Editors summary

Compact early fruiting tomato and groundcherry plants suitable for urban farming are produced using genome editing.

Rapid customization of Solanaceae fruit crops for urban agriculture

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Cultivation of crops in urban environments might reduce the environmental impact of food production[1–4]. However, lack of available land in cities and a need for rapid crop cycling to yield quickly and continuously mean that so far only lettuce and related 'leafy green' vegetables are cultivated in urban farms[5]. New fruit varieties with architectures and yields suitable for urban farming have proven difficult to breed[1,5]. We identified a regulator of tomato stem length (*SlER*) and devised a trait-stacking strategy to combine mutations for condensed shoots, rapid flowering (*SP5G*), and precocious growth termination (*SP*). Application of our strategy using one-step CRISPR-Cas9 genome editing restructured vine-like tomato plants into compact, early yielding plants suitable for urban agriculture. Field data confirmed yields were maintained, and we demonstrated cultivation in indoor farming systems. Targeting the same stem length regulator alone in groundcherry, another Solanaceae plant, also enabled engineering to a compact stature. Our approach can expand the repertoire of crops for urban agriculture.

The loss of arable land, driven by population growth, diminishing water resources and climate change poses a substantial challenge for the future of agriculture. Part of the solution will require increasing yields of the staple crops that feed humans and their livestock, such as corn, rice, soybean, and wheat, which are bred for high productivity in large-scale field conditions. A complementary approach, that might contribute to sustainable agriculture, is to grow more food in urban environments[1,2]. For example, although initial infrastructure costs can be high, rooftop farms and climate-controlled automated vertical farming systems optimize land use and are designed to be more environmentally friendly and sustainable than traditional farming[1,3,4]. However, the benefits of urban agriculture and its expansion are limited by the few crops that can be cultivated under highly restrictive growth parameters. Crop varieties that are both compact and rapid cycling are needed to optimize efficiency and productivity, and for these reasons, urban agriculture is currently limited to lettuce and related leafy green vegetables[1,5].

There is much interest in urban agriculture of fruits and berries but developing crop varieties suitable to the restrictive growth parameters of urban agriculture farming systems requires considerable modification. For example, commercial varieties were (and continue to be) bred for maximum productivity under typical greenhouse and field parameters. As an important component of the human diet and a major fruit crop, tomato is a promising candidate for growth

in urban farms. We previously showed that mutating two regulators of flowering in the universal florigen hormone system can convert tall, continuously growing "indeterminate" tomato plants into early yielding, compact "determinate" varieties. Natural and CRISPR-Cas9-induced mutations in the classical flowering repressor gene *SELF PRUNING* (*SP*) confer a determinate growth habit, and mutating its paralog *SP5G* in the *sp* background accelerates flowering and enhances plant compactness[6,7]. While these *sp sp5g* "double-determinate" genotypes are rapid cycling and productive when grown at high density in greenhouses and fields[7], even smaller plants that produce commercially viable yields would be better suited to urban agriculture. Specifically, although per plant fruit yield may be lower on smaller plants, this reduction can be compensated by growing more plants at higher density, thereby maintaining productivity in a limited growth space.

We reasoned that decreasing stem length between leaves and flowers (internodes) would further increase the compactness of *sp sp5g* double-determinate plants without likely compromising productivity. In a previous ethyl methanesulfonate (EMS) mutagenesis experiment with the standard plum tomato variety "M82"[8], we identified a dwarf mutant with shortened internodes and extremely compact inflorescences that form tight clusters of fruits (**Fig. 1a-d**). This mutant, designated *short internodes* (*si*), showed good fruit set and high fertility, and all vegetative and reproductive internodes and flower/fruit stems (pedicels) were substantially shorter than wild-type (WT) plants and *si*/+ heterozygotes (**Fig. 1a-d**, **Supplementary Fig. 1a**). These phenotypes closely resembled a monogenic recessive mutant called *short pedicel 1* (*spd1*) that was isolated in a separate mutagenesis experiment with M82[9,10]. We confirmed allelism, and mapping-by-sequencing positioned *si*/*spd1* to a large interval on chromosome 8 (**Supplementary Fig. 1b; Online Methods**). This region included the tomato ortholog of the classical Arabidopsis *ERECTA* (*ER*) gene, which is known to control internode length[11]. Notably, three EMS alleles, including one from a mutagenesis in the dwarf "MicroTom" genotype[12], carried point mutations that caused splicing defects and a premature stop codon (**Fig. 1e** and **Supplementary Fig. 1c-g, Supplementary Fig. 2**). In addition, CRISPR-Cas9 mutagenesis of tomato (denoted with "*Sl*" prefix) *ER* (*SlER*) resulted in null mutants with identical phenotypes as *si*/*spd1*, and these alleles also failed to complement the EMS mutants (**Fig. 1e** and **Supplementary Fig. 1h-k**).

We also identified the gene underlying *spd2,* a short internode mutant in the same class as *sler*, but with additional developmental defects that make it unsuitable for agriculture, including

sterility[10]. Mapping and cloning showed three EMS alleles had mutations in the tomato homolog of Arabidopsis *SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1* (*SERK1*) on chromosome 4, which in Arabidopsis functions in a complex with *ER* (**Supplementary Fig. 3a; Online Methods**)[13]. We found *slserk1* mutants showed severe developmental defects including fused stems and inflorescences, and parthenocarpic fruits, and less complex leaves (**Supplementary Fig. 3b**). The expression patterns of *SlSERK1* were similar to those of *SlER*, and *sler slserk1* double mutants showed *slserk1* is epistatic to *sler* (**Supplementary Fig. 3c-d**). We mutagenized *SlSERK1* by CRISPR-Cas9 and obtained several T_0 individuals that were chimeric for large deletion mutations and showed a range of severity similar to the EMS alleles (**Supplementary Fig. 3f-h**). Finally, we mutated *SlER-like 1* (*SlERL1*), a paralog of *SlER* that shares a similar expression pattern (**Fig. 1f, g**). While CRISPR-Cas9 generated *slerl1* mutants were indistinguishable from WT plants, *sler slerl1* double mutants showed severe pleiotropic growth defects resembling *spd2*/*slserk1* (**Fig. 1h-j**, **Supplementary Fig. 3i-k**).

