGAGATGGATACACTCAATAT
GATGTTTCAGAGTTCGTCCAAGGAGAAGAAAACCGAAGTTCTCATGTGCGATCTCGA
TTTTGATAGAGATATCCCATCAAATATCGCAAATACAATGGATGCTCGAACAAAGAAT
TCGTGATAAACCAATGCATCAACAACATAAAACATGATTTAGTTGAACATATATGGCA
TAAGTTTGGAGGTGATGAAGACAACAACTAACTGAGATGGATCTTTCAAATAATTCT
CATGTATTTTCTAGTCTTTTGTTCATGTTTTTTTTTTAAACTATGTTTGAATATTGT
CTTGAATAAATTTTAAGTTTTCTATAATAAATTTTAAGTTAAAAAATAAATAAAAT
TTAAAATTGTTAAAATAAGCAACTTGCAATGGAAAAATAAAATTAACAAATTGCTTAG
CTAAGTTGCTTAACCCAAATAAATTATTAATACTATTAATTTTTTAATTAAGCAaCCCA
TAATGGGtGTTAGGATGAGGGTGCTC

Supplemental Figure 6. The sequence of the DNA transposon Sal-PIF inserted in the coding region of the *FAE1* allele e.

Sequence annotation of DNA transposon Sal-PIF was predicted using GenScan at GeniusNet (<http://genome.dkfz-heidelberg.de/cgi-in/GENSCAN/genscan.cgi>). ORF1: highlighted in grey; ORF2: highlighted in light blue. The start codon (ATG) and stop codon (TAA, TGA) are underlined. The terminal inverted repeats (TIRs) are highlighted in red.

Supplemental Data. Zeng and Cheng (2014). Plant Cell 10.1105/tpc.114.126631

7A

MTLTQKKQNTPSKRRIKVKRSKRRSFTRPIRGHRRPTIIELLLRRCERHHRAPPSPLFA
ERQQRFTGPPPTMRPTVRKTIIDHVDDKHRDRMTGRRLRRRAT

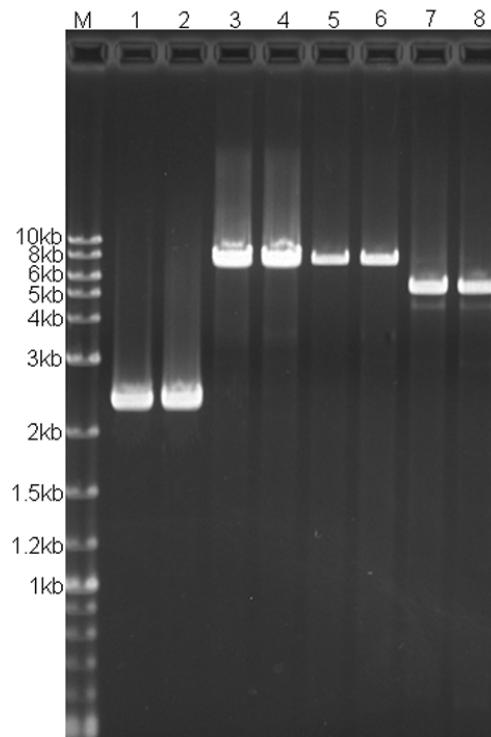
7B

MSSSENTFEDLFDDAFDQYFDQTFEKFTMGHQEPRKXKKKRAYIERNREEGHIRL
WISLSPLQKCTAAIRVLAYGCAGDMVDEYLRLGETTTRLCVENFVEGVINLFGDEYLRR
PTPTDLQRLLDVGEYRGFPGMIGSIDCMHWEWKNCPTAWKGQYSRGSKGPTIVLEAV
ASYDLWIWHAFFGPPGTLNDINVLDLRSPVFDDIIHGQAPPVTFVNGRQYHMAYYLT
GIYPKWATFIQSIRLPQVPKAVLFAQRQEAVRKDVERAFGVLQARFAIVKNPALFWDKV
KIGKIMRACIILHNMVVEDERDGYTQYDVSEFVQGEENRSSHVLDLDFDRDIPSNIANTM
DARTRIRDKPMHQQLKHDLVEHIWHKFGGDEDNN

Supplemental Figure 7. Deduced amino acid sequences of the DNA transposon *Sal-PIF* predicted using GenScan at GeniusNet (<http://genome.dkfz-heidelberg.de/cgi-in/GENSCAN/genscan.cgi>).

(A) The 103 amino acids of the deduced protein of ORF1. (B) The 383 amino acids of the deduced protein of ORF2.

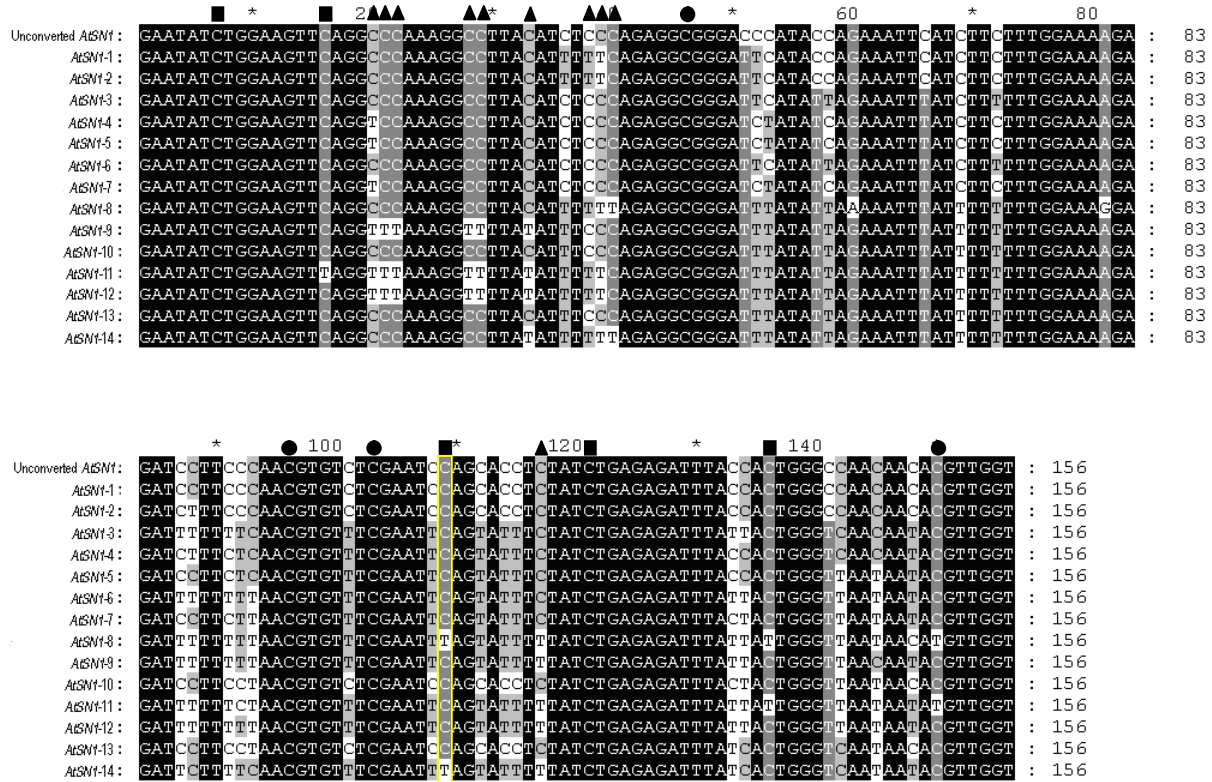
Supplemental Data. Zeng and Cheng (2014). Plant Cell 10.1105/tpc.114.126631



Supplemental Figure 8. PCR amplification of the entire *FAE1* alleles E^1 , E^2 , E^3 and *e* using the primer pair WF/WR No 14 (Supplemental Table 1 online).

