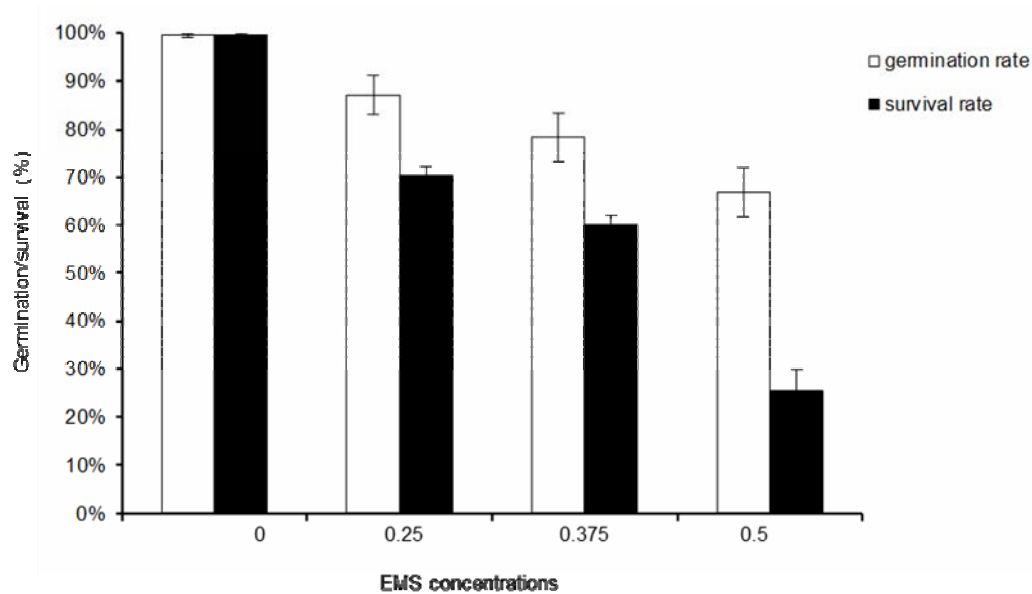


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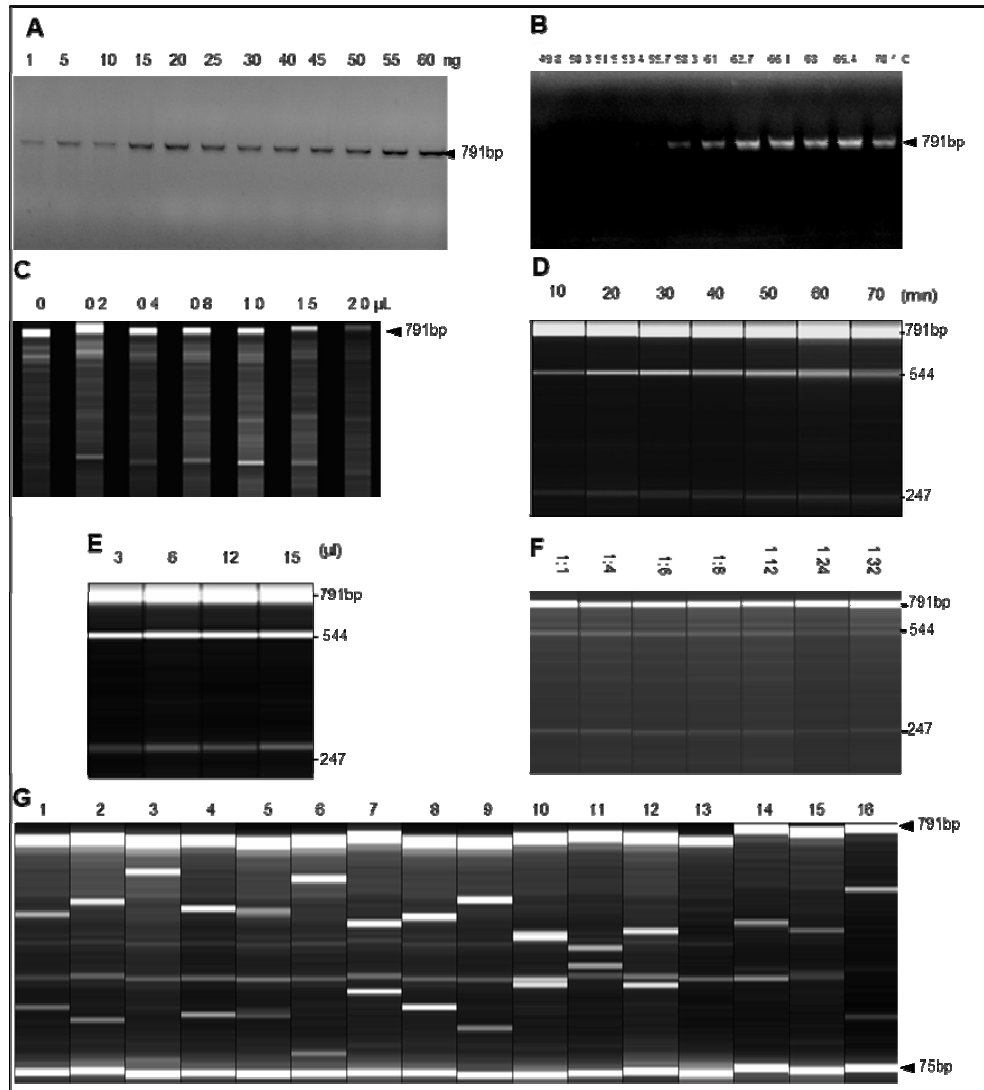
## An efficient TILLING platform for cultivated tobacco