Our results show conservation of function for *ER* and two of its interacting receptors, but for the purpose of agricultural application our primary interest was in the specific phenotype of short internodes caused by mutations in *SlER*. However, *ER* has been shown to have multiple roles in plant development, for example in meristem maintenance and stomatal patterning[14–18], which could impact agricultural productivity. To test agricultural performance of the *sler* mutant, specifically its potential to increase compactness of double determinate *sp sp5g* plants (**Fig. 2a**), we generated all combinations of double and triple *sp*, *sp5g*, and *sler* mutants in the M82 background and evaluated shoot architecture and yield components in greenhouses and agricultural fields (**Fig. 2; Online Methods**). Compared to *sp* determinate plants, *sp sler* plants produced condensed shoots with no yield loss (**Supplementary Fig. 4a-c**). Notably, the *sp sp5g sler* triple mutants were the most compact of all genotypes (**Fig. 2b-d**), and these "triple-determinate" plants were still early flowering and produced the same number of inflorescences and flowers as *sp sp5g* double-determinates (**Supplementary Fig. 4d**). Though a smaller fruit size caused a reduction in yield, harvest index (defined as the total yield per plant weight) of the triple-determinates exceeded *sp* determinate plants and matched *sp sp5g* double determinates (**Fig. 2d**). Together, these results suggest that CRISPR-Cas9 targeting of only three genes, controlling flowering time (*SP5G*), growth termination (*SP*), and stem length (*SlER*), can transform any tomato genotype into a compact, early yielding form.

Breeding medium and large-fruited varieties such as M82 for urban agriculture is not practical, because larger plants are needed to support the high metabolic and structural demands of fruits that also require more time to develop and ripen. We therefore focused on using CRISPR-Cas9 to generate a triple-determinate small-fruited variety. We targeted *SlER* in our previously generated "Sweet100" double-determinate plants[7], and as expected the resulting plants showed a triple-determinate form (**Fig. 3a, b** and **Supplementary Fig. 5a, b**). Important agronomic traits including flowering time, flower number, and sugar content (Brix) were the same as double-determinates, though fruit size was slightly decreased (**Supplementary Fig. 5c-e**). We tested if Sweet100 triple-determinate plants perform well under restricted space conditions by performing a high-density yield trial in agricultural fields (**Online Methods**). Less than 40 days after transplanting, both double-determinate and triple-determinate plants produced their first ripe fruits, providing early yield and rapid cycling (**Fig. 3c, d**). Importantly, triple-determinate plants had the smallest stature of all Sweet100 genotypes in all conditions, and yields were the same as double-determinates (**Fig. 3d** and **Supplementary Fig. 6**). We also found that the highly compact fruit clusters minimized fruit drop during harvest (**Supplementary Fig. 6c**). Finally, we demonstrated the first steps for cultivating our Sweet100 triple-determinate variety in both a light-emitting diode (LED) growth chamber and a self-contained, climate-controlled LED hydroponic vertical farm system (**Fig. 3e, f; see Methods**). Together, these results demonstrate that high performing triple-determinate small-fruited tomato varieties can be developed to accommodate the plant size and space restrictions of urban agriculture.

Mutations of *ER* cause similar effects on stem length in diverse plants (**Fig. 1a-d**)[11,19], suggesting that targeting this gene in other fruit crops could convert tall, bushy shoot architectures into more compact forms better adapted for both outdoor and indoor cultivation. We recently reported that CRISPR-Cas9 genome editing can be used to engineer domestication traits in the orphan Solanaceae fruit crop groundcherry (*Physalis grisea,* previously *Physalis pruinosa*)[20,21]. Groundcherry plants are typically large and bushy, with long stems between single-flower inflorescences, each of which produces a single sweet berry. To test if a compact groundcherry plant could be engineered by mutating the ortholog of *ER* (*PgER*), we identified both *PgER* and *PgERL1* using our genome and transcriptome assemblies[20], and targeted specifically *PgER* using two guide RNAs (**Fig. 4a, b**). Surprisingly, null *pger* mutants had a dwarf phenotype that was more severe than tomato *sler* mutants and resembled triple-

determinate plants (**Fig. 4c**). The shortened internodes and pedicels in the *pger* dwarf plants resulted in a remarkably high concentration of fruits on each shoot compared with WT plants (**Fig. 4c**). As for tomato, fruit size was reduced (**Fig. 4d**), but this could be compensated for by targeting regulators of fruit size, such as the homolog of the *CLAVATA1* gene[20]. Other important productivity traits, such as fruit number and sugar content, were the same as WT (**Fig. 4e**). We also compared the dwarf *pger* plants to a determinate groundcherry variety that we had produced by mutating the ortholog of *SP* (*PgSP*)[20]. Compared to WT, determinate (*pgsp*) groundcherry plants produce multiple flowers at each node, but growth terminates rapidly on all shoots, which limits fruit production[20]. We found that fruit number in *pger* plants was much higher than the determinate plants, making this new dwarf variety of groundcherry an attractive starting point for urban agriculture of this fruit (**Fig. 4f, g**).

Our strategy enables rapid engineering of two Solanaceae fruit crops to the most challenging agronomic parameters of urban agriculture: rapid cycling and compact plant size. Our CRISPR-Cas9 based approach will enable rapid modification of many other small-fruited tomato varieties into a triple-determinate growth habit by generating loss-of-function alleles of *SP, SP5G* and *SlER* in elite breeding lines. Alternatively, in cases where resources for genome editing are not available, the novel genetic diversity we have generated in these genes in a "plum" and "cherry" variety could easily be incorporated into traditional breeding programs.

Small-fruited tomato varieties have been bred for diverse colors, shapes, sizes, and flavor profiles in order to appeal to consumers; crossing these genotypes with our triple determinate plants would enable rapid selection for these highly desirable and heritable fruit quality traits. To demonstrate this, we generated F_2 populations between Sweet100 triple determinates and a "cocktail" and a "grape" tomato variety, and selected new triple determinate genotypes with larger and elongated fruits, respectively (**Supplementary Fig. 7**). Our alleles could also be used to customize plant compactness for specific agronomic needs. For example, *sp5g* and *sler* mutations could be combined to develop early yielding and shorter indeterminate varieties for urban greenhouses. In such cases, particularly when larger-fruited varieties are sought, a more subtle change in internode length might be beneficial, which could be achieved with weak *sler* alleles. Notably, one of our CRISPR-Cas9 alleles was a 6 bp in-frame mutation in the *SlER* LRR domain that resulted in a less severe effect on stem and pedicel length, and we also generated weak alleles by targeting the promoter of *SlER* (**Supplementary Fig. 8**)[22].

Our findings indicate that even closely-related species may require different genetic solutions to enable commercially viable growth in urban farms. Like groundcherry, the Solanaceae crop pepper is a bushy plant that produces single-flower inflorescences, and may benefit more from mutations in *ER* as opposed to *SP*[23]. Beyond the Solanaceae, reducing or eliminating *SP* function in cucumber and kiwifruit, respectively, also results in accelerated flowering and compact growth habits[24,25]. Targeting the coding sequence or regulatory regions of *ER* alone, or in combination with *SP,* could help customize these and other high-value fruit crops for urban agriculture.

