Auxin-driven ecophysiological diversification of leaves in domesticated tomato

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One sentence summary: Distribution of heterobaric and homobaric leaves is controlled by natural variation in an auxin-related transcription factor

Summary

The study of crop diversification has focussed mainly on the genetic changes underlying traits favoured by humans. However, the passage from natural habitats to agronomic settings probably operated changes beyond those comprising the classical domestication syndrome. A deeper understanding of these traits and their genetic signature would be valuable to inform conventional crop breeding and *de novo* domestication of crop wild relatives. Heterobaric leaves have bundle sheath extensions (BSEs) that compartmentalise the sub-stomatal cavity whereas homobaric leaves do not; BSE development is known to be controlled by the *OBSCURAVENOSA* (*OBV*) locus and the *obv* mutant lacks BSEs whereas leaves carrying the wild type allele have BSEs. Here we identify the *OBV* gene and the causative mutation, a non-synonymous amino acid change. This mutation exists as a rare balanced polymorphism in the natural range of wild tomatoes, but has increased in frequency in domesticated tomatoes suggesting that the latter diversified into heterobaric and homobaric leaf types. The mutation disrupts a C2H2 zinc finger motif in the *OBV* protein, resulting in the absence of BSEs in leaves and here we show that this and other pleiotropic effects, including changes in leaf insertion angle, leaf margin serration, minor vein density and fruit shape, are controlled by *OBV* via changes in auxin signalling. Loss of function of the transcriptional regulator AUXIN RESPONSE FACTOR (*ARF4*) also results in defective BSE development, revealing an additional component of a novel genetic module controlling aspects of leaf development important for ecological adaptation and subject to breeding selections.

Crop domestication was driven by artificial selection to create a loose suite of traits known as the ‘domestication syndrome’ (1). Considerable effort has been devoted to unveiling the genetic basis of the domestication syndrome traits. However, the passage from natural ecosystems to increasingly sophisticated, human-managed ones created new drivers for crop evolution that may have had impact beyond the domestication syndrome (2). These drivers include geographic expansion beyond the crops’ centre of origin, natural selection under cultivation in a highly managed environment, and indirect selection due to constraints caused by developmental trait correlations and physiological trade-offs (3). The extent and breadth of the genetic signature created by those processes in crops is not yet known. Identifying such genetic variants and characterizing their consequences to plant development and function would lead to a more integrated understanding of crop ecology and provide valuable support to crop breeding and to the effort of creating new crops with *de novo* domestication pipelines (4, 5).

Leaf functional traits are key for resource acquisition and thus strongly constrained by developmental co-variation (6). For instance, high photosynthetic rates require high transpiration capacity, which in turn depends on water transport capacity (7). Leaf hydraulic conductance is a fundamental driver of leaf diversity, as it is linked with leaf shape, longevity and venation architecture (8). Notably, many angiosperm species have translucent leaf veins due to the presence of bundle sheath extensions (BSEs), compact columns of chlorophyll-less cells that link the veins to the leaf epidermis (9, 10) (Fig. 1A). BSEs have considerable functional impact, as they can divide the leaf mesophyll into compartments, resulting in patchy stomatal opening and non-uniform photosynthesis (11). Leaves harbouring such compartments created by BSEs are called heterobaric, as opposed to homobaric ones, where the leaf lamina is topologically continuous. The effect of BSEs on leaf compartmentalization can be demonstrated by water infiltration in the lamina (12). The distribution of these leaf functional types in natural ecosystems is highly skewed: in a climax forest, most species from the upper strata are heterobaric, whereas those dwelling in the understorey are homobaric (13). Likewise, some crops (*e.g.* soybean, sunflower) are heterobaric, whereas others (*e.g.* coffee, cacao) are homobaric (14). What determines the distribution of these leaf functional types in natural and agricultural environments? Identifying the genetic basis of BSEs development would provide a stepping stone to answer this question.

We previously reported that the tomato monogenic recessive mutant *obscuravenosa* (*obv*) lacks BSEs in leaves, leading to reduced water transport capacity: both stomatal conductance (g_s) and hydraulic conductance (K_{leaf}) are lower in the mutant compared to wild-type (WT) plants (15). Analysis of *S. pennellii* introgression lines (ILs) in tomato showed that *OBV* resides on a chromosome 5 interval defined by the bin d-5E (Fig. 1B and C), which contains 37 genes (Table S1) (16). A genome wide association study (GWAS) using 25 wild-type and 12 *obv* mutant accessions revealed a significant single nucleotide polymorphism (SNP) in Solyc05g054030 (Fig. 1D and fig. S1). The SNP is an A→G nucleotide change in the third exon of the gene coding sequence (CDS), resulting in a predicted histidine to arginine substitution on position 135 (H135R) of the protein (Fig. 1E), which is present in different tomato cultivars harbouring the *obv* mutation (Table S2). This genetic identity for *obv* was reported recently during the preparation of this manuscript (17). Combining genomic and passport information we analysed the geographic distribution of the mutation in the natural range of tomato wild relatives, including the ancestral species *S. pimpinellifolium* and found a sympatric cluster of mutant accessions in the lowlands of Ecuador and northern Peru (Fig. 1F). Nucleotide diversity analysis on 360 wild and domesticated tomato accessions (available on solgenomics.net) showed that *OBV* resides within a tomato domestication sweep (Fig. 1G) (18). We also found that the mutation increases in frequency between accessions along the wild-domesticated continuum (Fig. 1H).

To confirm the genetic identity of *OBV*, we transformed the *obv* mutant in tomato cv. Micro-Tom (MT-*obv*) (Figure 2A) with an overexpression (OE) vector for the CDS of Solyc05g054030. We observed correct BSE development leading to translucent veins in all *obv* plants harbouring the construct (Fig. 2B, and fig. S2). To assess whether the defect in BSE development in *obv* is indeed caused by a single, non-synonymous nucleotide change we constructed another OE vector with the *OBV* CDS but containing a A404G SNP and transformed it into the *obv* mutant. All lines expressed the transgene, but none reverted the dark-vein mutant phenotype lacking BSEs (Fig. 2C and fig. S3). We further confirmed that the *obv* mutant could be phenocopied by knocking down Solyc05g054030 expression with an RNA interference (RNAi) construct (Fig. 2D). We further crossed two homobaric cultivars (M82 and VFN8) with either the *obv* mutant in the MT background or the *obv* mutant complemented with the *OBV*-OE transgene (MT-*obv* *OBV*^{OE}). Hybrid plants derived

from M82 × MT-*obv* or VFN8 × MT-*obv* lacked BSEs leading to dark veins, but those derived from the cross with MT-*obv* *OBV*^{OE} showed phenotypic reversion, displaying translucent veins and BSEs (Fig. 2E and fig. S4). These results demonstrated that Solyc05g054030 is *OBV*, the gene responsible for BSE development in tomato leaves, and that a single amino acid change causes the *obv* mutation, which is responsible for the switch from heterobaric to homobaric leaves in tomato (14).

To better understand the nature of the genetic change underlying the *obv* mutation, we conducted *in silico* analyses of the gene and protein sequences of *OBV* (Fig. 2F). The complete sequence of the *OBV* gene encodes a 381 amino acid protein containing three Cys₂-Hys₂ (C2H2) Zn finger domains, which are associated with DNA-binding capability (19), an ethylene-responsive element binding factor-associated amphiphilic repression (EAR) domain, defined by the LxLxL motif and associated with transcriptional repression (20), and a carboxy-terminal coiled coil domain, which may function as molecular spacer or macromolecular scaffold (21). Gene ontology (GO) terms associated with this protein are nucleic acid binding (GO:0003676) and metal ion binding (GO:0046872) (Table S2). Using 324 protein sequences of the *OBV* orthologous group from 39 plant species (Table S3) we identified the H135R amino acid change of the *obv* mutant to a highly conserved motif of the second Zn finger domain (Fig. 2G). Protein modelling showed that the C2H2 motif is contained within a ββα structure, forming a functional unit stabilized internally by chelation of a single zinc ion (Fig. 2F). This suggests that the *obv* mutation leads to complete loss of protein function via disruption of its tertiary structure. Targeted phylogenetic analysis in relevant crop and model species revealed an ancient evolutionary origin of the *OBV* gene with great expansion due to duplication events (Fig. 2G and fig. S6). Protein sequence alignment of the Zn finger motif that contains the *obv* mutation in tomato showed that even the most phylogenetically distant species displayed high sequence identity across the domain, suggesting a conserved protein function (Fig. 2G). The closest *Arabidopsis* orthologs (*INDETERMINATE DOMAIN 14*, *15* and *16*), encode proteins involved in shoot gravitropism and regulation of auxin biosynthesis and transport (Table S4 and fig. S6) (22).