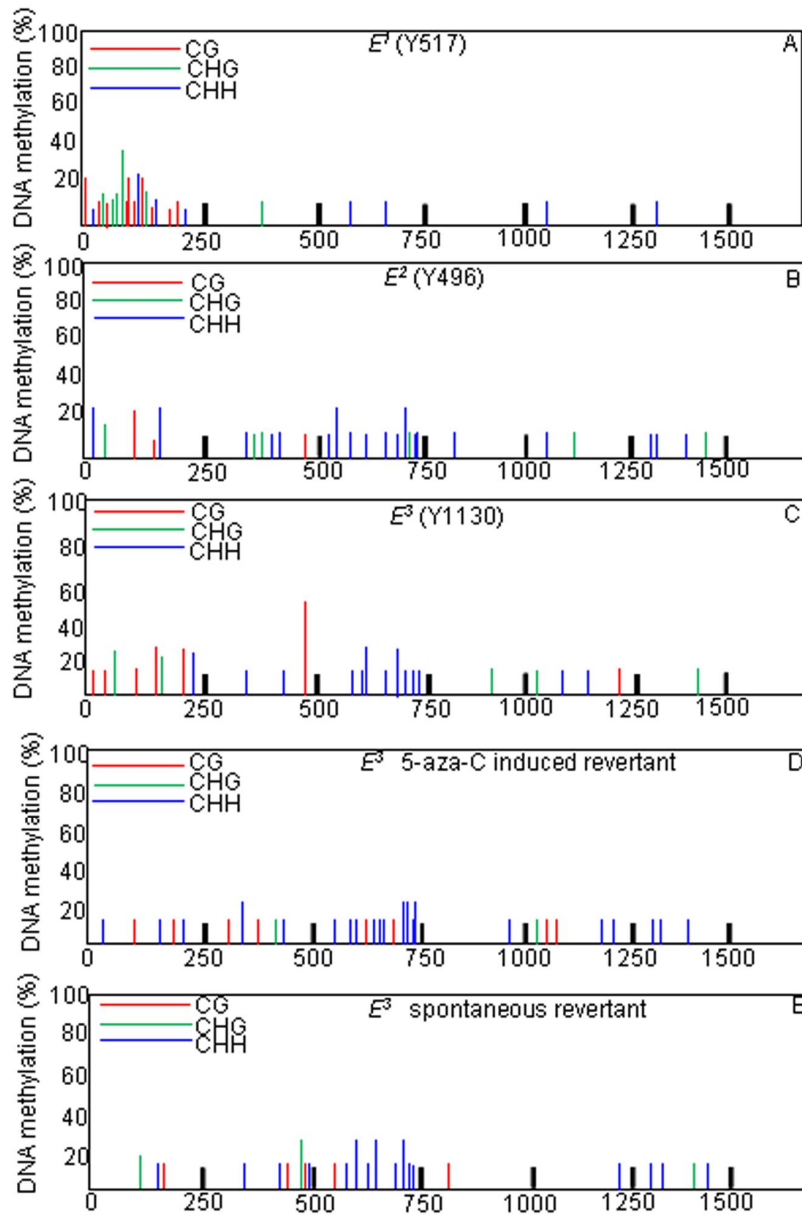
M: 1 kb DNA ladder; Lanes 1 and 2: 2524 bp fragment of E^1 ; Lanes 3 and 4: 7424 bp fragment of E^2 ; Lanes 5 and 6: 7424 bp fragment of E^3 ; Lane 7 and 8: 5369 bp of *e*.

Supplemental Data. Zeng and Cheng (2014). Plant Cell 10.1105/tpc.114.126631



Supplemental Figure 9. DNA methylation in the internal region between the long terminal repeats of *AtSN1* (positive control).

The known eighteen methylated cytosines as reported by Kuhlmann and Mette (2012), and one additional methylated cytosine (highlighted as yellow) were detected in the internal region between the long terminal repeats of *AtSN1* (positive control). Circle: CG; Rectangle: CHG; Triangle: CHH.



Supplemental Figure 10. DNA methylation in the coding regions of the *FAE1* alleles E^1 , E^2 and E^3 .

DNA methylation was not detected in the 1515 bp coding region (nucleotides 1-1515) of E^1 in Y517 (A), E^2 in Y496 (B) and E^3 in Y1130 (C), E^3 in 5-azaC induced revertants of Y1130 (D) and E^3 in spontaneous revertant of Y1130 (E).

11A

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Unconverted ATP1 : TGAATGAGATTAAAGCTGGGAAATGTTTCTTTTTGCCAACGGTGTGAAAGGAATGCCCTTGAATCTTGAGAATGAGAATG : 81
ATP1-Y517-1 : TGAATGAGATTAAAGCTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-2 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-3 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-4 : TGAATGAGATTAAAGCTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-5 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-6 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-7 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-8 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-9 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
ATP1-Y517-10 : TGAATGAGATTAAAGTGGGAAATGTTTCTTTTTGTAAAGGTGTGAAAGGAATGGTTTGAATTTTGAGAATGAGAATG : 81
    
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Unconverted ATP1 : TCGGGATTGTTGTTTGGTGGTGATACCCCTATAAAAAGAGGAGATCTTGTCAAGCCACTGGATCTATTGTGGATGTTT : 162
ATP1-Y517-1 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-2 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-3 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-4 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-5 : TCGGGATTGTTGTTTGGTGGTGATTTTCTTATAAAAAGAGGATTTTGTAAAGCCCTCGGGCTTTTGTGCAATGTTT : 162
ATP1-Y517-6 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-7 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-8 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-9 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
ATP1-Y517-10 : TCGGGATTGTTGTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGTATTGGATTTATTGTGGATGTTT : 162
    
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Unconverted ATP1 : CCGGGGAAAAGGCTATGCTAAGGCTGTGGTGCACCCGATGGGAGTACCTATTGATGAAAGAGG : 227
ATP1-Y517-1 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-2 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-3 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTACTTATTGATGAAAGAGG : 227
ATP1-Y517-4 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-5 : TTCTGGGAAAAGGGCTATGCTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-6 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTACTTATTGATGAAAGAGG : 227
ATP1-Y517-7 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-8 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-9 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTATTTATTGATGAAAGAGG : 227
ATP1-Y517-10 : TTCTGGGAAAAGTTATGTTAAGGCTGTGGTGCATCTGATGGGAGTACTTATTGATGAAAGAGG : 227
    
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11B

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Unconverted P1 : TCAAAGAAGATCTTTGTAGTTAGAGATAGAAATCTACATACTCTTTATTTGGAGTAACAATGGAGATATTTAAGCAATGA : 81
PI-E'(Y517)-1 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-2 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-3 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-4 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-5 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-6 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-7 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-8 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-9 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
PI-E'(Y517)-10 : TCAAAGAAGATTTTGTAGTTAGAGATAGAAATTTATATATTTTATTTGGAGTAACAATGGAGATATTTAAGTAATGA : 81
    
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Unconverted P1 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGCATTATTTTATCGAAATGAAATCTAGTATATAA : 162
PI-E'(Y517)-1 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-2 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-3 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-4 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-5 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-6 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-7 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-8 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-9 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
PI-E'(Y517)-10 : ATTTATGACCTATATTTATATAGTTTATTTGATTTTAAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
    
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      *           180           *           200           *           220           *           240
Unconverted P1 : TATAATGTTTTATCATCAGGATACTTTTCCTATTTTTGGCCACCTTTCATAGGACTACTGATTTATTTCAATGTGTATGCCT : 243
P1-E'(Y517)-1 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-2 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-3 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-4 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-5 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-6 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-7 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-8 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-9 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-E'(Y517)-10 : TATAATGTTTTATRATTAGGATATTTTTTTTTATTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTT : 243