Looking to the future, the gene targets and engineering strategies we have described, together with additional promising genetic targets for modifying flowering and plant size in other crops[26,27], are a key step towards the development of agricultural systems for space travel[28,29].

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Author contributions

C.-T.K. and Z.B.L designed the research and performed the experiments, J. H. and S.J.P. performed the MicroTom experiments and tomato transformation. Z.H.L. performed mapping analysis. Y.C. generated tomato CRISPR mutants. S.F.H. contributed to the tomato yield trial. J.V.E. performed tomato and groundcherry transformations. C.-T.K. and Z.B.L wrote the manuscript with editing contributed from all authors.

Competing interests

Z.B.L. is a paid consultant for and a member of the Scientific Strategy Board of Inari Agriculture, and he is also a named inventor on a number of patents and patent applications

(Patent Application Publications WO/2017/180474; WO/2014/081730A1; WO/2018/213547) directed to related technology that have been exclusively licensed from CSHL to Inari Agriculture.

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Figure legends

Fig. 1 Condensed shoots of the tomato *short internode* (*si*) mutant and identification of the underlying gene. a, Shoots and inflorescences of WT and *si* mutants. Arrowheads indicate inflorescences. **b**, Quantification of shoot lengths in WT, *si* and *si* heterozygotes (*si*/+). Prim., primary shoot and Axil, basal axillary shoot (Length between 1st inflorescence and 1st leaf); Symp., sympodial shoot (Length between 1st and 2nd inflorescence); n, number of plants. **c**, Inflorescences and mature fruits. DP, distal section of 1st pedicel; PP, proximal section of 1st pedicel; INT, 1st inflorescence internode; AZ, abscission zone. **d**, Quantification of inflorescence stem sections. n, number of inflorescences. **e**, The tomato *ERECTA* gene (*SlER*) and various ethyl methanesulfonate (EMS) and CRISPR-Cas9 generated alleles having identical phenotypes. **f**, Normalized expression (RPKM) for *SlER* and its paralog *SlER-like 1* (*SlERL1*) in meristems and major tissues. Sym. inflo., sympodial inflorescence; Sym. shoot; sympodial shoot. **g**, The *SlERL1* gene and CRISPR-Cas9 generated mutations. Guide RNA and protospacer-adjacent motif (PAM) sequences are highlighted in red and bold underlined, respectively. Blue dash and letter indicate deletion and insertion. Numbers in parentheses indicate gap lengths. **h**, Shoots and inflorescences of *slerl1* mutants compared to WT and *sler.* Arrowheads indicate inflorescences. **i**, Quantification of WT and *slerl1* inflorescence stem sections. n, number of inflorescences. **j**, *sler slerl1* double mutants. DAT, days after transplanting in **a**, **h**, and **j**. Box plots, 25th–75th percentile; center line, median; whiskers, full data range in **b**, **d** and **i**. *P* values (two-tailed, two-sample *t*-test) in **b**, **d** and **i**. The exact sample sizes (n) for each experimental group/conditions are given as discrete numbers in each panel.

Fig. 2 Creating highly compact, rapid flowering tomatoes by genome editing. a, A trait stacking strategy that combines mutations that cause precocious growth termination, rapid flowering, and shorter stems to create "triple-determinate" tomato varieties. **b**, A comparison of double (*sp sp5g*) and triple (*sp sp5g sler*) determinate tomato genotypes. Basal axillary shoots of *sp sp5g* and *sp sp5g sler*. Arrowheads indicate inflorescences. **c**, Mature plants and fruits (left) and associated shoots and inflorescences (right) from field-grown plants of double and triple determinate genotypes. Leaves were removed to expose fruits. Arrowheads indicate

inflorescences. **d**, Quantification of primary shoot height (length between first leaf and last inflorescence of primary shoot) and a field-based productivity trial comparing all three determinate genotypes. Harvest index, total yield/plant weight. n, number of plants, or inflorescences (for flower number). Box plots, 25^{th}–75^{th} percentile; center line, median; whiskers, full data range. *P* values (two-tailed, two-sample *t*-test). The exact sample sizes (n) for each experimental group/conditions are given as discrete numbers in each panel.

Fig. 3 CRISPR-Cas9 generation of a rapid cycling, highly compact cherry tomato variety. **a**, Shoots and inflorescences comparing double and triple determinate cultivars of cherry tomato variety Sweet100. Arrowheads indicate inflorescences. **b**, Quantification of shoot lengths and inflorescence stem sections, as in Figure 1. n, number of plants and inflorescences. **c**, Field-grown plants of Sweet100 *sp* determinate, *sp sp5g* double-determinate and *sp sp5g sler* triple-determinate plants at 50 days after transplanting. Both the double-determinate and triple-determinate plants show ripe fruits, but not determinate plants. **d**, Days after transplanting to first ripe fruit, primary shoot height and total yield in all three genotypes. **e**, Sweet100 triple-determinate plants producing ripe fruits in an LED growth chamber at 51 days after transplanting. **f**, More than 1000 Sweet100 triple-determinate plants cultivated in a hydroponic vertical farm system (**see Methods**). The triple-determinate plants produced open flowers 20 days after transplanting. n, number of plants. Box plots, 25^{th}–75^{th} percentile; center line, median; whiskers, full data range in **b** and **d**. the numbers represent *P* values (two-tailed, two-sample *t*-test) in **b** and **d**. The exact sample sizes (n) for each experimental group/conditions are given as discrete numbers in each panel.

Fig. 4 CRISPR-Cas9 generated compact groundcherry. a, Phylogenetic tree of the *ER* gene family in Arabidopsis, tomato, rice, *Amborella* (*A. trichopoda*) and groundcherry (*P. grisea*). Contigs for groundcherry (*Pg-t*) are from a previously published *de novo* transcriptome assembly. Tomato and groundcherry *ER* homologues are highlighted in red and orange, respectively. Bootstrap values from 100 replicates are represented on each node. **b**, Sequences of *pger* mutant alleles generated by CRISPR-Cas9. **c**, Shoots, inflorescences and fruit husks of WT

and *pger*. Arrowheads in insets indicate pedicels. **d**, Fruits of WT and *pger*. **e**, Pedicel length, shoot length, fruit weight, fruit number and sugar content of WT and *pger*. **f**, Representative field-grown *pgsp* and *pger* plants. Leaves were removed to expose fruits with husks. **g**, Total fruit number from *pgsp* and *pger* plants. n, number of inflorescences, plants and fruits in **e** and **g**. Box plots, 25^{th}–75^{th} percentile; center line, median; whiskers, full data range in **e** and **g**. The numbers represent *P* values (two-tailed, two-sample *t*-test) in **e** and **g**. The exact sample sizes (n) for each experimental group/conditions are given as discrete numbers in each panel.