We next characterized the expression profile of *OBV*: the gene is highly expressed in meristems leaf primordia, expanding and mature leaves and flowers (fig. S7A and B). Increased *OBV* expression levels in transgenic plants led to changes on leaf insertion angle,

leaf margin serration and fruit shape (fig. S7C-E), which hinted at a potential role for auxin as a mediator of BSE development. Auxins, like other plant hormones, exert their effect through alterations in metabolism, transport, and sensitivity (23). We found no differences either in auxin content (fig. S9A) nor in polar auxin transport (fig. S9B) between wild type, *obv* mutant and *OBV^{OE}* lines in leaf primordia. However, GUS expression driven by the *DR5* auxin-induced promoter was increased in the *obv* mutant (Fig. 3A) but decreased in the *OBV^{OE}* lines (fig. S10). All the lines responded to exogenous auxin application with high GUS expression (Fig. 3A and fig. S10). Exogenous auxin application also led to higher inhibition of hypocotyl elongation and stimulation of *in vitro* rhizogenesis in *obv* explants, compared to WT or *OBV^{OE}* lines (fig. S11). Taken together, the results suggested that *OBV* could be responsible for changes in auxin signalling. Thus, we assessed the expression profiles of *AUXIN RESPONSE FACTOR* (*ARFs*) and *AUXIN/INDOLE-3-ACETIC ACID* (*Aux/IAAs*) transcriptional regulators, which are components of auxin signal transduction (24). We found consistent upregulation of some *ARFs* (*ARF3*, *9B*, *10B*, *19* and *AUX/IAA4* and *14*) and downregulation of some *Aux/IAAs* (*Aux/IAA1A*, *1B*, *2*, *3*, *11*, *13*, *26* and *35*) in *OBV^{OE}* lines compared to WT (Fig. 3B). Regarding *obv*, expression differences were found for *ARF4* and *Aux/IAA15*, both of which were clearly upregulated in the *obv* mutant compared to WT (Fig. 3B).

We then tested the potential role of *ARF4* in BSE development using a CRISPR/Cas9-generated knockout mutant (*arf4-CR*) and a transcriptionally silenced line harbouring an *ARF4*-antisense (*ARF4-as*) transgene (25). We confirmed the lack of BSEs and the associated dark vein phenotype in leaves of both *arf4-CR* and *ARF4-as* plants (Fig. 3C and fig S11). Further, *OBV* expression was not altered in *arf4* and *ARF4-as* leaves (Fig. 3D) but increased four-fold in the *obv* mutant, suggesting the existence of a hypothetical negative feedback mechanism at the transcriptional level. *ARF4* expression was increased in the *obv* mutant but not in the *OBV^{OE}* lines (Fig. 3E). Taken together, these results suggest that *OBV* controls BSE development via interaction with the auxin signalling machinery.

As *OBV* modulates auxin sensitivity and signaling, we tested whether allelic variation in *OBV* also determines the link between leaf structure and function. We first determined that leaf vein density (vein length per leaf area, VLA) was reduced in *obv* plants (Fig 4A and B). Vein architecture plays a key role in carbon assimilation rate and water distribution within

the leaf (26). K_{leaf} is a measure of how efficiently water is transported through the leaf: We found that the *obv* mutant showed reduced K_{leaf} , which was restored to wild-type levels in *OBV*^{OE} complemented lines (Fig. 4C). A regression analysis between VLA and K_{leaf} showed a strong positive correlation ($r=0.811$, $p<0.001$), with a coefficient of determination (R^2) of 0.657 (Fig. 4D). This suggests that variation in VLA caused by *OBV* is a major determinant of variation in K_{leaf} . Interestingly, an auxin biosynthesis mutant with reduced VLA showed reduced K_{leaf} and photosynthetic rate in pea (*Pisum sativum*) (27). Net assimilation of CO₂ (A_n)-chloroplastic CO₂ concentration curves (C_c) (Fig. 4E) revealed no difference in maximum Rubisco carboxylation rate (V_{cmax}) (Table S6) between genotypes. However, the maximum rate of light-saturated net CO₂ assimilation (A_{max}) was lower in the *obv* mutant (Fig. 4E and F and Table S7). This suggests that the presence of BSEs overrides diffusive limitations to photosynthesis through their effect on the water transport capacity of the plants (28, 29).

Leaf BSEs fulfill significant roles in leaf function with large ecophysiological impact. Firstly, by connecting the vascular bundles to the leaf epidermis, BSEs minimize the extra-xylematic path length and favor a greater hydraulic integration in the leaf lamina. The stomata of heterobaric leaves may therefore operate closer to the point of embolism, while responding faster to sudden changes in xylem water potential (30). To some extent, this could explain the distribution of heterobaric wild tomato accessions in more arid habitats with erratic rainfall patterns (31). Secondly, photosynthetic assimilation rates are higher in heterobaric leaves due to the optimization of light transmission within the leaf lamina. BSEs can function as ‘transparent windows’ that enrich neighbouring mesophyll cells, or, in the case of C₄ plants, the bundle sheath itself, with high levels of photosynthetically active radiation (400-700 nm) (32, 33). Lastly, the presence of BSEs increases the plasticity of minor vein density in response to growth light intensity, adjusting water supply and photosynthetic rate to specific environmental conditions (12). The sum of these effects on leaf function suggests that the presence of BSEs has adaptive value. This raises the question of what the main driver for the loss of BSEs in many domesticated tomato cultivars could be. One possible explanation is that the heterobaric/homobaric ecological distribution logic was broken down by the transition to an agricultural setting and, in this case, the homobaric phenotype is functionally more advantageous due a trade-off with the structural and functional costs of

BSEs (34). Furthermore, since the *obv* mutation increases sensitivity to auxin, we cannot exclude the selection of a favorable pleiotropic effect of this hormone on plant development and productivity (35).

In this report we have identified an allelic variant in *OBV*, a gene controlling the development of BSEs, which impacts leaf anatomy and function in tomato and other important crop species. We show that the OBV protein is highly conserved among angiosperms and that it is a key determinant of leaf functional type. BSE development is strongly linked to auxin signalling, involving *ARF4*, whose loss of function also impairs BSE formation. Our findings represent an entry point to unravel leaf functional design through a gene-focused approach and provide a molecular anchor for the analysis of phenotypic co-variation in leaf anatomical and physiological traits. Our results also suggest that leaf functional type results from a trade-off probably conditioned by environmental factors. The conservation of *OBV* in angiosperms could be leveraged to explore its roles in other plant major crop species and potentially tailor different leaf types to specific agronomic settings. For instance, BSEs were recently shown to provide a pathway for P uptake following foliar fertilization in spring barley (*Hordeum vulgare*) (36). Thus, our discovery of a simple genetic switch between leaf functional types could have important ecological implications and agricultural applications.