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      *
Unconverted P1 : GCATGAGCATGAGT : 257
P1-E'(Y517)-1 : GCATGAGTATGAGT : 257
P1-E'(Y517)-2 : GCATGAGTATGAGT : 257
P1-E'(Y517)-3 : GCATGAGTATGAGT : 257
P1-E'(Y517)-4 : GCATGAGTATGAGT : 257
P1-E'(Y517)-5 : GCATGAGTATGAGT : 257
P1-E'(Y517)-6 : GCATGAGTATGAGT : 257
P1-E'(Y517)-7 : GCATGAGTATGAGT : 257
P1-E'(Y517)-8 : GCATGAGTATGAGT : 257
P1-E'(Y517)-9 : GCATGAGTATGAGT : 257
P1-E'(Y517)-10 : GCATGAGTATGAGT : 257

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11C

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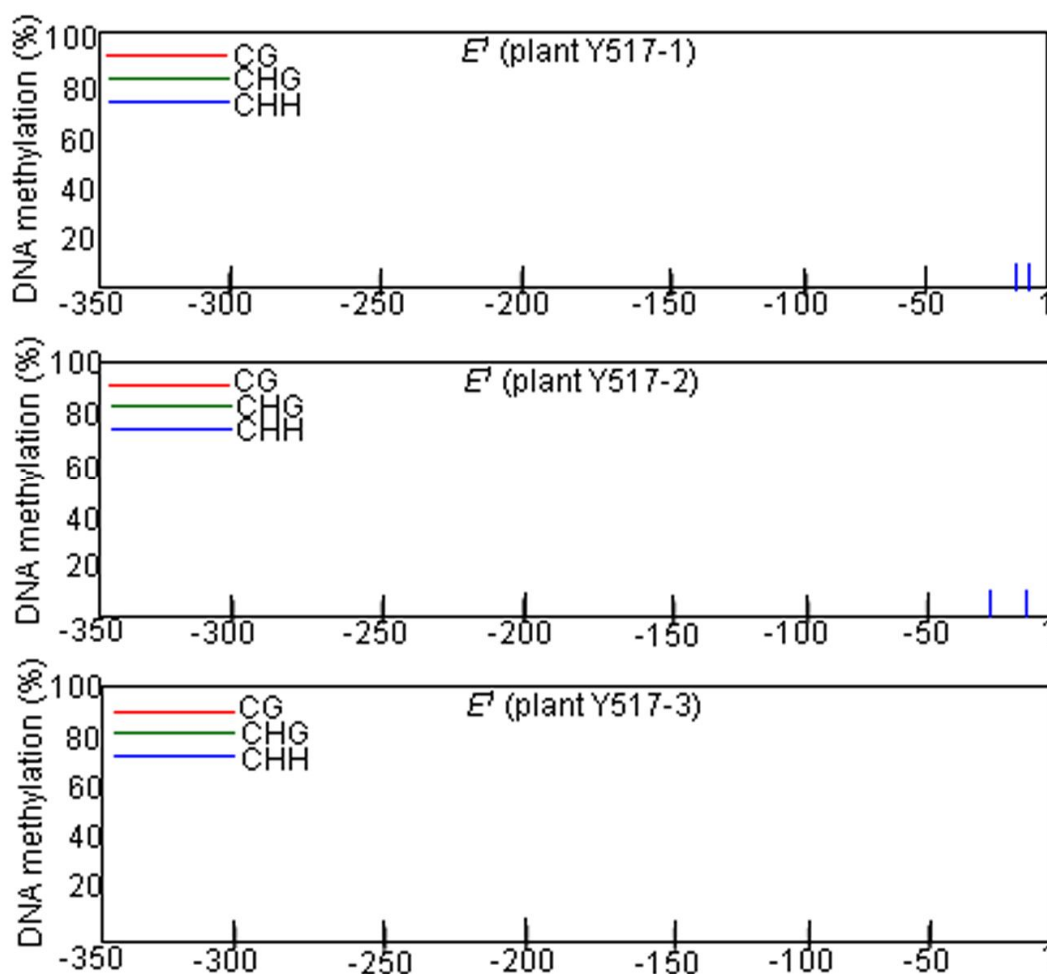
      *           20           *           40           *           60           *           80
Unconverted P2 : ATTTTTTGGCCACCTTTCATAGGACTACTGATTTATTTCAATGTGTATGCCTGCATGAGCATGAGTATACACATGTCTTTTAAAA : 83
P2-E'(Y517)-1 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-2 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-3 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-4 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-5 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-6 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-7 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-8 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-9 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83
P2-E'(Y517)-10 : ATTTTTTGGTATTTTTRATAGGATTATTGATTTATTTAATGTGTATGTTTCATGAGTATGAGTATATATATGTTTTTTAAAA : 83

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      *           100           *           120           *           140
Unconverted P2 : ATGCATGTAAAGTGTAAAGGACCCACAAAAGAGGATCTATACAAAATACATCTCATCACTTC : 143
P2-E'(Y517)-1 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-2 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-3 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-4 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-5 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-6 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-7 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-8 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-9 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143
P2-E'(Y517)-10 : ATGCATGTAAAGTGTAAAGGATTATAAAAAGAAGATTTATAAAAAATATATTTTATTTATTTT : 143

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Supplemental Figure 11. Bisulfite sequence alignment of *ATP1* (control gene) and the *FAE1* allele *E*¹ in line Y517.

(A) The control gene *ATP1* had 100% conversion of C to T in the 10 clones analyzed. (B) DNA methylation was not detected in the promoter region (nucleotides -335 to -79) of *E*¹. (C) DNA methylation was not observed in the partial promoter region (nucleotides -143 to 1) of *E*¹. Unconverted genomic sequence is shown on the top. P1: The promoter region (nucleotides -335 to -79) was amplified using primer pair No 30. P2: The promoter region (nucleotides -143 to 1) was amplified using primer pair No 28. (D) DNA methylation was not detected in the promoter region (-335 to 1) of the *FAE1* allele *E*¹ in the three plants Y517-1, -2 and -3. Ten clones were analyzed for each bisulfite experiment.


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*           180           *           200           *           220           *           240
Unconverted P1 : TATAATGTTTTATCATCAGGATACCTTTCCATTTTTTTGGCACCTTTATAGGACTACTGATTATTTCAATGTGTATGCCT : 243
P1-E(Y496)-1 : TATAATGTTTTATATAGGATACCTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243
P1-E(Y496)-2 : TATAATGTTTTATATCAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243
P1-E(Y496)-3 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243
P1-E(Y496)-4 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243
P1-E(Y496)-5 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243
P1-E(Y496)-6 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTGAAAAAAG-GTGTATGTTT : 242
P1-E(Y496)-7 : TATAATGTTTTATCATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATT-AAAAG-GTGTATGTTT : 241
P1-E(Y496)-8 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGGTATGTTT : 243
P1-E(Y496)-9 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243
P1-E(Y496)-10 : TATAATGTTTTATATAGGATATTTTTTATTTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTT : 243

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Unconverted P1 : GCATGAGCATGAGT : 257
P1-E(Y496)-1 : GCATGAGTATGAGT : 257
P1-E(Y496)-2 : GCATGAGTATGAGT : 257
P1-E(Y496)-3 : GCATGAGTATGAGT : 257
P1-E(Y496)-4 : GCATGAGTATGAGT : 257
P1-E(Y496)-5 : GCATGAGTATGAGT : 257
P1-E(Y496)-6 : GCATGAGTATGAGT : 256
P1-E(Y496)-7 : GCATGAGTATGAGT : 255
P1-E(Y496)-8 : GCATGAGTATGAGT : 257
P1-E(Y496)-9 : GCATGAGTATGAGT : 257
P1-E(Y496)-10 : GCATGAGTATGAGT : 257