Online Methods

Plant materials and growth conditions. Seeds of tomato cultivar M82, Sweet100, MicroTom, *short internode* (*si*) and groundcherry (*Physalis grisea* is the correct name for a North American plant that had been misnamed *P. pruinosa*)[21] were from our own stocks. The *short pedicel 1* (*spd1*) and *short pedicel 2* (*spd2*) mutants were obtained from Dani Zamir and Naomi Ori at Hebrew University, Israel. Seed of *sler* mutant in the MicroTom background (TOMJPE5066-1) was provided by the University of Tsukuba, Gene Research Center, through the National Bio-Resource Project (NBRP) of the AMED, Japan (http://tomatoma.nbrp.jp/).

Both tomato and groundcherry seeds were sown directly in soil in 96-cell plastic flats and grown to ~4 week-old seedling stage according to standard protocols. Seedlings were transplanted to pots in the greenhouse or fields 28-40 days after sowing. Briefly, plants were grown in a greenhouse under long-day conditions (16 h light, 26–28°C /8 h dark, 18–20°C; 40–60% relative humidity) supplemented with artificial light from high-pressure sodium bulbs (~250 μmol m^{-2} s^{-1}), the agricultural fields at Cold Spring Harbor Laboratory, the Cornell Long Island Horticultural Experiment Station, Riverhead, New York, and the Gulf Coast Research and Education Center, Wimauma, Florida. Plants were grown under drip irrigation and standard fertilizer regimes. Damaged or diseased plants were marked and excluded from data analyses.

CRISPR-Cas9 mutagenesis and plant transformation. CRISPR–Cas9 mutagenesis for tomato and groundcherry were performed as described previously[20,30–32]. Briefly, gRNAs were designed using the CRISPRdirect software[33] (https://crispr.dbcls.jp/) and binary vectors were built through Golden Gate cloning as described[34,35]. The final binary plasmids were introduced into the tomato cultivar M82, Sweet100 and groundcherry seedlings by *Agrobacterium tumefaciens*–mediated transformation as described previously[31,32]. Transplanting first-generation (T0) transgenic plants and genotyping of CRISPR-generated mutations were performed as previously described[20,36]. Guide RNA and primer sequences for genotyping can be found in the Supplementary Data File.

Plant phenotyping. Quantification data on tomato and groundcherry shoots and inflorescences were obtained from the individual plants grown in greenhouses and fields at Cold Spring Harbor Laboratory. Prior to phenotyping, all CRISPR-generated null mutants were backcrossed at least once to the M82 or Sweet100 cultivar, and genotyped by PCR and sprayed by 400 mg/liter

kanamycin to confirm absence of the transgene. All phenotyping was conducted on non-transgenic homozygous plants from selfing or backcrossing with WT plants. We manually measured pedicels, peduncles and inflorescence internodes when at least half of the flowers were opened in the inflorescences. Mature red fruits were used for measurement of fruit size and mass. All measurements were taken with an electronic digital caliper (Fowler). Shoot lengths and heights were evaluated with standard 30 cm and 100 cm rulers. Fruit mass was quantified by a digital scale (OHAUS). Data for flowering time, flower, inflorescence and fruit number were quantified from matched staged plants and inflorescences. For analyses of flowering time, we counted leaf numbers on the primary shoot before initiation of the first inflorescence as described previously[7]. Exact numbers of individuals for the quantification are indicated in all figures.

Mapping-by-sequencing. To map the locus underlying condensed shoot and inflorescence of *spd1*, we generated an F_2 segregating population by crossing *spd1* with the wild progenitor of tomato, *S. pimpinellifolium*. From a total of 96 *spd1* x *S. pimpinellifolium* F_2 plants, we selected 16 segregating *spd1* mutants and 12 WT siblings for tissue collection and DNA extraction. Tissue collection, library preparation, whole genome sequencing, mapping-by-sequencing and data analyses were followed as previously described[36]. The difference in allele frequency (ΔSNP index) between WT and *spd1* was evaluated for all pairwise comparisons. By plotting across the 12 tomato chromosomes, one large genomic region on chromosome 8 surpassed a genome-wide 95% cut-off in SNP index. Despite a large mapping interval, *SlER* was the top candidate gene.

The mapping of *spd2* was performed with an *spd2* x *S. pimpinellifolium* F_2 population. Bulked and individual mutant and WT sibling plants were used for mapping with a core set of PCR markers that scanned the genome. The candidate region was narrowed down to 564kbp in chromosome 4, and the *SlSERK1* candidate gene was sequenced from all EMS alleles, which revealed coding sequence mutations.

RNA extraction, cDNA synthesis and transcriptome profiling. For RNA extraction, leaf tissue was collected and immediately flash-frozen in liquid nitrogen. Total RNA from leaves was extracted using the RNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. 1 μg of total RNA was used for reverse transcriptase PCR using the SuperScript III First-Strand Synthesis System (Invitrogen).

Tissue-specific expression patterns for *SlER*, *SlERL1* and *SlSERK1* were obtained from the tomato tissue RNA sequencing database. All data from different tissues and meristems were procured from the tomato genome project transcriptome profiling data sets deposited in the Sequence Read Archive (SRA) under accession SRP010775 and our own tomato meristem maturation expression atlas[37].

Yield trials under agricultural field conditions. Tomato yield trials were performed as previously described with slight modification[7]. The yield trial for M82 *sp*, s*p sp5g*, *sp sp5g sler*, sp^{CR} and sp^{CR} $sler^{CR-1}$ was conducted on plants grown in the fields of the Gulf Coast Research and Education Center, Wimauma, Florida (May 21, 2019). The yield trial for Sweet100 *sp*, *sp sp5g* and *sp sp5g sler* was conducted on plants grown in the field of Cornell Long Island Horticultural Experiment Station, Riverhead, New York (August 9, 2019). Seeds were germinated in 96-cell flats in greenhouses and grown for 40 days in the greenhouse (Florida) or 30 (New York). Yield trials for this project were performed under higher-density planting of 2 plants/m^2 (Florida and New York) and 4 plants/m^2 (New York), with standard fertilizer regimes and drip irrigation. Each genotype was represented by ten biological replicates (Florida), and twelve biological replicates for yield per individual plant (New York). For block yield (randomized replicated block design), eight plants were planted in each block, and eight replicated blocks (2 plants/m^2 and 4 plants/m^2) were analyzed (New York). To evaluate fruit yield and plant weight, fruits and plants were manually separated from the plant and the soil, respectively. Total fruit yield was the sum of green and mature fruits (Red and breakers) from each plant. Harvest indices were calculated by dividing the total fruit weight by the vegetative biomass. Sugar content in fruit juice was determined by measuring the Brix value (percentage) with a digital Brix refractometer (ATAGO Palette). Exact numbers for individuals (n) of the yield trials are presented in all figures.