Figures

Figure 1 – Mapping and identification of a candidate gene for the *obscuravenosa* (*obv*) mutation. (A) Schematic cross-sectional comparison of heterobaric and homobaric leaves. In the heterobaric leaves (*e.g.* the tomato wild relative *Solanum pennellii*) the bundle sheath extensions (BSEs) are visible as a translucent network of veins, whereas in homobaric leaves (*e.g.* cultivars of tomato, *S. lycopersicum*, harbouring the *obv* mutation, such as cv. M82) the leaf lamina appears uniformly green. (B) Leaf phenotype of tomato cv. M82 and the *S. pennellii* introgression lines (IL) 5-3, 5-4 and 5-5. Scale bars, 1 cm. (C) Bin-mapping of the genomic region containing the *obv* mutation using ILs sequences. (D) Association plot of the Genome-Wide Association Study (GWAS) for the *obv* phenotype: the x-axis represents the chromosome region where Solyc05g054030 is located, and the y-axis represents the negative log₁₀ of *p*-values per SNP derived from the association analysis. The red horizontal line represents the Bonferroni-corrected genome-wide significance threshold ($p=1.554 \cdot 10^{-8}$). The boxes represent the open reading frame of Solyc05g054030. The SNP (an A-to-G transition) located in the third exon of Solyc05g054030 is strongly associated with the *obv* phenotype. (E) Gene structure of Solyc05g054030 showing exons (boxes) and introns (lines) and the position of the SNP (red arrowhead) in the third exon and DNA and amino acid sequence of the region around the SNP, showing that it represents a non-synonymous mutation leading to the substitution of a Histidine (H) in the wild-type (WT) for an Arginine (R) in the *obv* mutant. (F) Geographic distribution of accessions of wild tomatoes on the Pacific coast of Peru, Ecuador and the Galapagos Islands (inset) harbouring either the WT or mutant *obv* allele. Scale bar, 100 km. (G) Nucleotide diversity (π) of the ancestral wild species *S. pimpinellifolium* (PIN), *S. lycopersicum* var. *cerasiforme* (CER) and big-fruit cultivars (BIG) of domesticated tomato. The horizontal lines indicates genome-wide top 5% cutoff ratio for domestication sweeps. (H) Frequency of the *obv* mutation in four different categories of tomato accessions (*S. pimpinellifolium*; *S. lycopersicum* var. *cerasiforme*; *S. lycopersicum* fresh or processing cultivars).

group of 324 proteins from 39 plant species. The total height of the letters depicts the information content of the position in bits. Right, 3D model of ZnF2 showing the relative positional arrangement of the C2H2 residues and the Zn ion ligand. (G) Left, maximum likelihood protein sequence tree of the *OBV* gene in selected model and crop species. Symbols on nodes represent bootstrap support values: full circles >0.75, open circles <0.75. Right, partial protein sequence alignment focused on the second ZnF and showing the Histidine (H) residue affected by the *obv* mutation in tomato (red). Shading indicates similarity to consensus (according to Blosum62 score matrix with a threshold of 1): black 100%; dark grey 80-99%; light grey 60-79%; white <60%. The bar on the top indicates the mean pairwise identity over all pairs in the column: green 100%; brown 30-99%, red <30%.

Figure 3 – Interaction between *OBV* and auxin signaling in the control of leaf functional type. (A) Histochemical GUS analysis in transgenic lines harbouring a homozygous *DR5::GUS* construct in either wild type (WT) Micro-Tom or *obv* mutant background. Seedlings were either pre-treated (+IAA) or not (mock) with 20 μ M of indol-3-acetic acid (IAA). Scale bar = 1 cm. (B) Transcriptional profile of *AUXIN RESPONSE FACTOR (ARF)* and *AUXIN/INDOLE-3-ACETIC (Aux/IAA)* genes from leaf primordia. Heat map represents the transcript profiles in two *OBV* overexpression (OE) homozygous transgenic lines and the *obv* mutant. Values represent means of four biological replicates normalized against the corresponding WT sample. Statistically significant increases (red) or decreases (blue) in comparison with MT are represented by colored squares ($p < 0.05$). (C) Representative leaves and terminal leaflets of MT, a CRISPR *ARF4* mutant (*arf4*-CR) and an antisense *ARF4* knockdown line (*arf4*-as). Scale bars = 1 cm. (D) Relative *OBV* mRNA levels in young leaves of MT, *arf4*-CR, *arf4*-as and *obv*. p -values show significant differences to the *obv* mutant. (E) Relative *ARF4* mRNA levels in young leaves of MT, homozygous transgenic lines overexpressing (OE) *OBV* and the *obv* mutant. p -values show significant differences to the *obv* mutant. Bars represent mean $\pm$ s.e.m. ($n=4$). A one-way analysis of variance (ANOVA) test compared the means, where significant ($p < 0.05$), values are shown.

Figure 4 - *OBV* controls vein development and leaf hydraulic conductance for hydrated leaves ($K_{\text{leaf}}^{\text{max}}$). (A) Representative micrographs of cleared terminal leaflets of wild-type (WT) tomato cv. Micro-Tom (MT), the *obscuravenosa* (*obv*) mutant, and two independent *OBV* overexpression (OE) lines sections in either WT or *obv* background. Scale bars, 100 μ m. (B) Leaf vein density in WT, *obv* and the transgenic lines. (C) values for tomato cv. M82 (*obv* mutant), a cv. M82 line harbouring the wild-type *OBV* allele introgressed by conventional breeding and a transgenic *OBV*-OE line; and (D) in WT, *obv* and the transgenic lines. (E) Regression analysis of $K_{\text{leaf}}^{\text{max}}$ and vein density for WT, *obv* and the transgenic lines. Each point corresponds to an individual measurement on a different plant. (F) Net photosynthetic assimilation (A_n) response curve to CO₂ concentration in the chloroplasts (C_c), each point represents mean $\pm$ s.e.m. (n=8). (G) Maximum assimilation rate at ambient CO₂ and saturating irradiance derived from the curves in F. Boxes in box plots represent interquartile range (IQR), center line the mean, and the ends of the whisker are set at 1.5*IQR above and below the third and first quartiles, respectively. Asterisks show outliers. p -values for significant differences ($p < 0.05$) are shown.

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396 **Data and materials availability**

397 All data are available in the main text or the supplementary materials. No restrictions are
 398 placed on the materials, such as material transfer agreements.

399

400 **Supplementary materials**

401 Materials and Methods
 402 Figs. S1 to S12
 403 Tables S1 to S7

404 References

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Supplementary Materials for

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Materials and Methods

Figs. S1 to S12

Tables S1 to S7

References

Materials and Methods

Plant material, growth conditions and breeding

Tomato seeds (*Solanum lycopersicum* cv Micro-Tom, MT and cv M82) were obtained from Prof Avram Levy (Weizmann Institute, Israel) and the Tomato Genetics Resource Center (TGRC, University of California, Davis), respectively. TGRC accession numbers: MT, LA3911; M82, LA3475. Seeds were sown on polyethylene trays containing Tropostrato[®] commercial substrate (São Paulo, Brazil) and grown in a greenhouse in Viçosa (642 m asl, 20°45'S; 42°51'W), with an average irradiance of $\sim 800 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod 12/12-h and air temperature 26/18°C day/night. Upon appearance of the first true leaf, seedlings of MT and M82 were transplanted to pots with a capacity of 350 mL and 3000 mL, respectively. Soil was fertilized with 2 g L⁻¹ 10-10-10 NPK and 4 g L⁻¹ limestone. For *in vitro* cultivation, seeds were sown in flasks containing 30 mL of half-strength MS medium (36) gellified with agar 4 g L⁻¹, pH 5.7 ± 0.05 . The seedlings were kept under controlled conditions: photoperiod of 16-h/8-h day/night, light intensity of $45 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature of $25 \pm 1^\circ \text{C}$.

The tomato cv. M82 harbours the recessive *obv* mutant allele. To generate the tomato cv. M82 near-isogenic lines carrying the *OBV* functional allele from MT (M82-*OBV*), we performed the introgression of both the wild-type *OBV* allele and the *OBV*^{OE} transgene from either wild-type MT or MT plants harbouring the *obv* mutant allele and an *OBV*^{OE} transgene, respectively, following the scheme illustrated in fig. S14. Briefly, plants were grown in greenhouse under conditions described above. M82 and MT were crossed using the former as female parental and the latter as pollen donor. F₁ plants were self-pollinated, generating a segregating F₂ progeny. The F₂ seedlings resulting were screened for translucent veins and the bigger stature of M82. Selected plants were backcrossed (BC) with M82 parental, producing BC₁ F₂ seeds. This procedure of crossing and screening was carried until the BC₃ generation, where plants were manually self-pollinated and the progeny screened for homozygous plants (BC₃F_n).

GWAS analysis

The online repository of Solanaceae genetics and genomics information Sol Genomics Network (solgenomics.net) was used to access the database of 360 genomes of tomato cultivars and wild species (16). We focussed on a discrete region of chromosome 5 based

on the previously mapped location of *OBV* (15) and analysed genetic variations present in all 37 genes contained in the bin d-5E (Table S1). We then used the extensive database from the Tomato Genetics Resource Center, TGRC (tgrc.ucdavis.edu) to retrieve phenotypic data for different accessions of tomato cultivars and wild species and conduct the genotype $\times$ phenotype association.