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12C

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*           20           *           40           *           60           *           80
Unconverted P2 : ATTTTTTGGCACCTTTATAGGACTACTGATTATTTCAATGTGTATGCCTGCATGAGCATGAGTATACACATGCTTTTAAAA : 83
P2-E(Y496)-1 : ATTTTTTGGTATTTTTATAGGATATTGATTGAAAAAAG-GTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 82
P2-E(Y496)-2 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83
P2-E(Y496)-3 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83
P2-E(Y496)-4 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83
P2-E(Y496)-5 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83
P2-E(Y496)-6 : ATTTTTTGGTATTTTTATAGGATATTGATTGAAAAAAG-GTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 82
P2-E(Y496)-7 : ATTTTTTGGTATTTTTATAGGATATTGATT-AAAAG-GTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 81
P2-E(Y496)-8 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83
P2-E(Y496)-9 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83
P2-E(Y496)-10 : ATTTTTTGGTATTTTTATAGGATATTGATTATTTTAAATGTGTATGTTTGTATGAGTATGAGTATATAATGTTTTAAAA : 83

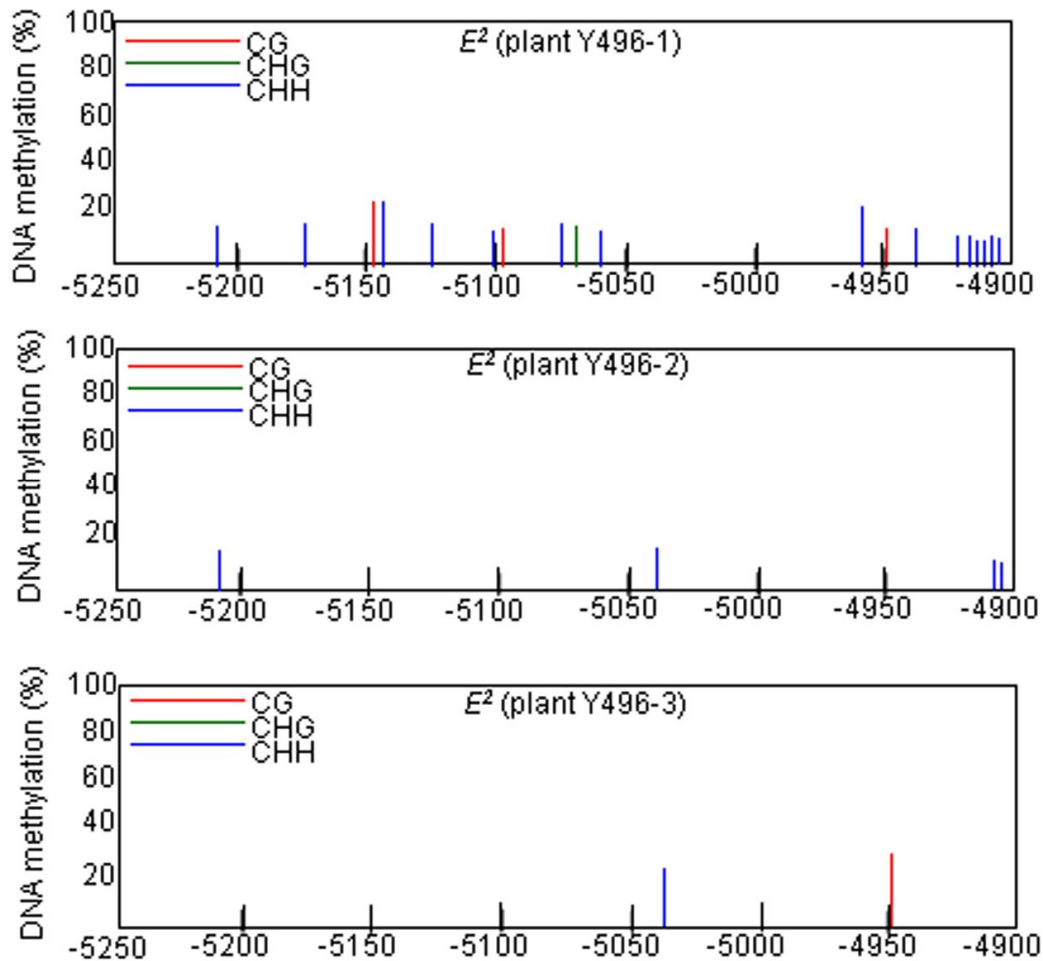
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*           100           *           120           *           140
Unconverted P2 : ATGCANGTAAAGTGTAAACGGACCAAAAAAGAGGATCTATACAAATACATCTCATCACTTC : 143
P2-E(Y496)-1 : AAGCATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 142
P2-E(Y496)-2 : ATGTATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 143
P2-E(Y496)-3 : ATGTAAGTAAAGTGTAAATGGATTAAAAAGAGGATTTATACAAATACATCTCATCACTTC : 143
P2-E(Y496)-4 : AAGCATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 143
P2-E(Y496)-5 : ATGTATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 143
P2-E(Y496)-6 : AAGCATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 142
P2-E(Y496)-7 : AGCAATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 141
P2-E(Y496)-8 : ATGTATGTAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 143
P2-E(Y496)-9 : ACC-ANGTAAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 142
P2-E(Y496)-10 : AACAAGTAAAGTGTAAATGGATTAAAAAGAGGATTTATATAAATAATTTTATTTTT : 143

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12D



Supplemental Figure 12. Bisulfite sequence alignment of *ATP1* (control gene) and the *FAE1* allele *E*² in line Y496.

(A) The control gene *ATP1* had 97% conversion of C to T in the 10 clones analyzed.

(B) DNA methylation was not detected in the promoter region (nucleotides -5234 to -4978) of *E*². (C) DNA methylation was not observed in the promoter region

(nucleotides -5042 to -4900) of *E*². Unconverted genomic sequence is shown on the top. P1: The promoter region (nucleotides -5234 to -4978) was amplified using

primer pair No 30. P2: The promoter region (nucleotides -5042 to -4900) was amplified using primer pair No 29. (D) DNA methylation was not detected in the

promoter region (-5234 to -4900) of the *FAE1* allele *E*² in the three plants Y496-1, -2 and -3. Ten clones were analyzed for each bisulfite treatment.

13A

Unconverted *ATP1* : TGAACGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰CCAAACGGTGTGAAAGGAATGGCC⁴⁰TGAATCTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-1 : TGAACGATGTATTTAAGCTGGGAAATGGTTCTTTTTC²⁰CAAAAGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 80
ATP1-Y1130-2 : TGAACGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-3 : TGAATGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰TAAACGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-4 : TGAATGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-5 : TGAATGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-6 : TGAACGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-7 : TGAATGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-8 : TGAATGAG--TTTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-9 : TGAATGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79
ATP1-Y1130-10 : TGAATGAG--ATTCAAGCTGGGAAATGGTTCTTTTTC²⁰GTTAATGGTGTGAAAGGAATGGTTTGAATTTT⁶⁰GAGAATGAGAA : 79

Unconverted *ATP1* : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATACCGCTATAAAAAGAAGGAGATCTTGTCAAGCC¹²⁰ACTGGATCTATTGTGGATG : 160
ATP1-Y1130-1 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 161
ATP1-Y1130-2 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-3 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATCTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-4 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-5 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-6 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-7 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-8 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-9 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160
ATP1-Y1130-10 : TGTGGGATGTTGTCTTTGGTGGT¹⁰⁰GATATTGTTATAAAAAGAAGGAGATTTGTTAAGTGTATTGGATTTATTGTGGATG : 160