### SUPPORTING INFORMATION



**Figure S1.** Germination and survival rates after EMS treatments.

Germination rates of M1 seeds (white bars) and survival rates of M1 plants (black bars) are shown as percentages. Data are shown as mean  $\pm$  SD ( $n \geq 3$ ).



**Figure S2.** Optimization of mutation detection using capillary electrophoresis

**(A)** PCR amplification with 1 to 60 ng (left to right) of genomic DNA. **(B)** PCR with annealing temperature of 50 to 70°C (left to right). **(C-G)** Virtual gel pictures from capillary electrophoresis. **(C)** CEL1 from 0 to 2 µL in a 6-µL reaction volume (left to right). **(D)** Reaction time of 10 to 70 min at 45°C (left to right). **(E)** CEL1 reaction volume of 3 to 15 µL (left to right). **(F)** Pools of 1:1 to 1:32 of mutant : wild-type DNA (left to right) were amplified, digested by CEL1, and detected by capillary electrophoresis. **(G)** Screening of a 791-bp fragment of *CHS* by TILLING; various mutations were identified by capillary electrophoresis (see Table S3). The 791-bp *CHS* gene fragment with an G/A mismatch was used as PCR template in (A) to (F).

\* 20 40 60 80  
 CYP82E10 : MVSPVEAIVGLVTITLFFYFIRKKKQKPSKPLPPKIPGGWPVIGHLFYFDDDSDDRPLARKLGDLDADKYGPVFTFRLGL : 80  
 CYP82E5 : MVSPVEAIVGLVTITLFFYFLPKKQKPSKPLPPKIPGGWPVIGHLFYFDDDSDDRPLARKLGDLDADKYGPVFTFRLGL : 80  
 W41G P42L  
 CYP82E4 : MLSPIEAIVGLVTITLFFFLWTKKQKPSKPLPPKIPGGWPVIGHLFYFNDGDDRPLARKLGDLDADKYGPVFTFRLGL : 80  
 M6SP6EAIVGLVTITLFF5F6wtKKsQkPSKPLPPKIPGGWPVIGHLFyF1DDgDDRPLARKLGDLDADKYGPVFTFRLGL