The Genome-Wide Association Study (GWAS) was performed using data of 37 tomato accessions (25 wild-type and 12 mutants for *obv* phenotype, Table S2) and the Plink (v.1.07) software (37). A total of 3,218,302 single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) greater than 5% were used for association analysis. The whole-genome significant cutoff was defined as $P=1.554e-08$ based on the Bonferroni correction method. A Manhattan plot was generated using the R package qqman (38). Gene annotation was obtained from International Tomato Annotation Group (ITAG, v.2.4) and visualized using the R package Gviz (39).

Structural modeling analysis

The analyses of functional domains in the OBV protein were done using the functional domain prediction tool from Interpro (www.ebi.ac.uk/interpro/), and the Logo plot was obtained by comparing of 324 protein sequences from the ortholog group of 39 plant species, using EVcouplings (evcouplings.org). The 3D model of the wild-type OBV protein was generated on SwissModel (swissmodel.expasy.org).

OBV homologs identification and phylogenetic inference

To identify *OBV* homologs in tomato and other plant species, we retrieved the ortholog group of proteins using the Plant Transcription Factor Database (planttfdb.gao-lab.org). We next aligned the sequences from the model species *A. thaliana* with MUSCLE (40). The alignment was submitted to trimAl (41) for alignment trimming and then submitted to FastTree (42) for tree inference. Trees were visually inspected using FigTree (tree.bio.ed.ac.uk/software/figtree/). Further phylogenetic inference using only *OBV* homologs were performed using MUSCLE, for sequence alignment, trimAl, for alignment trimming, SMS (43), for evolutionary model selection, and PHYML (44), for maximum likelihood tree inference. Final trees were annotated with taxonomic information from NCBI Taxonomy using TaxOnTree (bioinfo.icb.ufmg.br/taxontree).

The plant proteomes used in this work were retrieved from Sol Genomics Network, for tomato (ITAG v.4.0) (45), and from Uniprot (www.uniprot.org), for other species.

Molecular cloning

The coding sequence of *OBV* gene (1,149 pb) was reverse transcribed and amplified by PCR (Polymerase Chain Reaction) from RNA extracted from leaves of *Solanum lycopersicum* cv. Micro-Tom (wild-type) or the *obv* mutant, using the primers listed in Table S4. Young leaves (~2.0 cm) were collected for extraction of total RNA using Trizol[®] (Ambion, Life Technology), according to the manufacturer's recommendations. The cDNA synthesis was performed with SuperScript[™] III First-Strand Synthesis System (Invitrogen). The amplicons were purified and cloned independently in the plasmids PCR8/GW/TOPO[®] (Invitrogen). Subsequently, each fragment was recombined in the overexpressing vector pK7WG2D,1 through Gateway[®] technology (46). The correct assembly of the construct was confirmed by PCR and Sanger sequencing.

For post-transcriptional gene silencing we used an interference RNA (RNAi) construct. A specific fragment of 223 pb (Appendix 1) from coding sequence of *OBV* gene was selected using BLAST queries against the Sol Genomics database (solgenomics.net/, ITAG release 2.40). The amplified fragment was purified and cloned into the plasmids PCR8/GW/TOPO[®] (Invitrogen), and then recombined in the gene silencing vector pK7GWiWG2 (I) (46). The assembly was confirmed by PCR and Sanger sequencing. The confirmed plasmids for overexpression or silencing were used to transform *Agrobacterium tumefaciens* (EHA105) by heat shock as previously described (47).

Plant transformation

A. tumefaciens EHA105 harbouring the plasmids described above was used to perform tomato plants transformation as described (47). Briefly, seeds were sterilized by agitation in 30% (v/v) commercial bleach (2.7% w/v sodium hypochlorite) for 15 min and rinsed with distilled water. 30 seeds of Micro-Tom or *obv* mutant were inoculated into flasks containing half-strength MS medium (36), vitamins B5, sucrose 30 g L⁻¹ and agar 4 g L⁻¹. The pH of the medium was adjusted to 5.7 ± 0.05 and sterilized by autoclaving. The seeds were kept four days in the dark, 25 ± 1 °C, followed by four days under photoperiod of 16-h/8-h day/night and intensity 45 ± 3 µmol m⁻² s⁻¹. The cotyledons of 8-days-old seedlings were sectioned and used in co-cultivation with *A. tumefaciens* in Root Inducing Medium (RIM) for 2 days, 25 ± 1 °C in the dark. Then, the explants were transferred to

Shoot Inducing Medium (SIM) and were kept in 25 ± 1 °C under photoperiod of 16-h/8-h and light intensity of $10\text{-}20 \mu\text{mol m}^{-2} \text{s}^{-1}$, for four weeks. Explants were replated to fresh medium twice during this period. Well-developed shoots (≥ 5 cm) were transferred to flasks containing hormone-free MS medium, for shoot elongation and rooting. At all *in vitro* stages, the medium was supplemented with 300 mg L^{-1} timentin to suppress *A. tumefaciens* growth and 100 mg L^{-1} kanamycin for *in vitro* selection of transgenic plants.

Selection of overexpressing and silencing lines

Regenerated overexpressing and silencing T_0 plants were acclimatized under controlled conditions, photoperiod 16-h/8-h day/night, irradiance of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature of 25 ± 1 °C, using autoclaved Tropostrato® substrate and vermiculite (1:1) for four weeks. Subsequently, T_0 transgenic plants were transferred to greenhouse for genotyping and seed production.

To verify the transgene insertion, genomic DNA was extracted from young leaflets (48). First, the presence of the resistance gene *NEOMYCIN PHOSPHOTRANSFERASE II* (*neo*) was confirmed by PCR, for both constructs. A second confirmation was made to identify the transgene using a forward primer for *pCaMV 35S* and reverse specific for the construct of interest. The primers used are listed in Table S4.

To obtain homozygous plants, overexpressing and silencing lines were manually self-pollinated producing T_1 , T_2 , T_3 and T_4 seeds. Progeny tests were carried out on seeds derived from single plants through foliar spray of antibiotic (kanamycin 400 mg L^{-1} for five consecutive days). The plant populations ($n=30$) were 100% plants were antibiotic resistant (assessed visually as absence of leaf yellowing following kanamycin application) were considered homozygous. The assay was repeated on at least two subsequent generations.

Real-time quantitative PCR (RT-qPCR)

To assess the *Aux/IAA* transcriptional profile, leaf primordia (≤ 0.5 cm) were collected from Micro-Tom (wild-type), *obv* mutant and overexpressing lines. RNA was purified using ReliaPrep™ RNA Miniprep Systems (Promega), according to the manufacturer recommendations. The cDNA synthesis was performed with SuperScript™ III First-Strand Synthesis System (Invitrogen). Real-Time quantitative PCR reactions (qRT) were performed in a thermocycler Real-Time StepOnePlus PCR (Applied Biosystems) with a

final volume of 14 μ L using reagent SYBR Green Master Mix (Thermo Fisher Scientific). The melting curves were analyzed for nonspecific amplifications and dimerization of primers. Absolute fluorescence data were analyzed using the software LinRegPCR (46) to obtain the values of quantification cycle (Cq) and calculate the primer efficiency. The abundance of transcripts was normalized against the geometric mean of two reference genes, *TIP4* and *EXPRESSED* (49). All the primers used are listed in Table S4. To analyse the *OBV* expression profile in wild-type MT plants, the following tissues were collected: germinated seeds, root tips (≤ 2 cm), hypocotyls, leaf primordia (≤ 0.5 cm), expanding leaf 1 (≤ 2 cm), expanding leaf 2 (≤ 3 cm), mature leaf, flowers (petals + anthers), immature green fruits (IG3) and red ripe fruits (RR). RNA extraction and analyses was conducted as described above.

In situ hybridization

The *OBV* expression analyzed by *in situ* hybridization assay was performed as described previously (50). Apical meristems and leaf primordia of wild-type MT plants were collected and processed.