Unconverted *ATP1* : TCCGCCGGAAAGGCTATGCTAGGGCTGTGGCTCGACCGC¹⁸⁰GATGGGAGTACCTATTGATGGAAGAGGG : 227
ATP1-Y1130-1 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGAGGGGAGTACTTCTTCTGATCTTGTGGGG : 228
ATP1-Y1130-2 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGAGGGGAGTAACTACTAGTCCGGCCGCC : 227
ATP1-Y1130-3 : TTTTGTGGAAAGGCTATGCTAGGGTGTGGTGTGATGTGAGGGGAGTAACTACTAGTCCGGCCGCC : 226
ATP1-Y1130-4 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGCTGATGTGATGGGAGTATTTATTGCTGGAAGAGGG : 227
ATP1-Y1130-5 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGATGGGAGTACTTATTGATGGAAGAGGG : 227
ATP1-Y1130-6 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGATGGGAGTATTTATTGATGGAAGAGGG : 227
ATP1-Y1130-7 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGATGGGAGTACTTATTGATGGAAGAGGG : 227
ATP1-Y1130-8 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGATGGGAGTACTTATTGATGGAAGAGGG : 227
ATP1-Y1130-9 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGATGGGAGTACTTATTGATGGAAGAGGG : 227
ATP1-Y1130-10 : TTTTGTGGAAAGGTTATGTTAGGGTGTGGTGTGATGTGATGGGAGTATTTATTGATGGAAGAGGG : 227

13B

Unconverted *P1* : TCAAAGAAGATCTTTGTAGTTAGAGACAGAAATCTACATACTCTTTATTTGGAGTAA²⁰CAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-1 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-2 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-3 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-4 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-5 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-6 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-7 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-8 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-9 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81
P1-E³(Y1130)-10 : TTAAGAAGATTTTTGTAGTTAGAGATAGAAATTTATATATTTTTATTTGGAGTAAATGGAGATATTTAAGCAATGA : 81

Unconverted *P1* : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGCATTATTTTATCGAAATGAAATCTAGTATATAA : 162
P1-E³(Y1130)-1 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-2 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATCTAGTATATAA : 162
P1-E³(Y1130)-3 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-4 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-5 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-6 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-7 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-8 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-9 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162
P1-E³(Y1130)-10 : ATTTATGAGTTATATTTATATAGTTTATTT⁻⁵¹⁴⁶CAATTTTAATTGAAAGTATTATTTTATCGAAATGAAATTTAGTATATAA : 162

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      *           180           *           200           *           220           *           240
Unconverted P1 : TATAATGTTTTATCATTAGG-ATATTTTCTT-ATTTTTTGGCACCTTTC-ATAGGACTACTGATTTATTTCAATGTGTATG : 240
P1-E3(Y1130)-1 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-2 : TATAATGTTTTATCATTAGG-ATATTTTCTT-ATTTTTTGGTACCTTTC-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-3 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-4 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-5 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-6 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-7 : TATAATGTTTTATATTAGGATATTTTTTTTATTTTTTGGTATTTTTTTATAGGATTATTGATTTATTTAATGGGGAG : 243
P1-E3(Y1130)-8 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-9 : TATAATGTTTTATCATTAGG-ATACTTTCTT-ATTTTTTGGCACCTTTC-ATAGGATTATTGATTTATTTAATGTGTATG : 240
P1-E3(Y1130)-10 : TATAATGTTTTATATTAGG-ATATTTTCTT-ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATG : 240

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      *           260
Unconverted P1 : CCTGCATGAGCATGAGT : 257
P1-E3(Y1130)-1 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-2 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-3 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-4 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-5 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-6 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-7 : GTTGGAGAGTATGAGT : 260
P1-E3(Y1130)-8 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-9 : TTTGTATGAGTATGAGT : 257
P1-E3(Y1130)-10 : TTTGTATGAGTATGAGT : 257

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13C

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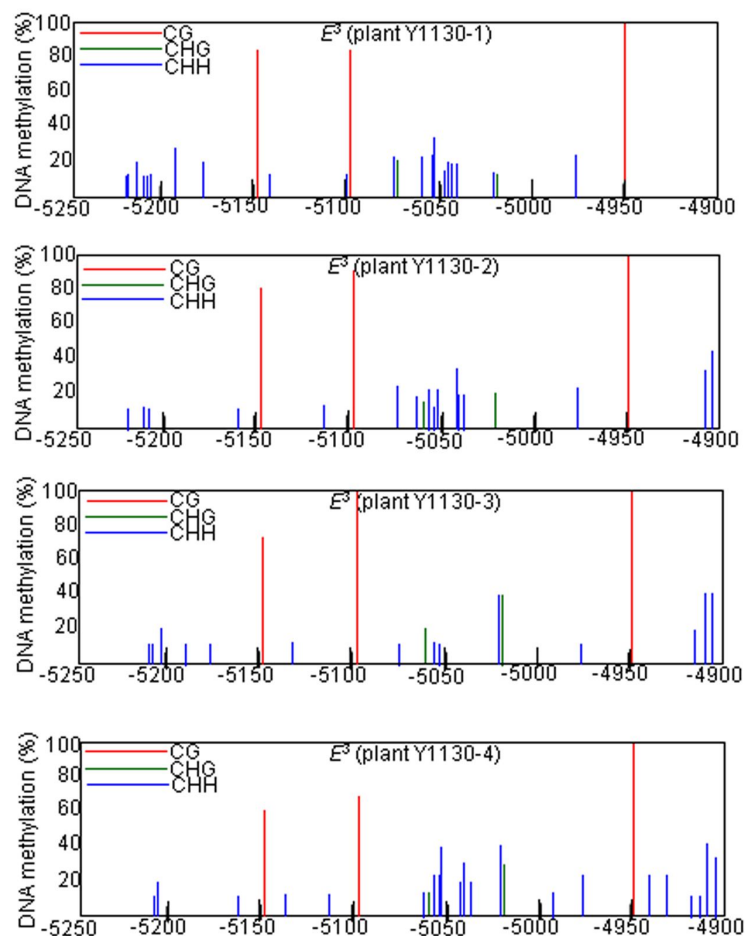
      *           20           *           40           *           60           *           80
Unconverted P2 : ATTTTTTGGCACCTTTC-ATAGGACTACTGATTTATTTCAATGTGTATGCTTGCATGAGCATGAGTATACACATGCTTTTAAA : 82
P2-E3(Y1130)-1 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-2 : ATTTTTTGGTATTTTTT-ATAGGATTACTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-3 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-4 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-5 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-6 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-7 : TTTTTTGGTATTTTTTATAGGATTATTGATTTATTTAATGGGCAAGCTTGCAGAGTATGAGTATACATATGCTTTTAAA : 83
P2-E3(Y1130)-8 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 80
P2-E3(Y1130)-9 : ATTTTTTGGCACCTTTC-ATAGGACTACTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82
P2-E3(Y1130)-10 : ATTTTTTGGTATTTTTT-ATAGGATTATTGATTTATTTAATGTGTATGTTTGCATGAGTATGAGTATACATATGCTTTTAAA : 82

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      *           1004943           *           120           *           140
Unconverted P2 : AATGATGTAAGTGTAAACGGACCACAAAAGAGGATCTATACAAATACATCTCATCAGCTTC : 143
P2-E3(Y1130)-1 : AAACAAGCAAAAGTGTAAACGGATTATAAAAAGAGGATTTATATAATGTATTTTTATTATTTT : 142
P2-E3(Y1130)-2 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143
P2-E3(Y1130)-3 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143
P2-E3(Y1130)-4 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143
P2-E3(Y1130)-5 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143
P2-E3(Y1130)-6 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143
P2-E3(Y1130)-7 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 144
P2-E3(Y1130)-8 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 141
P2-E3(Y1130)-9 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143
P2-E3(Y1130)-10 : AATGATGTAAGTGTAAACGGATTATAAAAAGAGGATTTATATAAATATATTTTATTATTTT : 143

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Supplemental Figure 13. Bisulfite sequence alignment of *ATP1* (control gene) and the *FAE1* allele E^3 in line Y1130.