D100N L115F G116D P129L H153P  
 10 120 140 160  
 CYP82E10 : PLVLVSSSYEAVKDCFSSTND AIFS NRPAFLYGEYLGYNNAMFLTKYGPYWRKNRKLVIQEVLSASRLEKIKHVRFGHIC : 160  
 CYP82E5 : PLVLVSSSYEAVKDCFSSTND AIFS NRPAFLYGEYLGYNNAMFLTKYGPYWRKNRKLVIQEVLSASRLEKIKHVRFGHIC : 160  
 L125I W131\* V138I L151M  
 CYP82E4 : PLVLVSSSYEAVKDCFSSTND AIFS NRPAFLYGEYLGYNNAMFLTKYGPYWRKNRKLVIQEVLSASRLEKIKHVRFGHIC : 160  
 PLVLVSSSYEAVKDCFSSTND AIFS NRPAFLYGEYLGYNNAMFLTKYGPYWRKNRKLVIQEVLSASRLEKIKHVRFGHIC : 160  
 L190Q F226L W229\* P235L  
 180 200 220 240  
 CYP82E10 : TSIRKLYTRIDGNSSTINLTDWLEELNFGILIVKMIAGKNYESGKGDEQVERFKAFKDFIILSMFVLDADFPIPLFKWV : 240  
 CYP82E5 : TSIRKLYTRIDGNSSTINLTDWLEELNFGILIVKMIAGKNYESGKGDEQVERFKAFKDFIILSMFVLDADFPIPLFKWV : 240  
 D218  
 R169\* T180I G189D E225K A231T V240M  
 CYP82E4 : ASIRKLYTRIDGNSSTINLTDWLEELNFGILIVKMIAGKNYESGKGDEQVERFKAFKDFIILSMFVLDADFPIPLFKWV : 240  
 tSIRKLYTRIDGNSSTINLTDWLEELNFGILIVKMIAGKNYESGKGDEQVERF4KAFKDF6ILSMFVLDADFPIPLFKWV

\* 260 280 300 320  
 CYP82E10 : DFQGHVKAMKRTFKDIDSVFQNWLEEHVKKREKMEVNAEGNEQDFIDVLSKMSNEYLDGYSRDTVIKATVFSVLVDAA : 320  
 CYP82E5 : DFQGHVKAMKRTFKDIDSVFQNWLEEHVKKREKMEVNAEGNEQDFIDVLSKMSNEYLDGYSRDTVIKATVFSVLVDAA : 320  
 E272K E275K  
 CYP82E4 : DFQGHVKAMKRTFKDIDSVFQNWLEEHVKKREKMEVNAEGNEQDFIDVLSKMSNEYLDGYSRDTVIKATVFSVLVDAA : 320  
 DFQGHVKAMKRTFKDIDSVFQNWLEEH6K4EKMEVNA2GNEQDFIDVLSKMSNEYLDGYSRDTVIKATVFSVLVDAA

