A collection of enhancer trap insertional mutants for functional genomics in tomato

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Figure S1. Schematic representation of the T-DNA insertional mutagenesis programme described in this work. (a) Development of the enhancer trap collection using *Agrobacterium*-mediated transformation protocol with the binary vector pD991. (b) Phenotypic and GUS histochemical characterization of enhancer trap lines. (c) Molecular characterization of T-DNA integration sites.
Figure S2. Graphical representation of the distribution of T-DNA insertions (orange arrows) on tomato chromosomes (Ch). Black ovals on the chromosomes indicate the centromere and horizontal lines represent the size in megabases (Mb). Green plots represent the percentage of heterochromatin (% nucleotides per 500kb) and blue plots display the percentage of euchromatin (% nucleotides per 500kb).
Figure S3. Complementation test of 1381ETMM and lyrate mutations. F1 progeny obtained from a cross between wild-type heterozygous plants, one bearing the 1381ETMM mutation (female parent) and the other carrying the lyrate mutation (lyr2, accession number LA2923, male parent) showed the expected 3:1 segregation (18 WT : 8 mut; $\chi^2 = 0.50, P = 0.46$) of wild-type (WT) and mutant phenotypes. Mutant F1 plants were affected in the development of leaves (a), flowers (b) and fruits (c). Scale bar = 5 cm in (a); and 1 cm in (b) and (c).
**Figure S4.** Phenotypic characterization of RNA interference (RNAi) lines for the *Solyc11g011960*, which was the gene tagged by the T-DNA insertion in the 2477ETMM line. Leaves of the T1 RNAi - 2477ETMM plants displayed evident necrosis symptoms and a reduction of plant growth either under *in vitro* (a) or greenhouse (b) conditions, similar to those showed by the 2477ETMM insertional mutant. Scale bar = 1 cm in (a); and 10 cm in (b).
**Table S1.** Transformation efficiency in two tomato cultivars.

| Cultivar      | Inoculated explants | Transgenic plants \((2n + 4n)\) | Transgenic plants \((2n)\) | Ratio \(2N : 4N\) | Transf. frequency\(^a\) | % transgenic plants \((2n)\) |
|---------------|---------------------|----------------------------------|--------------------------|-------------------|-------------------------|-----------------------------|
| P73           | 4200                | 1816                             | 1021                     | 1 : 0.78          | 43.2%                   | 56.2%                       |
| Moneymaker    | 18500               | 6026                             | 4539                     | 1 : 0.33          | 32.6%                   | 75.3%                       |
| Total         | 22700               | 7842                             | 5560                     | 1 : 0.41          | 34.6%                   | 70.9%                       |

\(^a\)Transformation frequency was estimated as the number of independent transgenic events divided by the total number of inoculated leaf explants, then multiplied by 100.
**Table S2. Summary of reporter GUS expression.**

| Sample       | Number of lines showing GUS expression in \(^a\) | Number of lines showing GUS expression restricted to \(^b\) |
|--------------|-----------------------------------------------|----------------------------------------------------------|
| **Vegetative structures** |                                               |                                                          |
| Root         | 16                                            | 4                                                        |
| Stem         | 141                                           | 23                                                       |
| Rachis       | 117                                           | 4                                                        |
| Petiole      | 151                                           | 4                                                        |
| Leaflet      | 164                                           | 14                                                       |
| **Flowers**  |                                               |                                                          |
| Sepal        | 56                                            | 1                                                        |
| Petal        | 48                                            | 3                                                        |
| Stamen       | 359                                           | 219                                                      |
| Pistil       | 158                                           | 24                                                       |
| Stigma       | 38                                            | 4                                                        |
| Style        | 43                                            | 9                                                        |
| Ovary        | 56                                            | 7                                                        |
| Ovule        | 25                                            | 2                                                        |
| **Immature fruits** |                                           |                                                          |
| Pericarp     | 227                                           | 78                                                       |
| Placenta     | 103                                           | 13                                                       |
| Mucilage     | 49                                            | 6                                                        |
| Embryo       | 199                                           | 92                                                       |

\(^a\)Number of lines showing GUS expression in the evaluated tissue.

\(^b\)Number of lines displaying GUS staining restricted to the evaluated tissue.
**Table S3.** Primer sequences used for anchor PCR, genotyping and qRT-PCR analyses.

**A. Primers used for anchor PCR analysis**

| Primer name | Primer sequence (5’-3’) |
|-------------|-------------------------|
| Ad1         | CTAATACGACTCACTATAGGC   |
| Ad2         | CTATAGGGCTCGAGCGGC      |
| Ad3         | AGCGGCGGGGAGGT          |
| ARB-1       | ACAGTTTTTCGCGATCCAGAC   |
| ARB-2       | GGTCTTGCAGAGATAGTGG     |
| ARB-3       | CTGGCGTAATAGCGAAGAGG    |
| ALB-1       | TTGGCGTGTCAGCTATCTA     |
| ALB-2       | ATCGGTCgCTAATGCAAAAGG   |
| ALB-3       | ATAATAACCGCTGCGACATCTAC |

**B. Primers used for genotyping analysis**

| Primer name | Primer sequence (5’-3’) |
|-------------|-------------------------|
| Gt5-F       | AAGGAAGCTAGGAATCAACAAGA |
| Gt5-R       | ATTTCTCGGTGAAGGGGTTC    |
| Gt6-F       | TGCTCAATGAGTGTCGAAA     |
| Gt6-R       | TTGAATATATGGTCCCTGAA    |
| Gt11-F      | GAAGTGCGGGCAAGTGCTTTCA  |
| Gt11-R      | GAGGCGCGGGATCTATCTTTCC  |

**C. Primers used for qRT-PCR assays**

| Primer name  | Primer sequence (5’-3’) |
|--------------|-------------------------|
| 1381_Fz      | CATCCCCAACATGCTATTCTT   |
| 1381_Rz      | ATGCAGTGAAACCCCTCCATC   |
| 2477_Fz      | ATCCCGCGAAACGAAAGAGAG  |
| 2477_Rz      | GTGCATCCCATTGTTGTTC     |
| Ubiquitine3_Fz| CACACTTACCTTGCTTGCTGT  |
| Ubiquitine3_Rz| TAGTCTTTCCGGTGAGAGTCTTTCA |