(A) The control gene *ATP1* in Y1130 had 100% conversion of C to T in the bisulfite treatment. (B) Methylation occurred at positions -5146 and -5095. (C) Methylation occurred at position -4943. Unconverted genomic sequence is shown on the top. P1: The promoter region (nucleotides -5234 to -4978) was amplified using primer pair No 30. P2: The promoter region (nucleotides -5042 to -4900) was amplified using primer pair No 29. (D) DNA methylation was detected at positions -5146, -5095 and -4943 in the promoter region (-5234 to -4900) of the *FAE1* allele E^3 in the four plants Y1130-1, -2, -3 and -4. Ten clones were analyzed for each bisulfite experiment.

14A

Unconverted *ATP1* : TGAACGAGATTCAAGCTGGGGAATGGTCTTTTTGCCAACGGTGTGAAAGGAATGCCCTTGAATCTTGAGAATGAGAATG : 81
ATP1-Revertant1-1 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-2 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-3 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-4 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-5 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-6 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-7 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-8 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-9 : TGAATGAGATTAAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81
ATP1-Revertant1-10 : TGAACGAGATTCAAGCTGGGGAATGGTCTTTTTGTTAATGGTGTGAAAGGAATGGTTTTGAATTTGAGAATGAGAATG : 81

Unconverted *ATP1* : TCGGGATTGTTGCTTTTGGTGGTGATACCGCTATAAAAAGAGGAGATCTTGTCAAGCGCACTGGATCTATTGUGGATGTTG : 162
ATP1-Revertant1-1 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-2 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-3 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-4 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-5 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGCGCTTGGCTTTTTCGCAATGTTG : 161
ATP1-Revertant1-6 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-7 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-8 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-9 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162
ATP1-Revertant1-10 : TTGGGATTGTTGTTTTTGGTGGTGATATTGTTATAAAAAGAGGAGATTTTGTAAAGTGATTTGGATTTATTGUGGATGTTG : 162

Unconverted *ATP1* : CCGCGGGAAAGGCTATGCTAGGGCTGTGGTCCAGCCGATGGGAGTACCTATTGATGSAAGAGGE : 227
ATP1-Revertant1-1 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACCTATTGATGSAAGAGGA : 227
ATP1-Revertant1-2 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTATTATTGATGSAAGAGGE : 227
ATP1-Revertant1-3 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTATTATTGATGSAAGAGGE : 227
ATP1-Revertant1-4 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACCTATTGATGSAAGAGGE : 227
ATP1-Revertant1-5 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTATTATTGATGSAAGAGGE : 225
ATP1-Revertant1-6 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACTATTGATGSAAGAGGE : 227
ATP1-Revertant1-7 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACTATTGATGSAAGAGGE : 227
ATP1-Revertant1-8 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACTATTGATGSAAGAGGE : 227
ATP1-Revertant1-9 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACTATTGATGSAAGAGGE : 227
ATP1-Revertant1-10 : TTGTGGGAAAGGTTATGTTAGGGCTGTGGTTCAGTGTGATGGGAGTACTATTGATGSAAGAGGE : 227

14B

Unconverted *P1* : TCAAAGAAGATCTTTGTAGTTAGAGACAGAAATCTACATAGCTCTTTATTTGGAGTAACAAATGGAGATATTTTAAGCAATGA : 81
P1-Revertant1-1 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGTAATGA : 81
P1-Revertant1-2 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATACTTTTATTTGGAGTAACAAATGGAGATATTTTAAGLAATGA : 81
P1-Revertant1-3 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGLAATGA : 81
P1-Revertant1-4 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGTAATGA : 81
P1-Revertant1-5 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGLAATGA : 81
P1-Revertant1-6 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGLAATGA : 81
P1-Revertant1-7 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGTAATGA : 81
P1-Revertant1-8 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGLAATGA : 81
P1-Revertant1-9 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGLAATGA : 81
P1-Revertant1-10 : TTAAGAAGATTTTGTAGTTAGAGATAGAAATTTAATATAATTTTATTTGGAGTAATAATGGAGATATTTTAAGLAATGA : 81

Unconverted *P1* : ATTTATGAGCTTATATTTATATAGTTTATTTTCAATTTTAAATTGAAAGCATTATTTTTATTGAAATGAAATCTAGTATATAA : 162
P1-Revertant1-1 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-2 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-3 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-4 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-5 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-6 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-7 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-8 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-9 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162
P1-Revertant1-10 : ATTTATGAGCTTATATTTATATAGTTTATTTTATTTTAAATTGAAAGTATTATTTTTATTGAAATGAAATTTAGTATATAA : 162


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      *      180      *      200      *      220      *      240
Unconverted P1 : TATAATGTTTTATCATCAGGATACTTTCCATATTTTGGCACCTTTCATAGGACTACTGATTTATTTCAATGTGTATGCCCT : 243
P1-Revertant1-1 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-2 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 242
P1-Revertant1-3 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-4 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-5 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGACTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-6 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATCGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-7 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-8 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-9 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243
P1-Revertant1-10 : TATAATGTTTTATATAGGATACTTTTATATTTTGGTATTTTATAGGATTATTGATTTATTTAATGTGTATGTTT : 243

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      *
Unconverted P1 : GCATGACCATGAGT : 257
P1-Revertant1-1 : GTATGAGTATGAGT : 257
P1-Revertant1-2 : GTATGAGTATGAGT : 256
P1-Revertant1-3 : GTATGAGTATGAGT : 257
P1-Revertant1-4 : GTATGAGTATGAGT : 257
P1-Revertant1-5 : GTATGAGTATGAGT : 257
P1-Revertant1-6 : GTATGAGTATGAGT : 257
P1-Revertant1-7 : GAAAGAAATGAAA : 257
P1-Revertant1-8 : GTATGAGTATGAGT : 257
P1-Revertant1-9 : GTATGAGTATGAGT : 257
P1-Revertant1-10 : GTATGAGTATGAGT : 257