Phenotypic characterization

The morphological characterization was made in MT, *obv* mutant and overexpression lines in T₃ homozygous transgenic plants after anthesis. Plant height was measured from the soil level to the first inflorescence. The length and diameter of the internodes were obtained by measuring the fourth, fifth and sixth internodes counted from the bottom up. Stem diameter was measured from the cotyledons level using a mechanical pachymeter (Mitutoyo[®] Vernie Caliper model, Japan). Leaf number to the first inflorescence was obtained by counting of the leaves on main stem, from the bottom up. The leaf angle was determined based on the insertion of the fifth, sixth and seventh leaf, using a protractor. Biomass accumulation was obtained through root, stem and leaves dry mass by destructive analysis and oven-drying 60 days after germination. All measurements were conducted in at least 12 plants per genotype.

For leaf characterization, three terminal leaflets were selected per plant, in eight plants per genotype. The leaflets were digitized using an HP Scanjet G2410 scanner (Hewlett-Packard, Palo Alto, California, USA) Total leaf area was obtained digitizing all leaves and calculating the area in Image-Pro Plus. Specific leaf area (SLA) was calculated

through the ration between leaf area (LA) and leaf dry weight (LDW), as described by the equation: $SLA \text{ (cm}^2 \text{ g}^{-1}\text{)} = LA/LDW$.

Gas exchange, chlorophyll *a* fluorescence and *A/C_c* curves

Gas exchange analyses were performed in adult plants of cv. M82, M82-*OBV* (cv M82 harbouring the functional *OBV* allele) and M82-*OBV*^{OE} (M82 plants harbouring an *OBV* overexpression construct). All the evaluations described below were measured in fully expanded fifth leaf apical leaflets from base to apex. Gas exchange and chlorophyll *a* fluorescence parameters were determined simultaneously using an open-flow infrared gas analyzer (IRGA) system (model LI-6400XT, Li-Cor Inc., Lincoln, NE, EUA) equipped with an integrated fluorescence chamber of 2cm² (6400-40 Leaf Chamber, Li-Cor Inc.). The equipment was configured to provide a light intensity of 1000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$, with the air flow in the chamber regulated to 300 $\mu\text{mol s}^{-1}$. For evaluation of dark respiratory activity (R_d), a 6 cm² leaf chamber with air flow set at 100 $\mu\text{mol s}^{-1}$ was used. The effective quantum yield of photosystem II ($\phi PSII = (Fm' - Fs)/Fm'$) was determined by measuring the steady-state fluorescence (Fs) and the maximum fluorescence (Fm'), applying a saturating light pulse of 8000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ photons (51). Photochemical quenching ($qP = (Fm' - Fs)/(Fm' - F0')$) was calculated as described (52). The apparent rate of electron transport ($ETR = 0.5 \times 0.84 \times \phi PSII \times PAR$) was calculated as described in ref. 53, where 0.5 = fraction of the excitation energy distribution in the FSII; 0.84 = fraction of light absorbed by leaves; and PAR = photosynthetically active radiation.

The *A/C_i* response curves were measured under ambient O₂ and temperature, using PAR of 1000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ and injection of incremental CO₂ concentrations into the chamber (50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800 $\mu\text{mol mol}^{-1}$). Calculations of chloroplast concentrations of CO₂ (C_c) and mesophyll conductance (g_m) were performed using the Harley method (54). The CO₂ compensation point quantified previously for tomato (55) was used as reference to calculate g_m and C_c . The maximum Rubisco carboxylation velocity (V_{cmax}), the maximum capacity for electron transport rate (J_{max}), and the use of phosphate trioses (TPU) were estimated based on C_i and C_c and normalized to 25°C (56). Stomatal, mesophyll and biochemical limitations were calculated as described (57). CO₂ and water vapor leaks were corrected according to the methodology described in ref. 58.

Leaf hydraulic conductance ($K_{\text{leaf}}^{\text{max}}$) determinations

Leaf water potential (Ψ_{leaf}) was measured in the terminal leaflet of the fifth expanded leaf of MT, *obv* mutant and transgenic *OBV* overexpression lines, using a Scholander-type pressure chamber (model 1000, PMS Instruments, Albany, NY, USA). The leaf hydraulic conductance ($K_{\text{leaf}}^{\text{max}}$) was estimated using the transpiration rates and the water potential difference between the transpiring and non-transpiring leaflet. The non-transpiring leaflet consisted of the lateral leaflet of the same leaf, which was covered with plastic film in the night before the measurements. $K_{\text{leaf}}^{\text{max}}$ was calculated according to equation:

$$K_{\text{leaf}}^{\text{max}} = E / (\Psi_L - \Psi_X)$$

Where E is the transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$) determined during gas exchange measurements, and $(\Psi_L - \Psi_X)$ corresponds to the pressure gradient between the transpiring and non-transpiring leaflet (MPa) (8). The parameters were determined in six plants per genotype.

$K_{\text{leaf}}^{\text{max}}$ in the tomato cv. M82 background was determined in well-watered plants using the evaporative flow method (57) using a flowmeter. Healthy light-acclimated leaflets were cut underwater and immediately connected to the flowmeter. During connection with the equipment, the leaflet was maintained at an irradiance of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and at a constant airflow to maintain the transpiration rates (The leaf temperature varied between 26 and 32 °C). After flow stabilization, the leaflets were removed from the system and placed in the dark (in plastic bags under high humidity to avoid dehydration) for 5 minutes to balance the water potential, this variable being quantified after that time. to calculate K_{leaf} the following equation was used:

$$K_{\text{leaf}}^{\text{max}} = F / \Psi_{\text{leaflet}}$$

where F is the steady-state leaf water flow. In this case, the K_{leaf} values were normalized using leaf area and water viscosity at 25 °C.

Anatomical analyses

Expanded terminal leaflet from the fifth leaf were used for anatomical analyses. To obtain cross sections, a 1×0.5 cm fragment was excised from the leaf lamina and fixed in FAA70 (70% formalin-acetic acid-alcohol) for 24 h. Subsequently, the samples were dehydrated in an ethanol series (70%, 85% e 95%), under vacuum (-20 polHg). Pre-

infiltration was performed with resin and ethanol 95% (1:1) for 2 h under vacuum (-20 polHg), and infiltration was done in resin for one week. Cross-sections of 5 µm thickness were mounted on blades and stained with 0.05% toluidine blue. The images were analyzed under a light microscope (Zeiss AxioScope A1, Jena, Germany).

Vein density measurements

Vein density was measured in six terminal leaflets per genotype. The leaflets were collected and fixed in methanol 95% for 48 h, then the solution was changed to lactic acid 100% and incubated at 100°C until complete clarification (5-6 hours). The diaphanized leaflets were analyzed using a light microscope (Zeiss AxioScope A1, Jena Germany) and vein density was measured as length of veins per unit leaf area, using the software Image Pro-Plus® (imagej.nih.gov/ij/).

Histochemical GUS analysis

Seedlings of 20-days-old MT, *obv* mutant and overexpression lines were crossed to transgenic MT or *obv* lines carrying the auxin-responsive synthetic promoter (*DR5*) (59) fused to the reporter gene *UID* (encoding β glucuronidase). F₁ hybrid seeds were harvested and grown *in vitro* as described above. For each genotype, four three-week old seedlings were incubated in exogenous auxin (20 µM indole-3-acetic acid, IAA), or mock treated for 3 h. The seedlings were then incubated at 37 °C for 12 h in GUS staining solution (100 mM NaH₂PO₄, 10 mM EDTA, 0.1% Triton and 1 mM 5-bromo-4-chloro-3-indolyl-D-glucuronic acid; pH=7.2) to reveal GUS activity (60). After staining, the seedlings were washed in ethanolic series (70%, 80%, 95% e 100%), 1 h for each series at 37 °C to remove chlorophyll. Samples were then photographed using a using a light microscope (Zeiss AxioScope A1, Jena Germany).