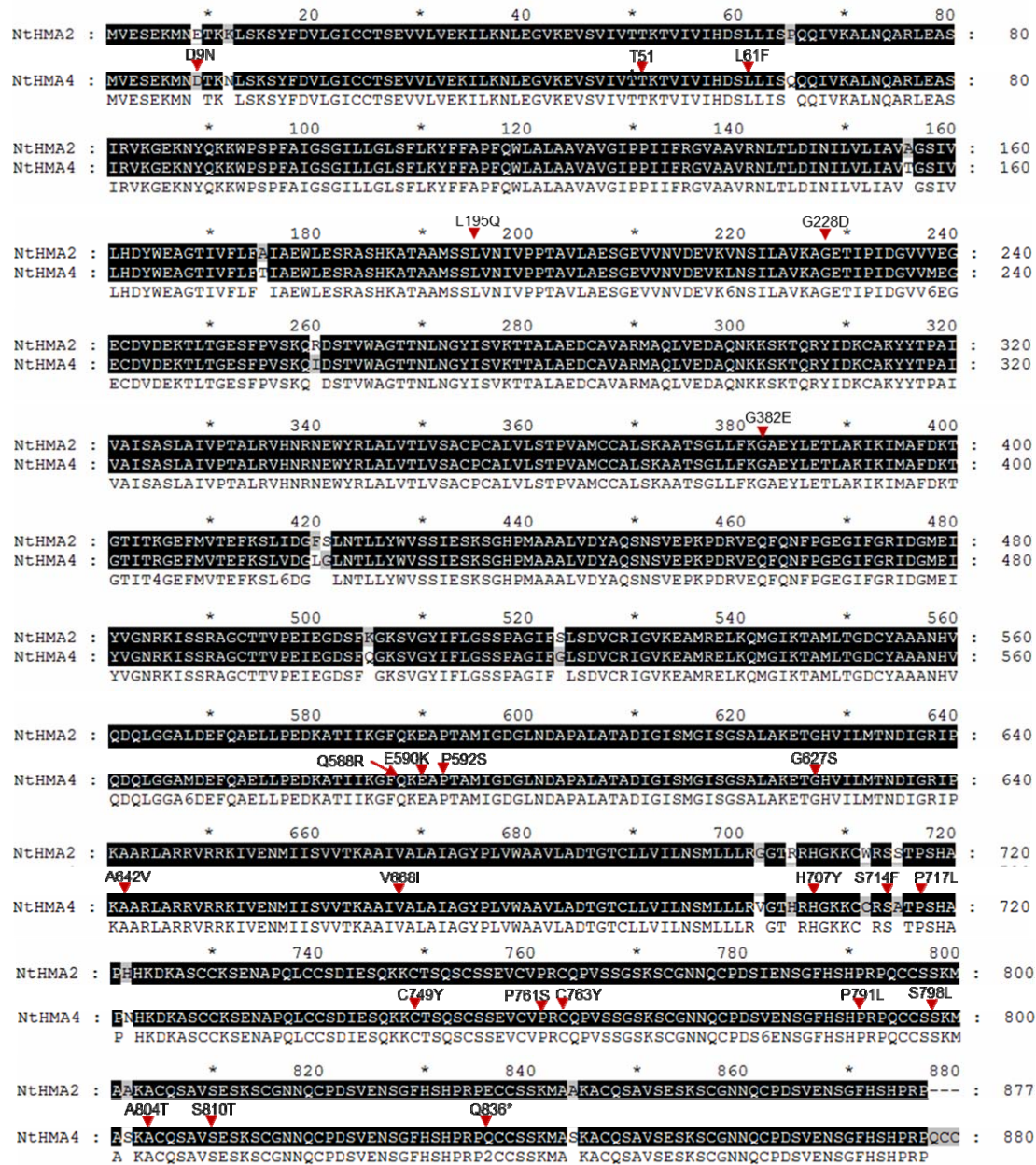
\* 340 360 380 400  
 CYP82E10 : DTVALHMNWGMALLINNQHALKKAQEEIDKKVKGDRWVEESDIKDLVYLQAIIVKEVLRLLYPPGPLLVPHENVEDCVVSGY : 400  
 CYP82E5 : DTVALHMNWGMALLINNQHALKKAQEEIDKKVKGDRWVEESDIKDLVYLQAIIVKEVLRLLYPPGPLLVPHENVEDCVVSGY : 400  
 CYP82E4 : DTVALHMNWGMALLINNQHALKKAQEEIDKKVKGDRWVEESDIKDLVYLQAIIVKEVLRLLYPPGPLLVPHENVEDCVVSGY : 400  
 DTVALH6NWGMALLINNQHALKKAQEEIDKKVKGDRWVEESDIKDLVYLQAIIVKEVLRLLYPPGPLLVPHENVEDCVVSGY

\* 420 440 460 480  
 CYP82E10 : HIPKGTRLFANVMKLRDPKLWSNPDKFDPERFFAIDIDFRGQHYEFIPFGSGRRSCPGMTYALQVEHLTIAHLIQGFNY : 480  
 CYP82E5 : HIPKGTRLFANVMKLRDPKLWSNPDKFDPERFFAIDIDYRGQHYEFIPFGSGRRSCPGMTYALQVEHLTIAHLIQGFNY : 480  
 CYP82E4 : HIPKGTRLFANVMKLRDPKLWSNPDKFDPERFFAIDIDFRGQHYEFIPFGSGRRSCPGMTYALQVEHLTIAHLIQGFNY : 480  
 HIPKGTRLFANVMKLRDPKLWS1PDkFDPERFFA ID5RGQhyE5IPFGSGRRSCPGMTYA6QVEHLT6AHLIQGFNY