Growth conditions of LED growth chamber and hydroponic vertical farm.

To grow Sweet100 triple-determinate tomatoes in an LED growth chamber, seeds were sown in soil in flats with 32-cell plastic inserts. Seedlings were transplanted to pots in the LED growth chamber 17 and 20 days after sowing. Briefly, plants were grown under long-day conditions (16 h light, 26-28°C /8 h dark, 18-20°C; ambient humidity) with artificial light from LED (475 μmol

460 m^{-2} s^{-1}) with 4000k color temperature at Cornell University, Ithaca, New York. The chamber
461 dimensions were 1.12 m (width) x 0.74 m (depth) x 1.32 m (height). A total 18 pots were evenly
462 distributed in the growth chamber for high-density planting (1 plant/0.05m^2). Plants were grown
463 under overhead watering and standard fertilizer regimes.
464 To demonstrate the potential of Sweet100 triple-determinate tomatoes for hydroponic
465 vertical farming, seeds were sown in both peat moss plugs (Grow-tech) and peat/coco plugs
466 (iHort) in flats with plastic 200-cell inserts. Seedlings were grown in a greenhouse at Cold
467 Spring Harbor Laboratory and also a self-contained hydroponic farm inside of an upcycled
468 insulated shipping container designed and manufactured by Freight Farms based in Boston, MA.
469 Seedlings were grown under long-day conditions (16 h light, 26–28°C /8 h dark, 18–20°C; 40–
470 60% relative humidity) and with sub-irrigation containing 50ppm of JR Peters 15-5-15 Cal-Mg
471 fertilizer. Seedlings in the hydroponic farm were grown with artificial light from red/blue LED
472 (150–200 μmol m^{-2} s^{-1}). Five-week old seedlings were transplanted into 128 adjacent vertical
473 growing columns for higher-density planting (1 plant/0.03 m^2) and 64 vertical growing columns
474 in an alternating pattern comprised of a column of plants next to a column with no plants for
475 lower-density planting (1 plant/0.06 m^2). Equal numbers of columns containing 6 or 7 evenly
476 spaced plants were transplanted into each section. Plants in the columns were grown with
477 artificial light from red/blue LED (200 μmol m^{-2} s^{-1}) and the same long-day conditions.
478 Automated irrigation systems were operated with JR Peters 15-5-15 Cal-Mg fertilizer (pH 6.0–
479 6.4) on a 45-90 min on/30 min off cycle during the day cycle and with one 30-minute irrigation
480 cycle in the middle of the night cycle. The concentrations of the fertilizer were gradually
481 increased from 150 ppm to 350 ppm in accordance with plant age and size.
482
483 **Phylogenetic analysis.** Coding and peptide sequences were obtained for tomato, Arabidopsis
484 and rice *ER* family members from the Phytozome v12.1 database (phytozome.jgi.doe.gov)[38].
485 Putative orthologs in groundcherry transcriptome were identified by BLAST as previously
486 described[20]. Full length peptide sequences of Arabidopsis, rice, tomato, *Amborella* and
487 groundcherry *ER* family members were aligned with MAFFT version 7 (L-ins-i algorithm)[39].
488 Model selection and phylogenetic inference were both conducted using IQTree as implemented
489 on CIPRES[40,41]. Full name of *AmTr_v1.0_scaffold00069.214* and *AmTr_v1.0_scaffold00024.267*

are *evm_27.model.AmTr_v1.0_scaffold00069.214* and *evm_27.model.AmTr_v1.0_scaffold00024.267*, respectively.

Statistical analyses. For quantitative analyses, exact numbers of individuals (n) are presented in all figures. Statistical calculations were performed using Microsoft Excel and R (RStudio (vl.1.442)[42]. Statistical analyses were performed using a two-tailed, two-sample *t*-test and a one-way analysis of variance (ANOVA) with Tukey test, whenever appropriate. All raw data and the the exact sample sizes (n; number of shoots, inflorescences, fruits, and plants) for each experimental group/conditions are given as discrete numbers in each figure panel and in the Supplementary Data File. Additional information is available in Nature Research reporting summaries, which includes statements on statistics, software used and data availability.

Data availability

Raw data for all quantifications and primer sequences are included in a Supplementary Data file. Raw Sanger sequencing data files confirming CRISPR-Cas9 generated mutations are available in the Supplementary information. Seeds may be requested by contacting Z.B.L.