Auxin quantification

The endogenous IAA levels were determined by gas chromatography-tandem mass spectrometry-selecting ion monitoring (Shimadzu model GCMS-QP2010 SE). First, the samples (50-100 mg fresh weight) were extracted and methylated (61). About 0.25 µg of the labeled standard [¹³C₆] IAA (Cambridge Isotopes, UK) was added to each sample as an internal standard. The chromatograph was equipped with a fused-silica capillary column (30 m i.d., 0.25 mm, 0.5-µm-thick internal film) DB-5 MS stationary phase using

helium as the carrier gas at a flow rate of 4.5 mL min⁻¹ in the following program: 2 min at 100°C, followed by a ramp of 10°C min⁻¹ to 140°C, 25°C min⁻¹ to 160°C, 35°C min⁻¹ to 250°C, 20°C min⁻¹ to 270°C and 30°C min⁻¹ to 300°C. The injector temperature was 250°C, and the following mass spectrometer operating parameters were used: ionization voltage, 70 eV (electron impact ionization); ion source temperature, 230°C; and interface temperature, 260°C. Ions with mass-to-charge ratios of 130 and 189 (corresponding to endogenous IAA) and 136 and 195 (corresponding to [¹³C₆]IAA) were monitored, and endogenous IAA concentrations were calculated based on extracted chromatograms at mass-to-charge ratios of 130 and 136.

Polar auxin transport assays (PAT)

Hypocotyl sections with 10-mm were excised from MT and *obv* seedlings 2-week-old and incubated in 5 mM phosphate buffer (pH 5.8) containing 1 μM IAA for 2 h at 25°C ± 2°C on a rotary shaker (200 rpm). The segments were placed between receiver blocks (1% [w/v] agar in water) and donor blocks (1% [w/v] agar in 5 mM phosphate buffer [pH 5.8] containing 1 μM IAA and 100 nM [³H] IAA) oriented with their apical ends toward the donor blocks. After 4 h of incubation inside a humid chamber at 25°C ± 2°C, the receiver blocks were removed and stored in a 3 mL scintillation cocktail (Ultima Gold; PerkinElmer). Receiver blocks in the scintillation cocktail were shaken overnight at 100 rpm and 28°C ± 2°C before analysis in a scintillation counter. For the negative control, hypocotyl segments were sandwiched for 30 min between NPA-containing blocks (1% [w/v] agar in water containing 20 μM NPA) an auxin transport inhibitor. ³H dpm was converted to fmol of auxin transported (62).

Auxin sensitivity assays

For hypocotyl elongation assays, MT and *obv* mutant seeds were grown on half-strength MS medium under photoperiod of 16-h/8-h day/night, 45 ± 3 μmol m⁻² s⁻¹ light intensity at 25 ± 1 °C. Hypocotyls from 8-days-old seedlings were excised in sections with 10-mm and preincubated in buffer (10 mM KCl; 1 mM MES-KOH [pH 6] and 1% [w/v] sucrose) for 2 h at 25°C in the dark to deplete endogenous auxin. Then, segments were incubated in the same buffer supplemented with α-naphthalene acetic acid (NAA) in increasing concentrations (0, 0.1, 1, 10, 100 e 1000 μM) for 24 h under agitation, light intensity of 45 ± 3 μmol m⁻² s⁻¹ at 25 ± 1 °C (60). For each genotype, three biological replicates were

conducted, each composed of 20 segments. Hypocotyls were photographed and the relative growth rate evaluated by Image Pro-Plus®.

For root regeneration from cotyledon explants, seeds of MT, *obv* mutant and overexpressing lines were germinated *in vitro* in half-strength MS medium under the conditions described above. Cotyledons from 12-days-old seedlings were excised and incubated in MS medium with or without IAA supplementation (0; 0.04; 0.4; 4 and 40 μM). The explants were kept in 25 ± 1 °C under photoperiod of 16-h/8-h and light intensity of 10-20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 15 days. The number of explants with visible roots was determined. Four replicates were conducted by genotype, each composed of 12 explants.

Statistical analysis

The experimental design was completely randomized. Data were submitted to analysis of variance (ANOVA) and the means were compared by Tukey test at 5% level of significance ($P \leq 0.05$).

Figure S1. Mapping and identification of the *OBV* candidate gene.

(A) Schematic depiction of the *Solanum pennellii* segments of the introgression lines in tomato (redrawn from ref. 63) showing the position of bin d-5E (15). (B) Manhattan plot of the GWAS for *obv* phenotype on d-5E region (63.73 to 64.03 Mb of chromosome 5). X-axis represents the chromosome position and the Y-axis represents the negative log10 of P-values per SNP derived from the association analysis. The red horizontal line represents the Bonferroni-corrected genome-wide significance threshold ($P=1.554e-08$). (C) Gene annotations surrounding SNPs with P-value below the significance threshold are shown in boxes.

Figure S3. Complementation of the *obv* mutant and knockdown of *OBV* gene.

(A) Representative cross-sections of primary and secondary veins of Micro-Tom (WT), *obv* mutant, overexpressing lines with functional (*OBV*^{OE} #3, #5, #10 and #12) and mutant (*obv*^{OE} #1 and #3) allele, and knockdown line in WT background (*OBV*^{RNAi} #3). BSEs (red arrow) are present in all overexpressing lines with *OBV* functional allele, but not in overexpressing lines with *obv* mutant allele. In knockdown line, the

reduction in *OBV* expression resulted in complete elimination of BSEs in WT leaves. Bars = 100 μ m. **(B)** Relative *OBV* mRNA levels in leaves of WT, *obv* mutant, overexpressing and RNAi lines. Values were normalized against the MT (wild-type) sample. Asterisk show significant differences to the *obv* mutant.

Figure S4. Morphology of M82, VFN8 and their respective hybrids with transgenic *OBV*-overexpressing (*OBV^{OE}*) plants in the Micro-Tom (MT) *obv* mutant genetic background. Representative F₁ generation plants from crossing of M82 × *obv*, M82 × *obv-OBV^{OE}* #10, M82 × *obv-OBV^{OE}* #12, VFN8, VFN8 × MT-*OBV^{OE}* #3 and VFN8 × *obv-OBV^{OE}* #10. Below are shown leaves and terminal leaflets. Cultivars M82 and VFN8 (both *obv* mutants) has dark veins and the complemented plants with *OBV^{OE}* allele recovered light veins phenotype. Plants were photographed 70 days after germination. Scale bars = 30 cm for plants; = 4 cm for leaves and leaflets.

Figure S5. Maximum likelihood tree of OBV family subclade comprising the tomato OBV (in red). The tree was inferred with PHYML (substitution model: JTT+G+I, bootstrap replicates: 1000). Branches are colored according to the taxonomic relationship between the organism which a sample belongs to with *S. lycopersicum*, which were determined by consulting their Lowest Common Ancestor (LCA) in the NCBI Taxonomy tree. Leaf labels are comprised of (1) LCA level with *S. lycopersicum* (e.g. n11), (2) accession number; (3) gene name when exists and (4) species name.

Figure S6. Phylogenetic reconstruction of OBV protein family.

Maximum likelihood tree of the OBV family from *S. lycopersicum*, subclade comprising the tomato OBV (in red) with its paralogs and orthologs in *Arabidopsis thaliana* and the basal angiosperm *Physcomitrella patens*, used as external group. The tree was inferred with PHYML (substitution model: JTT+G+I, bootstrap replicates: 1000). Branch support is shown on each node. Branches are colored according to the organism to which the protein sequence belongs to.

Figure S7. Expression patterns and pleiotropic effects of *OBV* in leaf insertion angle, leaf margin serration and fruit shape.

(A) Relative *OBV* mRNA levels in leaves (exp. = expanded), flowers, fruits (ripening stages represented by IG3 = immature green three; RR = red ripe), seeds and hypocotyls of tomato cultivar Micro-Tom (MT). ND = not detected. (B) *In situ* hybridization showing *OBV* expression patterns in a longitudinal section of the shoot apex, including the apical meristem and leaf primordia and a cross-section of an expanded terminal leaflet. Arrowheads show the specific regions where *OBV* transcripts accumulate. Scale bars = 100 μ m. Representative (C) plants, (D) fully expanded fifth leaf, and (E) mature fruits of MT, the *obv* mutant and homozygous T₃ transgenic lines overexpressing (OE) the *OBV* gene in either MT or *obv* mutant background. Scale bars = 10 cm (C) and 1 cm (D, E).

Figure S8. Influence of *OBV* on auxin concentration and transport.

(A) Auxin levels in leaf primordia (≤ 0.5 cm) of MT, *obv* mutant and *OBV*^{OE} lines, homozygous T₃. Data represent means of four biological replicates. Each biological replica was composed by a pool of three plants. Different letters indicate significant differences by Tukey's test at 5% probability. FW = Fresh weight. (B) [3H]IAA transport in detached hypocotyls of MT and *obv* mutant. The NPA is an auxin polar transport inhibitor and was used as negative control. Data are means (±SE) of at least ten biological replicates.