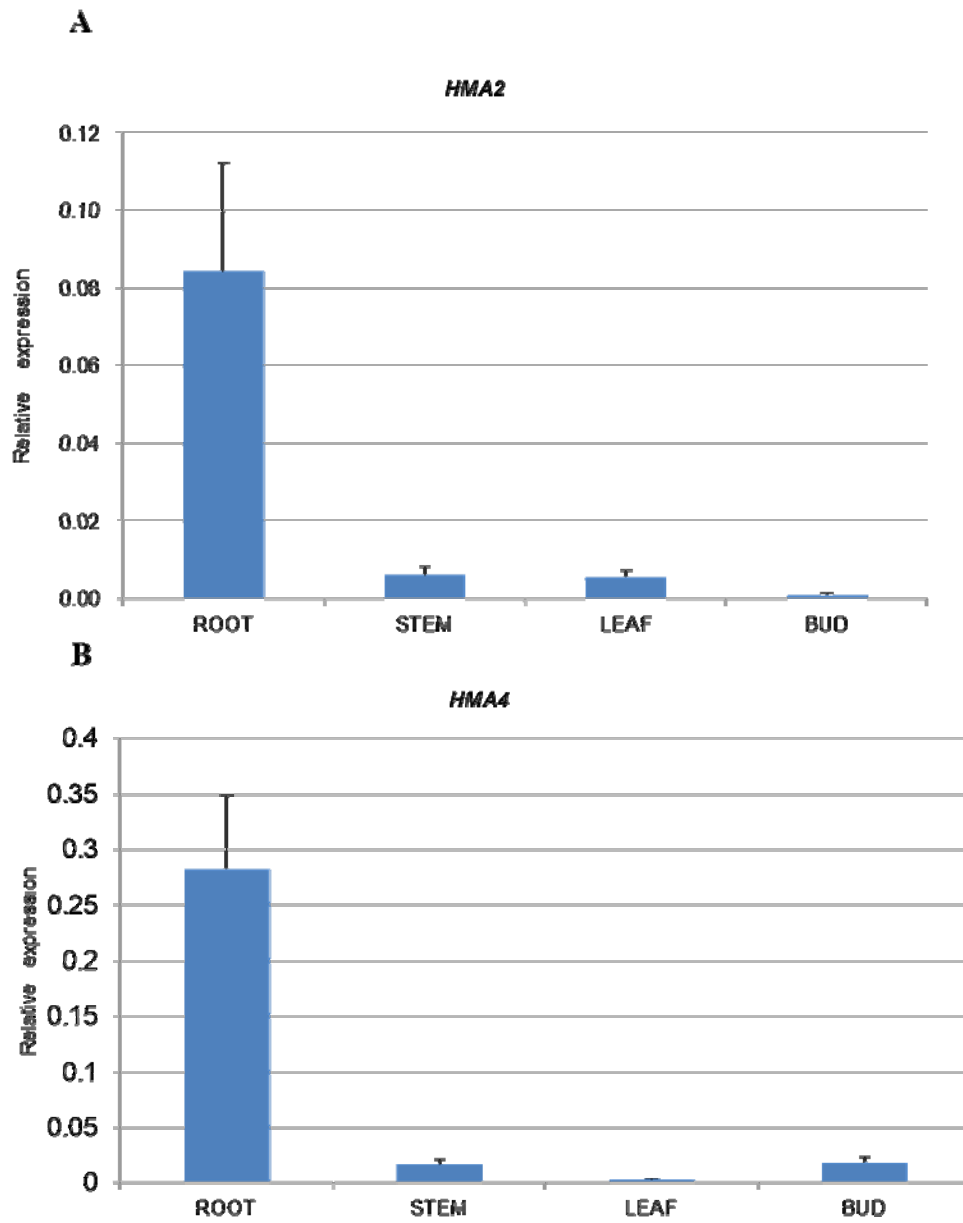
\* 500  
 CYP82E10 : KTPNDEPLDMKEGAGLTIRKVNPIEVVITRLTPELY : 517  
 CYP82E5 : KTPNDEPLDMKEGAGLTIRKVNPIEVVITRLTPELY : 517  
 CYP82E4 : KTPNDEPLDMKEGAGLTIRKVNPIEVVITRLTPELY : 517  
 4TPNDEPLDMKEGAG6TIRKVNPIEVVITRLTPELY