Online Methods References

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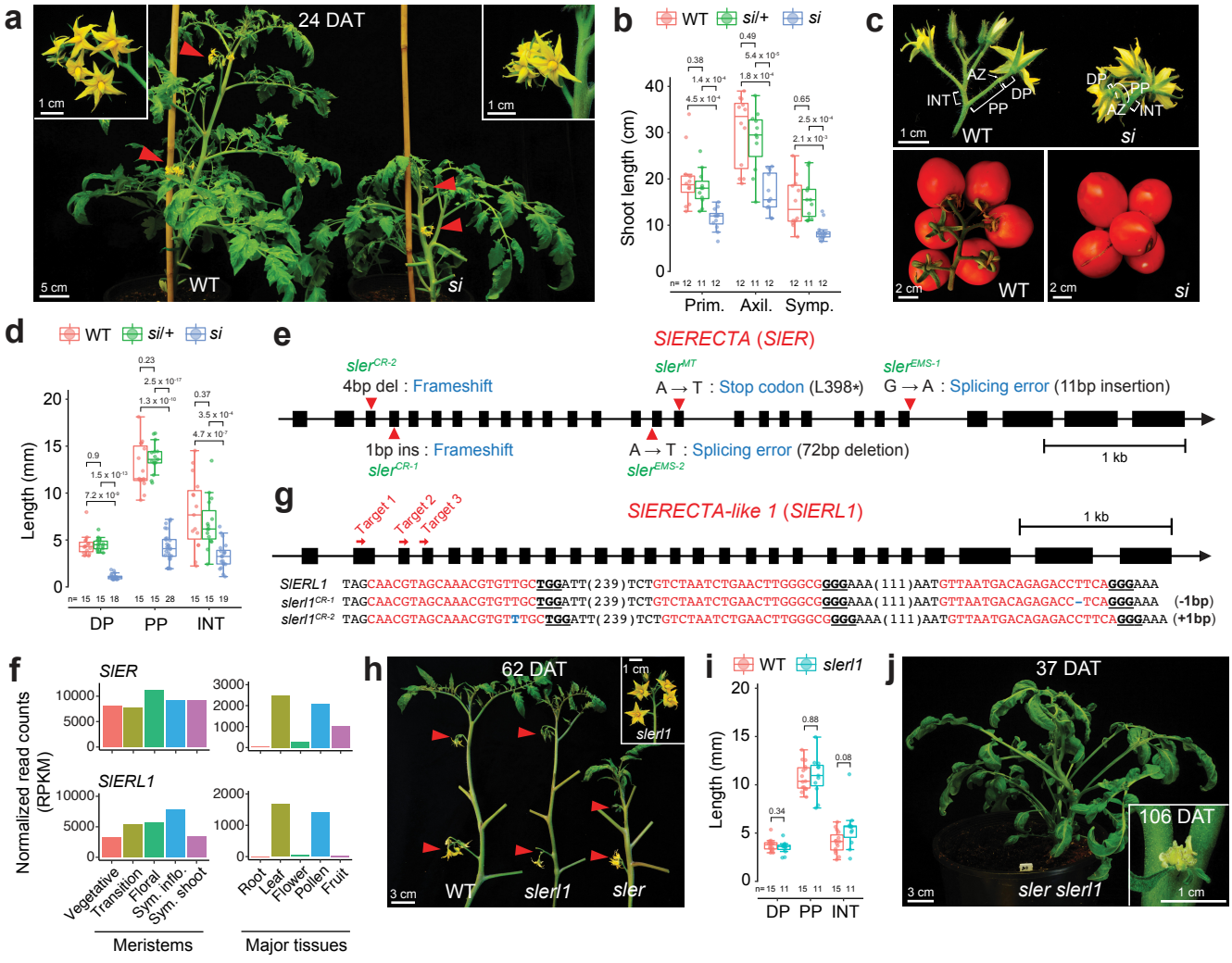
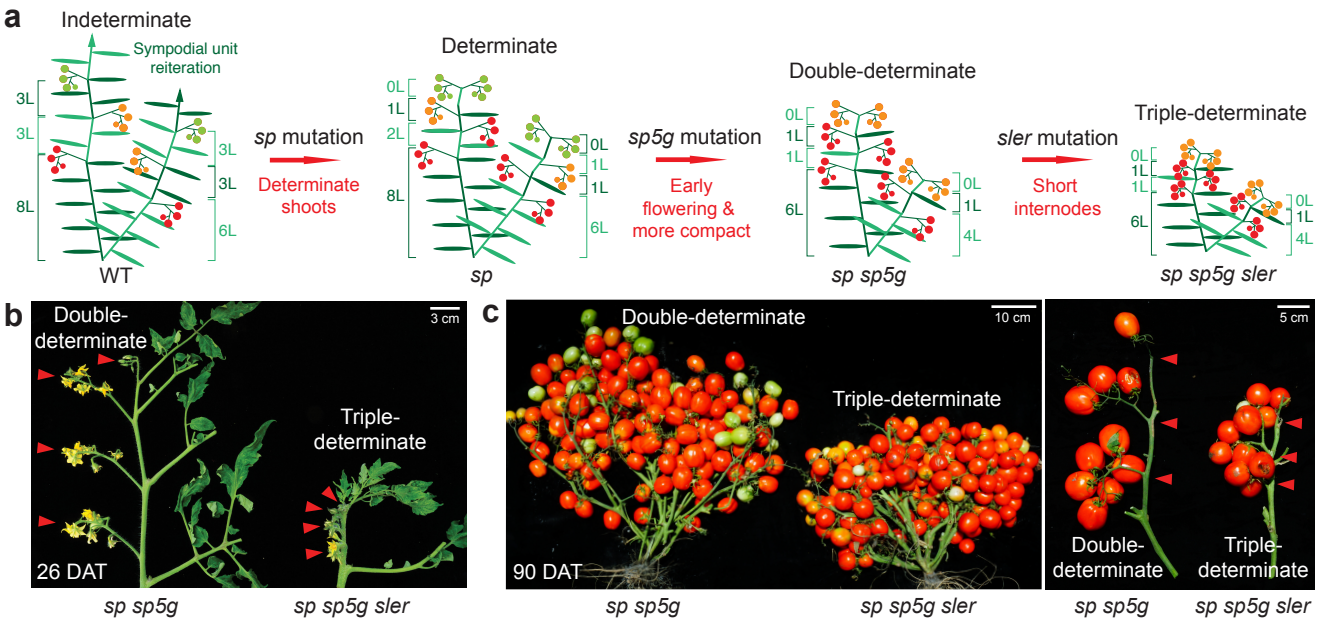
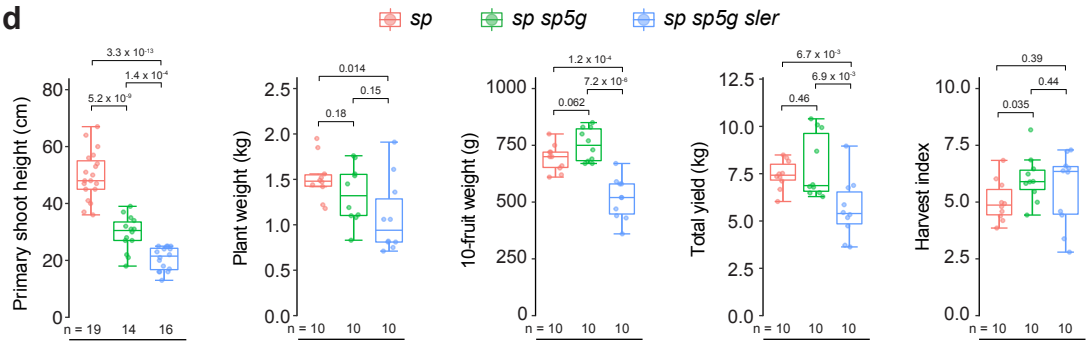
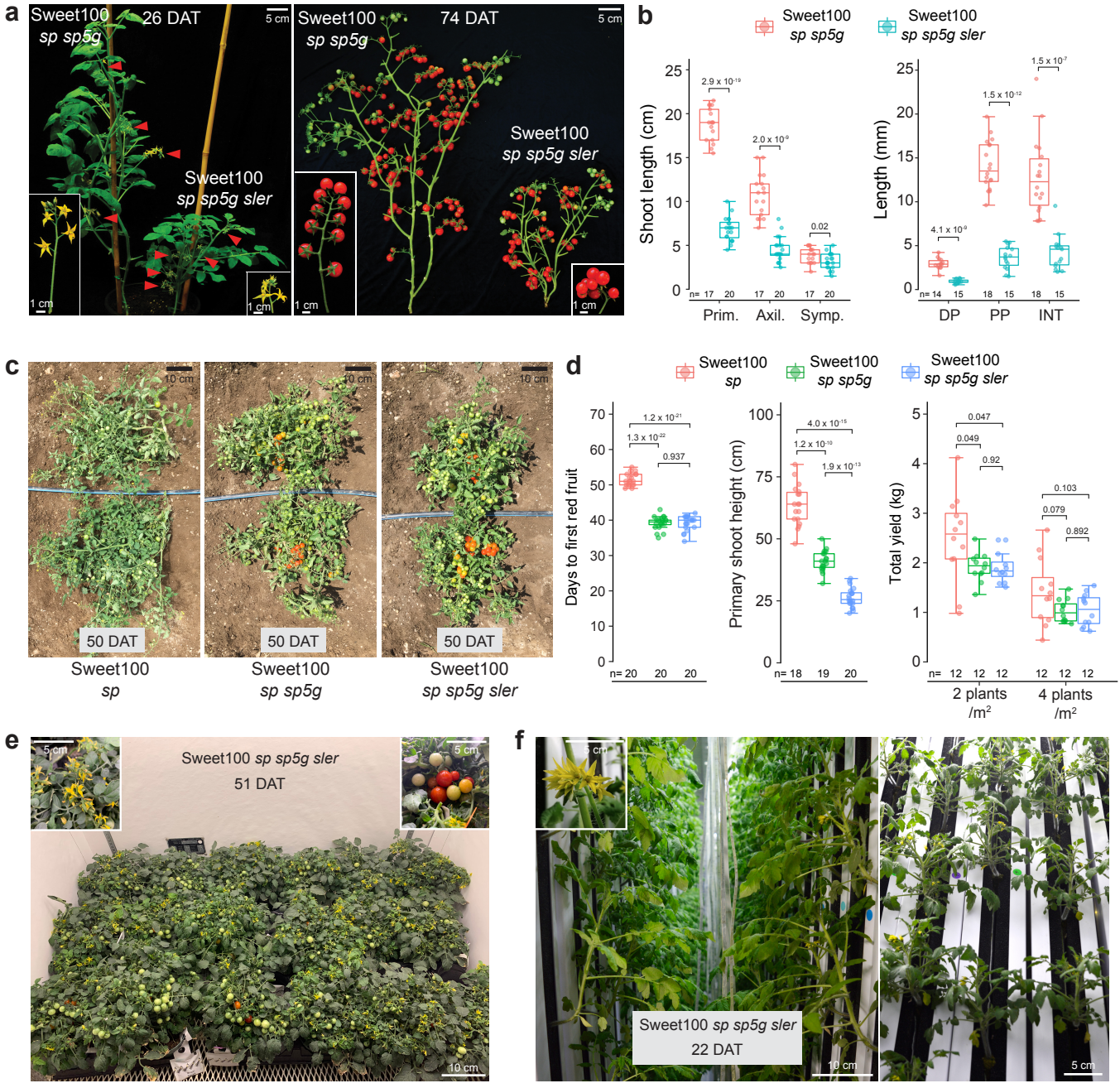
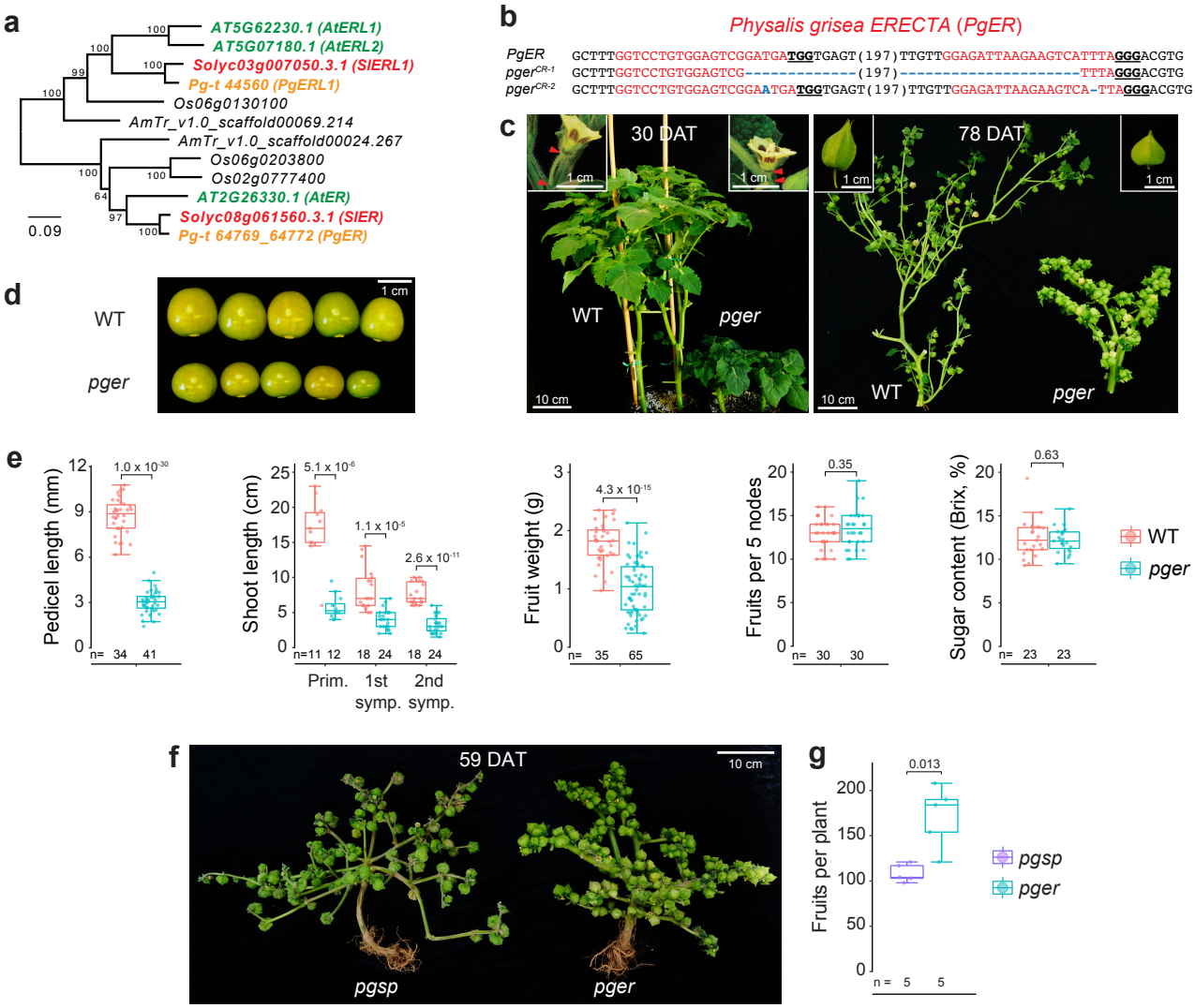