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14C

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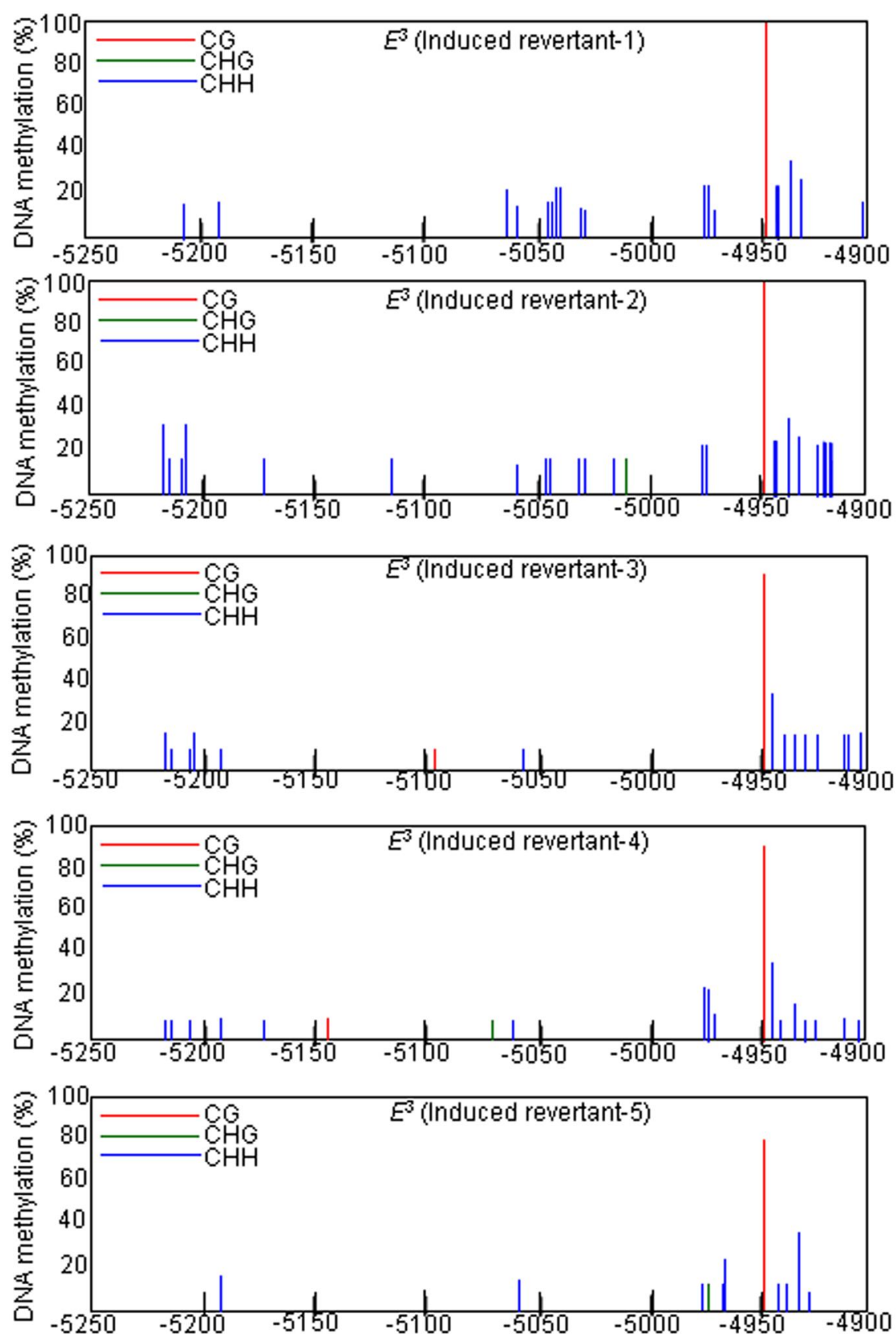
      *      20      *      40      *      60      *      80
Unconverted P2 : ATTTTTGGCACCTTTCATAGGACTACTGATTTATTTCAATGPGTATGCCGTGCATGACCATGACTATACACATGTCCTTTAAAAA : 83
P2-Revertant1-1 : ATTTTTGGTATTTTATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-2 : ATTTTTGGTATTTTATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-3 : ATTTTTGGTATTTTATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-4 : ATTTTTGGTATTTTATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-5 : ATTTTTGGTATTTTATAGGACTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-6 : ATTTTTGGTATTTTATAGGATTATCGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-7 : ATTTTTGGTACCTTTCATAGGATTATTGATTTATTTAATGPGCATGTGAAAGAAATAGAA-ATATATATGCTTTAAAAA : 82
P2-Revertant1-8 : ATTTTTGGCATTTTTATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-9 : ATTTTTGGTATTTTATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83
P2-Revertant1-10 : ATTTTTGGTATCTTTCATAGGATTATTGATTTATTTAATGPGTATGTTTGTATGACTATGACTATACACATGTTTAAAAA : 83

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      *      100      *      120      *      140
Unconverted P2 : ATGATGTAAAGTGTAAACGGACCACAAAAGGGATCTATACAAATACATCTCATCAGCTTC : 143
P2-Revertant1-1 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATCTATACAAATATATTTTATTATTTT : 143
P2-Revertant1-2 : ATGATGTAAAGTGTAAACGGATTGCAAAAAGGGATTATATAAAATATATTTTATTACTTT : 143
P2-Revertant1-3 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATCTATACAAATATATTTTATTATTTT : 143
P2-Revertant1-4 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATTATATAAAATATATTTTATTATTTT : 143
P2-Revertant1-5 : ATGATGTAAAGTGTAAACGGATTGCAAAAAGGGATTATATAAAATATATTTTATTACTTT : 143
P2-Revertant1-6 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATTATATAAAATATATTTTATTATTTT : 143
P2-Revertant1-7 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATCTATACAAATATATTTTATTATTTT : 142
P2-Revertant1-8 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATCTATACAAATATATTTTATTATTTT : 143
P2-Revertant1-9 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATTATATAAAATATATTTTATTATTTT : 143
P2-Revertant1-10 : ATGATGTAAAGTGTAAACGGATTATAAAAAGGGATTATATAAAATATATTTTATTATTTT : 143

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Supplemental Figure 14. Bisulfite sequence alignment of *ATP1* (control gene) and the *FAE1* allele E^3 in the 5-azaC induced revertants of Y1130.

(A) The control gene *ATP1* had 97% conversion of C to T in the bisulfite treatment. (B) Methylation was not detected at positions -5146 and -5095. (C) Methylation was detected at position -4943. Unconverted genomic sequence is shown on the top. P1: The promoter region (nucleotides -5234 to -4978) was amplified using primer pair No 30. P2: The promoter region (nucleotides -5042 to -4900) was amplified using primer pair No 29. Revertant 1: 5-azaC induced revertant of Y1130. (D) DNA methylation was detected at position -4943 in the promoter region (nucleotides -5234 to -4900) of *FAE1* allele E^3 in 5-azaC induced revertant-1, -2, -3, -4 and -5. Ten clones were analyzed for each bisulfite experiment.


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      *           180           *           200           *           220           *           240
Unconverted P1 : TATAATGTTTATCATCAGGATACCTTCCATTTTTTTGGCACCTTCCATAGGACTACTGATTTATTTCAATGTGTATGCC : 243
P1-Revertant2-1 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-2 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-3 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-4 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-5 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-6 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-7 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-8 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-9 : TATAATGTTTATTTATCAGGATACCTTCCATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243
P1-Revertant2-10 : TATAATGTTTATTTATCAGGATATTTTTTATTTTTTTGGCAATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTT : 243

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Unconverted P1 : GCATGACCATGAGT : 257
P1-Revertant2-1 : GTATGACTATGAGT : 257
P1-Revertant2-2 : GTATGACTATGAGT : 257
P1-Revertant2-3 : GTATGACTATGAGA : 257
P1-Revertant2-4 : GTATGACTATGAGT : 257
P1-Revertant2-5 : GTATGACTATGAGT : 257
P1-Revertant2-6 : GTATGACTATGAGT : 257
P1-Revertant2-7 : GTATGACTATGAGT : 257
P1-Revertant2-8 : GTATGACTATGAGT : 257
P1-Revertant2-9 : GTATGACTATGAGT : 257
P1-Revertant2-10 : GTATGACTATGAGT : 257

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15C

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      *           20           *           40           *           60           *           80
Unconverted P2 : ATTTTTTGGCACCTTCCATAGGACTACTGATTTATTTCAATGTGTATGCCCTGCATGACCATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-1 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-2 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-3 : ATTTTTTGGCATTTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-4 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-5 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-6 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-7 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-8 : ATTTTTTGGTATTTTTATAGGACTACTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-9 : ATTTTTTGGCATTTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83
P2-Revertant2-10 : ATTTTTTGGTATTTTTATAGGANTATTGATTTATTTAAATGTGTATGTTTGTATGATATGAGTATACACATGCTTTAAAA : 83

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      *           100           *           120           *           140
Unconverted P2 : ATCCATGTAAGTGTAACGGACCACAAAAGAGGATCTATACAAATACATCCTCATCAGCTTC : 143
P2-Revertant2-1 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-2 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-3 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-4 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-5 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-6 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-7 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-8 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-9 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143
P2-Revertant2-10 : ATCTATGTAAGTGTAACGGATTATAAAAAGAGGATTTATAATATGATTTTTTATTATTTT : 143

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Supplemental Figure 15. Bisulfite sequence alignment of *ATP1* (control gene) and the *FAE1* allele *E*³ in the spontaneous revertant Y1130-16.