**Figure S3.** Alignment of CYP82E4T, CYP82E5S, and CYP82E10S protein sequences and positions of mutated amino acids.

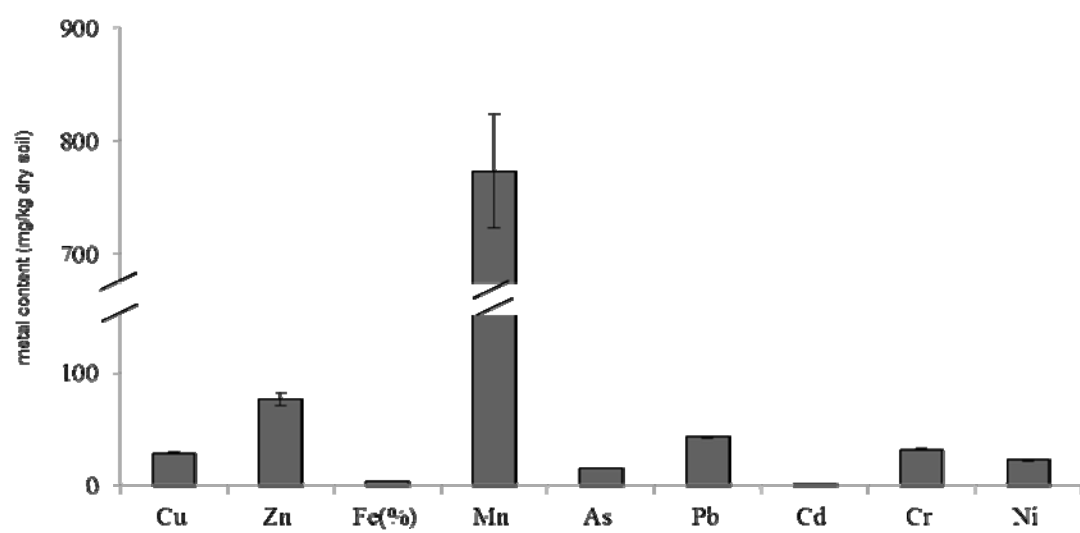




**Figure S5.** Alignment of HMA2S and HMA4T protein sequences and positions of mutated amino acids.



**Figure S6.** Expression pattern of *HMA2S* and *HMA4T* in different tobacco tissues.  
(A, B) Relative *HMA2S* and *HMA4T* gene expression levels determined by real-time PCR. Data are shown as mean  $\pm$  SD (n = 3).



**Figure S7.** Soil mineral contents.

**Table S1.** Summary of the EMS mutagenesis

| EMS concentration | Seeds treated       | Seeds germinated | M1 plants surviving | M1 plants transferred to field | Capsules harvested | Lines with seeds | M2 lines |
|-------------------|---------------------|------------------|---------------------|--------------------------------|--------------------|------------------|----------|
| 0.25%             | 20,000 <sup>a</sup> | 14,800 (74%)     | 11,784 (59%)        | 9,198                          | 3,926              | 3,014            | 2,847    |
| 0.375%            | 20,000 <sup>a</sup> | 10,200 (51%)     | ~2,020 (10%)        | NP                             | NP                 | NP               | NP       |
| 0.5%              | 20,000 <sup>a</sup> | 4,800 (24%)      | ~480 (2.4%)         | NP                             | NP                 | NP               | NP       |