a
24 DAT
1 cm
1 cm
5 cm
WT
si
b
WT
si/+
si
Shoot length (cm)
Prim.
Axil.
Symp.
c
AZ
INT
DP
PP
1 cm
WT
si
2 cm
2 cm
d
WT
si/+
si
Length (mm)
DP
PP
INT
e
SlERECTA (SlER)
sler^CR-2
4bp del : Frameshift
sler^MT
A → T : Stop codon (L398*)
sler^EMS-1
G → A : Splicing error (11bp insertion)
1bp ins : Frameshift
sler^CR-1
A → T : Splicing error (72bp deletion)
sler^EMS-2
1 kb
g
SlERECTA-like 1 (SlERL1)
Target 1
Target 2
Target 3
1 kb
SlERL1 TAGCAACGTAGCAAACGTGTTGCTGGAATT(239)TCTGTCTAATCTGAACTTGGGCGGGGAAA(111)AATGTTAATGACAGAGACCTTCAGGGAAA
slerl1^CR-1 TAGCAACGTAGCAAACGTGTTGCTGGAATT(239)TCTGTCTAATCTGAACTTGGGCGGGAAA(111)AATGTTAATGACAGAGACC-TCAGGGAAA (-1bp)
slerl1^CR-2 TAGCAACGTAGCAAACGTGTTTGCTGGAATT(239)TCTGTCTAATCTGAACTTGGGCGGGAAA(111)AATGTTAATGACAGAGACCTTCAGGGAAA (+1bp)
f
SlER
SlERL1
Normalized read counts (RPKM)
Vegetative
Transition
Floral
Sym. inflo.
Sym. shoot
Root
Leaf
Flower
Pollen
Fruit
Meristems
Major tissues
h
62 DAT
1 cm
slerl1
3 cm
WT
slerl1
sler
i
WT
slerl1
Length (mm)
DP
PP
INT
j
37 DAT
106 DAT
1 cm
3 cm
sler slerl1