(A) The control gene *ATP1* had 100% complete conversion of C to T in the bisulfite treatment. (B) Methylation was not detected at positions -5146 and -5095. (C) Methylation was detected at position -4943. Unconverted genomic sequence is shown on the top. P1: The promoter region (nucleotides -5234 to -4978) amplified using primer pair No 30. P2: The promoter region (nucleotides -5042 to -4900) amplified using primer pair No 29. Revertant 2: Spontaneous revertant Y1130-16. Ten clones were analyzed for each bisulfite experiment.

Supplemental Table 1 Primer pairs used in this study

No	Primer name	Sequence (5'-3')	Purpose
1	OF OR	ATGACGTCCGTTAACGTA TTAGGACCGACCGTTTTG	To clone the entire coding region of E^1 , E^2 , E^3
2	PF OR	TCGGTTCGGTTCTCTACATC TTAGGACCGACCGTTTTG	To clone the partial coding region of allele e
3	ZUP1-GSP-1* ZUP1-GSP-2**	GAAGGTAGCATGAGTACTCAACGAGGT GATGTAGAGAACCGAACCGAAGACGGT	To clone the remaining coding region of e by PCR walking
4	DOWN-GSP-1* DOWN-GSP-2**	TGGAGGCATCAAGATCAACGTTACATA TTCAGCTAAGTCAGAGACTCGTGTCCTA	To clone the 3' downstream sequences from the translation stop coding site for E^1 , E^2 , E^3 and e
5	HUP1-GSP-1* HUP1-GSP-2**	TGATAGGCTGAACCGACAGAGACTCTG ACCGACAGAGACTCTGACTAATTATGG	To clone the 5' upstream sequence of E^1 , E^2 , E^3
6	MUP1-GSP-1* MUP1-GSP-2**	ACGTCTATCGACCTCTGCCAATGTGTA CGATGACAAGGTCCACCAGTTCATAAT	Primer pairs 6-9: to clone further 5' upstream sequence of E^2 and E^3
7	MUP2-GSP-1* MUP2-GSP-2**	AATCTTGTCTCCATACCACCTTGTTTCG GTTCTATATGTGCCATCCTTGAAGTGC	
8	MUP3-GSP-1* MUP3-GSP-2**	TCACACCATCTCTGTTCCCTCAGTCCTC CGTCTTCGGTCCAAGGTAACACACTCT	
9	MUP4-GSP-1* MUP4-GSP-2**	TGCTTGGAGACTCGCCTATCAGTTGGA CAGGACACAGTATCTCACTCTTCCGCA	
10	CORE-F CORE-R	GCCTATCATTGCTAAGTACA CTCATGCAGGCATACACATT	To clone the 703 bp putative promoter sequences of E^2 , E^3 and e
11	GAP-F GAP-R	CAATGTGTATGCCTGCATGA TTGAAGGACAACATACGTGG	To clone the DNA fragment between the promoter and 5' flanking sequence of E^2 and E^3

12	MUP5-GSP-1* MUP5-GSP-2**	ATCCTCTTTTGTGGTCCGTTACTTTT TTTTGTGGTCCGTTACTTTACATGC	To clone further promoter sequences of E^2 and E^3
13	GAP0-F GAP0-R	CGACACACACACAGAGCA CACGGATGGCTGCAGTACAC	To clone the DNA fragment between the promoter and 5' flanking sequence of <i>e</i>
14	WF WR	GCCTATCATTGCTAAGTAGA TACTAGAACACCACTGCATT	To clone the entire gene sequences of E^1 , E^2 , E^3 and <i>e</i>
15	YF YR	GAGCAATGACGTCCGTTA GGACCGACCGTTTTG	To amplify the coding regions of E^1 and E^2 for yeast expression
16	ProF ProR	CGTAAGCTTCCTATCATTGCTAAGTAGAA AGCTCTAGACTCATGCAGGCATACACATT	To clone the 5' upstream sequence from the translation start site for E^1
17	FP1 FP2	CGTAAGCTTTCTTCTTGAACCTTGGGA AGCTCTAGATGCTCTGTGTGTGTGTCG	To clone the 5' upstream sequence from the translation start site for E^2 and E^3
18	OF RTR	ATGACGTCCGTTAACGTA AAGACTTGTCGTCAGCTCCA	RT-PCR
19	FAE1F1 FAE1R1	AGACGGTCCAAGTACGAGCTAGTTC TGCCGCTCTCATCATCTCCTT	Quantitative Real-time PCR
20	ActinF ActinR	ACGAGCTACCTGACGGACAAG GAGCGACGGCTGGAAGAGTA	RT-PCR, Quantitative Real-time PCR
21	ATP1-F ATP1-R	TGAAYGAGATTYAAGYTGGGGAAATGGT CCCTCTTCCATCAATARRTACTCCCA	To amplify the <i>ATP1</i> gene as bisulfite control
22	BSPCF1 BSPCR1	YGTTYGTTAAYGTAAAGTTTTTTTT TCCTCAAAAACCTCAAACCAAAAAC	To study DNA methylation of the coding region of position 1 to 200 of E^1 , E^2 and E^3
23	BSPCF2 BSPCR2	TTTTTATCGTTTTTCGGTTCGG CTACAACCCATACCACCAAAA	To study DNA methylation of the coding region of position 181 to 651 of E^1 , E^2 and E^3
24	BSPCF3 BSPCR3	TTTTGGTTTGAGTTTTTGAGG CAACAAATCCTTAACCAAAATCA	To study DNA methylation of the coding region of position 361 to 711 of E^1 , E^2 and E^3
25	BSPCF4	TGATTTGGTTAAGGATTTGTTG	To study DNA methylation of the coding region of position 689 to 1143

	BSPCR4	AAATAATCAATAACAAATTTA	of E^1 , E^2 and E^3
26	BSPCF5	TTTAAATTTGTTATTGATTATTTTTGT	To study DNA methylation of the coding region of position 1141 to 1411
	BSPCR5	CATTACTTAAAACCCACCCAA	of E^1 , E^2 and E^3
27	BSPCF6	GTTAGGGTTTAAGTGTAATAG	To study DNA methylation of the coding region of position 1365 to 1515
	BSPCR6	CCGACCGTTTTAAACACGAATC	of E^1 , E^2 and E^3
28	BSP1F	ATTTTTTGGTATTTTTTATAGGATTAT	To study DNA methylation of the promoter region of position -143 to 56
	BSP1R	AATAAACCRATAAACTTTTCC	of E^1
29	BSP1F	ATTTTTTGGTATTTTTTATAGGATTAT	To analyze DNA methylation of the promoter region of position -5042
	BSP2R	AATTCCTATTTATCRACAAAAAA	to -4843 of E^2 and E^3
30	BSP2F	TAAAGAAGATTTTTGTAGTTAGAGATAGA	To study DNA methylation of the promoter region of position -335 to -79
	BSP3R	ACTCATACTCATACAAACATACACA	and position -5234 to -4978 of E^2 and E^3
31	OF	ATGACGTCCGTTAACGTA	To produce marker specific for e
	GR	AAGGTGCCAGAACACTGCTC	
32	MF	CAATGTGTATGCCTGCATGA	To generate marker specific for E^2
	MR	CGAGTATCGAGCTATGTCTG	
33	AtSN1F	AGTGGTGGTTGTATAAGTTTAG	To amplify the <i>AtSN1</i> (positive control for bisulfite sequencing)
	AtSN1R	TAAACATAAAAAAAAAATTCCTTT	

*: *FAE1* gene-specific primer for the primary PCR. The adapter primer for the primary PCR is AP1 (5'-GTAATACGACTCACTATAGGGC -3') (Siebert et al., 1995).

** : *FAE1* gene-specific primer for the nested (secondary) PCR. The adapter primer for the nested PCR is AP2 (5'-ACTATAGGGCACGCGTGGT -3') (Siebert et al., 1995).