<sup>a</sup> 2 g, roughly equal to 20,000 seeds; NP, not performed

**Table S2.** Phenotypes observed in the M2 generation (22,776 M2 lines examined)

| Phenotype                                   | Lines | Percentage (%) |
|---------------------------------------------|-------|----------------|
| <b>Mutants with leaf phenotypes</b>         | 861   | 3.78           |
| Altered leaf color                          | 475   | 2.08           |
| Rolled leaves                               | 327   | 1.44           |
| Spinach-like leaves                         | 59    | 0.26           |
| <b>Mutants with flower phenotypes</b>       | 510   | 2.24           |
| White petals                                | 258   | 1.13           |
| Red petals                                  | 98    | 0.43           |
| Small stamen                                | 143   | 0.63           |
| No Anther                                   | 7     | 0.03           |
| Filamentary petals                          | 3     | 0.01           |
| Fused stamen and petal                      | 1     | 0.004          |
| <b>Increased height</b>                     | 18    | 0.08           |
| <b>No bolting (no inflorescence formed)</b> | 75    | 0.33           |
| <b>Defected root development</b>            | 684   | 3.00           |
| <b>Sterile</b>                              | 6351  | 27.88          |
| <b>Total</b>                                | 9870  | 43.32          |

**Table S3.** Mutation positions of the *chalcone synthase* gene

| <b>Lane No.</b> | <b>Transition</b> | <b>Position</b> |
|-----------------|-------------------|-----------------|
| 1               | C/T               | 264/527         |
| 2               | C/T               | 227/564         |
| 3               | C/T               | 671/120         |
| 4               | A/G               | 544/247         |
| 5               | C/T               | 244/547         |
| 6               | G/A               | 143/648         |
| 7               | G/A               | 490/301         |
| 8               | G/A               | 267/524         |
| 9               | G/A               | 577/214         |
| 10              | G/A               | 324/467         |
| 11              | G/A               | 369/422         |
| 12              | G/A               | 318/473         |
| 13              | G/A               | 139/652         |
| 14              | G/A               | 319/472         |
| 15              | T/A               | 458/333         |
| 16              | G/A               | 571/220         |

**Table S4.** Primers used in this study

| <b>Primer</b>        | <b>Sequence (5' to 3')</b>     |
|----------------------|--------------------------------|
| JAZS-F               | AAACTTGTAATCTTTTCGAGCCAATTC    |
| JAZS-R               | ATCTGCTGGAAAATCATCAAATACAA     |
| HMA4T-F2(sec exon)   | TTATCCAGAACGCAGTAAGCCG         |
| HMA4T-R2(sec exon)   | CTTGGAAGAACATACTAGAGAACTT      |
| IPT7T-F              | GGGTGCTACTGGTTCTGGAA           |
| IPT7T-R              | TCTCCTTCGCTGCTTCTTCA           |
| CYP82E4T-F           | TCTCTATTTACCATATTATGGAATTATGCC |
| CYP82E4T-R           | AGACAACATAATCTAATACAAGCATTAGT  |
| CYP82E5S-F           | GGTAATTTTGTATTTATTATATTATGCG   |
| CYP82E5S-R           | TCATCCTTAGTATTTAGATAATCTAATT   |
| CYP82E10S-F          | GTCAAATACCACCTCTTAATAGTAA      |
| CYP82E10S-R          | AAAAGTCCCTATTGGTAGGAAGTGC      |
| HMA4T-F1(first exon) | TAGAGTGTAGAGGAAAAATAGAAAGAAGAG |
| HMA4T-R1(first exon) | ATAAGCTGAGAGCTTAAGAAAAAAGAAACT |
| HMA2S-F              | AATATAGAGATGGCCAATGAAGGT       |
| HMA2S-R              | CTAAAATTTATATGACCAAGTACTT      |
| CHS-F                | ATGCACTTAACAGAGGAAATCTTG       |
| CHS-R                | TTACTTAATACTTTTCTTGTAGCC       |

**Table S5.** Gene sequence accession numbers from NCBI

| <b>Gene name</b>        | <b>Accession</b> |
|-------------------------|------------------|
| <i>HMA2S</i>            | HF675180.1       |
| <i>HMA4T</i>            | HF937053.1       |
| <i>CYP82E4S</i>         | EF370115.1       |
| <i>CYP82E5S</i>         | EU182719.1       |
| <i>CYP82E10T</i>        | HM802352.1       |
| <i>IPT7T</i> (T genome) | XM_009632496.2   |
| <i>IPT7S</i> (S genome) | XM_016578062.1   |
| <i>JAZT</i> (T genome)  | XM_009623141.2   |
| <i>JAZS</i> (S genome)  | XM_016641277.1   |