Figure S9. Reduction of auxin sensitivity in *OBV* overexpressing lines.

Histochemical GUS analysis in MT (MT x *DR5::GUS* F1) and overexpression lines (*OBV^{OE}* x *DR5::GUS* F1) seedlings at 30 days-old. (A, C, E, G, I) Expression pattern of *GUS* reporter gene fused to the auxin-inducible *DR5* promoter in wild-type seedlings and *OBV^{OE}* lines. (B, D, F, H, J) Expression pattern of *GUS* in MT and *OBV^{OE}* lines treated with exogenous auxin (20 μ M IAA). Bars = 1 cm.

Figure S10. *OBV* alters auxin sensitivity.

(A) Auxin dose response in hypocotyl segments. Elongation is given as increase in percentage at final length over the initial length after 24 h incubation in a solution containing the NAA under increasing concentrations. Data are means ($\pm$ SE) of three biological replicates with ≥ 20 segments for each replicate. Statistically significant differences compared with the MT were determined using Tukey's test: * $P < 0.05$. (B) Auxin dose-response assay in 12-day-old cotyledon explants showing a significant reduction in the emission of roots in *OBV*^{OE} lines. (C) Total number of emitted roots induced from the concentration of 0.4, 4 and 40 μ M IAA, after 15 days of cultivation. Different letters indicate statistically significant differences by Tukey's test, $P < 0.05$. Uppercase letters compare doses within the same genotype, lowercase letters compare dose effect between different genotypes. Bar = 1 cm.

Figure S12. Pipeline for introgression of *OBV* functional allele into M82 background.

This pipeline shows the steps performed to obtain a near-isogenic line of M82 carrying *OBV* functional allele from MT (wild-type). The same steps were performed to obtain introgression lines with transgene (*obv-OBV^{OE}* generation T₄, homozygous). The selection of plants was made by choosing plants with clear veins and larger size. Progeny test was conducted at the end of introgression process and populations with 100% of individuals presenting clear veins were considered homozygous. BC = backcross.

Table S1. *OBV* candidate genes contained in bin d-5E of the *S. pennellii* introgression lines.

ITAG gene ID	Begin	End	Order	Annotation
Solyc05g053680.1.1	62878570	62882485	15862	Pectinesterase (AHRD V1 **** B9RWI7_RICCO); contains Interpro domain(s) IPR000070 Pectinesterase, catalytic
Solyc05g053690.1.1	62884728	62888637	15863	Ubiquitin carboxyl-terminal hydrolase (AHRD V1 **** B9GFW8_POPTR); contains Interpro domain(s) IPR001394 Peptidase C19, ubiquitin carboxyl-terminal hydrolase 2
Solyc05g053700.1.1	62890386	62893341	15864	Xyloglucan endotransglucosylase/hydrolase 1 (AHRD V1 **** D5HRN0_FRAAN); contains Interpro domain(s) IPR016455 Xyloglucan endotransglucosylase/hydrolase
Solyc05g053710.2.1	62894088	62900846	15865	TBC1 domain family member 13 (AHRD V1 ***- D3BTH5_POLPA); contains Interpro domain(s) IPR000195 RabGAP/TBC
Solyc05g053720.2.1	62901420	62908425	15866	Os12g0425600 protein (Fragment) (AHRD V1 ***- Q0INN2_ORYSJ); contains Interpro domain(s) IPR004348 Protein of unknown function DUF246, plant
Solyc05g053730.2.1	62909468	62913284	15867	Genomic DNA chromosome 5 TAC clone K21P3 (AHRD V1 *- *- Q9FJ19_ARATH)
Solyc05g053740.2.1	62913758	62915753	15868	Alkaline ceramidase (AHRD V1 ***- Q17KM2_AEDAE); contains Interpro domain(s) IPR008901 Alkaline phytoceramidase
Solyc05g053750.1.1	62920546	62926504	15869	Lipase (AHRD V1 **** Q1XBG1_RICCO); contains Interpro domain(s) IPR006693 AB-hydrolase associated lipase region
Solyc05g053760.2.1	62934657	62937298	15870	Chaperone protein dnaJ 20 (AHRD V1 ***- B6U349_MAIZE); contains Interpro domain(s) IPR001623 Heat shock protein DnaJ, N-terminal
Solyc05g053770.2.1	62942781	62950268	15871	At5g48610-like protein (Fragment) (AHRD V1 *- *- Q6XWA2_ARATH)
Solyc05g053780.2.1	62958028	62959831	15872	RNA binding protein-like protein (AHRD V1 ***- Q3HVL3_SOLTU); contains Interpro domain(s)

				IPR015465 RNA recognition motif, glycine rich protein
Solyc05g053790.1.1	62960914	62962215	15873	UDP-D-glucuronate 4-epimerase 4 (AHRD V1 **** D7LCH5_ARALY); contains Interpro domain(s) IPR016040 NAD(P)-binding domain
Solyc05g053800.2.1	62969281	62993468	15874	DNA repair protein Rad50 (AHRD V1 ***- A8IE27_TRITU); contains Interpro domain(s) IPR004584 Recombination/repair protein Rad50
Solyc05g053810.2.1	62993932	62998448	15875	Serine hydroxymethyltransferase (AHRD V1 **** D2D306_GOSHI); contains Interpro domain(s) IPR001085 Serine hydroxymethyltransferase
Solyc05g053820.2.1	63001106	63003424	15876	UDP-glucosyltransferase family 1 protein (AHRD V1 **** C6KI44_CITSI); contains Interpro domain(s) IPR002213 UDP-glucuronosyl/UDP-glucosyltransferase
Solyc05g053830.2.1	63004325	63011970	15877	Protein transport protein Sec23 (AHRD V1 ***- Q9LUG1_ARATH); contains Interpro domain(s) IPR006896 Sec23/Sec24 trunk region
Solyc05g053850.2.1	63036304	63038329	15878	SELF PRUNING 5G
Solyc05g053860.2.1	63042284	63044388	15879	Solute carrier family 22 member 5 (Predicted) (AHRD V1 ***- B3RF59_SORAR); contains Interpro domain(s) IPR016196 Major facilitator superfamily, general substrate transporter
Solyc05g053870.2.1	63050870	63056412	15880	Copine I-like protein (AHRD V1 ***- Q6YYC5_ORYSJ); contains Interpro domain(s) IPR010734 Copine
Solyc05g053880.2.1	63058550	63062825	15881	cDNA FLJ42396 fis clone ASTRO2001107 (AHRD V1 *- *- B3KW72_HUMAN)
Solyc05g053890.1.1	63063554	63065233	15882	Glucosyltransferase-like protein (AHRD V1 *- *- Q9LXV0_ARATH); contains Interpro domain(s) IPR002213 UDP-glucuronosyl/UDP-glucosyltransferase
Solyc05g053900.2.1	63066251	63072361	15883	Aspartic proteinase nepenthesin-2 (AHRD V1 ***- B6TNP6_MAIZE); contains Interpro domain(s) IPR001461 Peptidase A1