a
Indeterminate
Sympodial unit reiteration
3L
3L
8L
3L
3L
6L
WT
sp mutation
Determinate shoots
Determinate
0L
1L
2L
8L
0L
1L
1L
6L
sp
sp5g mutation
Early flowering & more compact
Double-determinate
0L
1L
1L
6L
0L
1L
4L
sp sp5g
sler mutation
Short internodes
Triple-determinate
0L
1L
1L
6L
0L
1L
4L
sp sp5g sler
b
Double-determinate
3 cm
Triple-determinate
26 DAT
sp sp5g
sp sp5g sler
c
Double-determinate
10 cm
Triple-determinate
90 DAT
sp sp5g
sp sp5g sler
5 cm
Double-determinate
Triple-determinate
sp sp5g
sp sp5g sler


d
sp
sp sp5g
sp sp5g sler
Primary shoot height (cm)
3.3 x 10⁻¹³
1.4 x 10⁻⁴
5.2 x 10⁻⁹
n = 19 14 16
Plant weight (kg)
0.014
0.15
0.18
n = 10 10 10
10-fruit weight (g)
1.2 x 10⁻⁴
7.2 x 10⁻⁶
0.062
n = 10 10 10
Total yield (kg)
6.7 x 10⁻³
6.9 x 10⁻³
0.46
n = 10 10 10
Harvest index
0.39
0.44
0.035
n = 10 10 10


a
Sweet100 sp sp5g
26 DAT
5 cm
Sweet100 sp sp5g sler
1 cm
Sweet100 sp sp5g
74 DAT
5 cm
Sweet100 sp sp5g sler
1 cm
b
Sweet100 sp sp5g
Sweet100 sp sp5g sler
Shoot length (cm)
2.9 x 10-19
2.0 x 10-9
0.02
n= 17 20 17 20 17 20
Prim. Axil. Symp.
Length (mm)
4.1 x 10-9
1.5 x 10-12
1.5 x 10-7
n= 14 15 18 15 18 15
DP PP INT
c
10 cm
50 DAT
Sweet100 sp
Sweet100 sp sp5g
Sweet100 sp sp5g sler
d
Sweet100 sp
Sweet100 sp sp5g
Sweet100 sp sp5g sler
Days to first red fruit
1.2 x 10-21
1.3 x 10-22
0.937
n= 20 20 20
Primary shoot height (cm)
4.0 x 10-15
1.2 x 10-10
1.9 x 10-13
n= 18 19 20
Total yield (kg)
0.047
0.049
0.92
0.103
0.079
0.892
n= 12 12 12 12 12 12
2 plants /m2
4 plants /m2
e
Sweet100 sp sp5g sler
51 DAT
5 cm
10 cm
f
Sweet100 sp sp5g sler
22 DAT
10 cm
5 cm


a
AT5G62230.1 (AtERL1)
AT5G07180.1 (AtERL2)
Solyc03g007050.3.1 (SlERL1)
Pg-t 44560 (PgERL1)
Os06g0130100
AmTr_v1.0_scaffold00069.214
AmTr_v1.0_scaffold00024.267
Os06g0203800
Os02g0777400
AT2G26330.1 (AtER)
Solyc08g061560.3.1 (SlER)
Pg-t 64769_64772 (PgER)
0.09
b
Physalis grisea ERECTA (PgER)
PgER GCTTTGGTCCTGTGGAGTCGGATGATGGTGAGT(197)TTGTTGGAGATTAAGAAGTCATTTAGGGACGTG
pger CR-1 GCTTTGGTCCTGTGGAGTCG----------(197)----------TTTAGGGACGTG
pger CR-2 GCTTTGGTCCTGTGGAGTCGGAATGATGGTGAGT(197)TTGTTGGAGATTAAGAAGTCA-TTAGGGACGTG
c
30 DAT
78 DAT
1 cm
WT
pger
10 cm
d
WT
pger
1 cm
e
Pedicel length (mm)
1.0 x 10-30
n= 34 41
Shoot length (cm)
5.1 x 10-6
1.1 x 10-5
2.6 x 10-11
n=11 12 18 24 18 24
Prim.
1st symp.
2nd symp.
Fruit weight (g)
4.3 x 10-15
n= 35 65
Fruits per 5 nodes
0.35
n= 30 30
Sugar content (Brix, %)
0.63
n= 23 23
WT
pger
f
59 DAT
10 cm
pgsp
pger
g
Fruits per plant
0.013
n = 5 5
pgsp
pger