Solyc05g053910.1.1	63083195	63084367	15884	Lipase family protein (AHRD V1 ***-Q2RAM0_ORYSJ); contains Interpro domain(s) IPR002921 Lipase, class 3
Solyc05g053920.1.1	63091393	63092700	15885	Lipase (Fragment) (AHRD V1 ***-Q9ZTW1_DIACA); contains Interpro domain(s) IPR002921 Lipase, class 3
Solyc05g053930.2.1	63100530	63103607	15886	ATP binding / serine-threonine kinase (AHRD V1 **** C5DB71_VITVI); contains Interpro domain(s) IPR002290 Serine/threonine protein kinase
Solyc05g053940.2.1	63104823	63109660	15887	Ras-related protein Rab-1A (AHRD V1 ***- B6RB23_HALDI); contains Interpro domain(s) IPR003579 Ras small GTPase, Rab type
Solyc05g053950.2.1	63111561	63115533	15888	Plasmid partition ParA protein (AHRD V1 ***- B8KJV3_9GAMM); contains Interpro domain(s) IPR010775 Protein of unknown function DUF1365
Solyc05g053960.2.1	63115904	63122815	15889	Cysteine-rich extensin-like protein-2 (AHRD V1 *- *- Q08195_TOBAC)
Solyc05g053970.2.1	63122564	63124254	15890	Amino acid transporter (AHRD V1 **** B9HY05_POPTR); contains Interpro domain(s) IPR013057 Amino acid transporter, transmembrane
Solyc05g053980.1.1	63124718	63125086	15891	NBS-LRR resistance protein-like protein (AHRD V1 ***- A1Y9Q9_SOLLC)
Solyc05g053990.2.1	63124981	63127903	15892	Cc-nbs-lrr, resistance protein
Solyc05g054000.2.1	63127954	63129059	15893	Late blight resistance protein Rpi-blb2 (AHRD V1 ***- Q38QB6_SOLBU)
Solyc05g054010.2.1	63132655	63137053	15894	Cc-nbs-lrr, resistance protein
Solyc05g054020.1.1	63138387	63142845	15895	Cc-nbs-lrr, resistance protein
Solyc05g054030.2.1	63157458	63160584	15896	C2H2L domain class transcription factor (AHRD V1 *- *- D9ZIU3_MALDO); contains Interpro domain(s) IPR007087 Zinc finger, C2H2-type
Solyc05g054040.2.1	63167515	63172773	15897	Transmembrane 9 superfamily protein member 1 (AHRD V1 **** B6TZ24_MAIZE); contains Interpro domain(s) IPR004240 Nonaspanin (TM9SF)
Solyc05g054050.2.1	63173933	63177785	15898	Glutamate decarboxylase (AHRD V1 **** Q8LKR4_TOBAC); contains

Interpro domain(s) IPR010107
Glutamate decarboxylase

Table S2. *OBV* allele in different *Solanum lycopersicum* accessions and wild species.

Accession ID	TGRC ID	Genotype	Leaf phenotype*	aa 135
RF_000	LA3435	Heinz 1706	Mutant (<i>obv</i>)	Arginine
TS-49_SNPs	LA3238	Earliana	Mutant (<i>obv</i>)	Arginine
TS-8_SNPs	LA4024	E-6203	Mutant (<i>obv</i>)	Arginine
TS-3_SNPs	LA3475	M82	Mutant (<i>obv</i>)	Arginine
TS-121_SNPs	LA3846	NC EBR-6	Mutant (<i>obv</i>)	Arginine
TS-60_SNPs	LA2009	New Yorker	Mutant (<i>obv</i>)	Arginine
TS-128_SNPs	LA0012	Pearson	Mutant (<i>obv</i>)	Arginine
TS-133_SNPs	LA3528	Peto 95-43	Mutant (<i>obv</i>)	Arginine
TS-186_SNPs	LA3214	Rowpac	Mutant (<i>obv</i>)	Arginine
TS-151_SNPs	LA2399	T-5	Mutant (<i>obv</i>)	Arginine
TS-171_SNPs	LA1706	UC-82	Mutant (<i>obv</i>)	Arginine
TS-1_SNPs	LA0490	VF36	Mutant (<i>obv</i>)	Arginine
--	LA1022	VFN8	Mutant (<i>obv</i>)	Arginine
RF_003	EA06086	Gardener's Delight	Wild-type (<i>OBV</i>)	Histidine
RF_001	LA2706	Moneymaker	Wild-type (<i>OBV</i>)	Histidine
RF_077	TR00018	Red Cherry	Wild-type (<i>OBV</i>)	Histidine
RF_055	LA1401	<i>S. galapagense</i>	Wild-type (<i>OBV</i>)	Histidine
RF_074	LA0716	<i>S. pennellii</i>	Wild-type (<i>OBV</i>)	Histidine
RF_047	LA1578	<i>S. pimpinellifolium</i>	Wild-type (<i>OBV</i>)	Histidine
TS-195_SNPs	LA0516	Ace	Wild-type (<i>OBV</i>)	Histidine
TS-9_SNPs	LA2838A	Ailsa Craig	Wild-type (<i>OBV</i>)	Histidine
TS-155_SNPs	LA0533	Condine Red	Wild-type (<i>OBV</i>)	Histidine
TS-278_SNPs	LA0517	Early Santa Clara	Wild-type (<i>OBV</i>)	Histidine
TS-5_SNPs	LA2711	Edkawi	Wild-type (<i>OBV</i>)	Histidine
TS-212_SNPs	LA3242	Flora-Dade	Wild-type (<i>OBV</i>)	Histidine
TS-4_SNPs	LA3856	Hawaii 7998	Wild-type (<i>OBV</i>)	Histidine
TS-200_SNPs	LA3320	Hotset	Wild-type (<i>OBV</i>)	Histidine
TS-264_SNPs	LA0025	King Humbert 1	Wild-type (<i>OBV</i>)	Histidine
TS-10_SNPs	LA0502	Marglobe	Wild-type (<i>OBV</i>)	Histidine

TS-163_SNPs	LA1504	Marmande	Wild-type (<i>OBV</i>)	Histidine
TS-239_SNPs	LA3845	NC EBR-5	Wild-type (<i>OBV</i>)	Histidine
TS-237_SNPs	LA3243	Platense	Wild-type (<i>OBV</i>)	Histidine
TS-206_SNPs	LA0089	Prince Borghese	Wild-type (<i>OBV</i>)	Histidine
TS-122_SNPs	LA1090	Rutgers	Wild-type (<i>OBV</i>)	Histidine
TS-6_SNPs	LA3008	San Marzano	Wild-type (<i>OBV</i>)	Histidine
TS-152_SNPs	LA1021	Santa Cruz B	Wild-type (<i>OBV</i>)	Histidine
TS-188_SNPs	LA1506	Stone	Wild-type (<i>OBV</i>)	Histidine
TS-97_SNPs	LA0154	Tiny Tim	Wild-type (<i>OBV</i>)	Histidine
--	LA3911	MT (Micro-Tom)	Wild-type (<i>OBV</i>)	Histidine

Table S3. GO Enrichment Analysis by Gene Ontology for the *OBV* gene. Source: <http://www.geneontology.org/page/go-enrichment-analysis>

GO-group	GO-ID	Term
Biological process	GO:0009965	leaf morphogenesis
	GO:0048573	photoperiodism, flowering
	GO:0010363	regulation of plant-type hypersensitive response
	GO:0009937	regulation of gibberellic acid mediated signaling pathway
	GO:0048765	root hair cell differentiation
	GO:0009959	negative gravitropism
	GO:0031348	negative regulation of defense response
	GO:0045604	regulation of epidermal cell differentiation
	GO:0010075	regulation of meristem growth
	GO:0010029	regulation of seed germination
	GO:0010260	organ senescence
	GO:0009867	jasmonic acid mediated signaling pathway
	GO:0048527	lateral root development
	GO:0009590	detection of gravity
	GO:0006612	protein targeting to membrane
	GO:0010167	response to nitrate
	GO:0016558	protein import into peroxisome matrix
	GO:0010310	regulation of hydrogen peroxide metabolic process

	GO:0010431	seed maturation
	GO:2000904	regulation of starch metabolic process
	GO:0048510	regulation of transition from vegetative to reproductive phase
	GO:0009862	salicylic acid mediated signaling pathway
	GO:0051302	regulation of cell division
	GO:0010031	circumnutation
	GO:0010106	cellular response to iron ion starvation
	GO:0045893	positive regulation of transcription, DNA-templated
	GO:0035304	regulation of protein dephosphorylation
	GO:0009958	positive gravitropism
	GO:0000165	MAPK cascade
	GO:0015706	nitrate transport
	GO:0006635	fatty acid beta-oxidation
	GO:0006826	iron ion transport
	GO:0009617	response to bacterium
	GO:0045449	regulation of transcription, DNA-templated
Cellular component	GO:0005634	nucleus
	GO:0005667	transcription factor complex
Molecular function	GO:0003700	sequence-specific DNA binding transcription factor activity
	GO:0042803	protein homodimerization activity
	GO:0044212	transcription regulatory region DNA binding
	GO:0043621	protein self-association
	GO:0008270	zinc ion binding
	GO:0046982	protein heterodimerization activity

Table S4 Sequences used for phylogenetic and alignment analysis.

See Supplemental Excel Spreadsheet

Table S5. Oligonucleotides DNA sequence for PCR primers and RNAi target sequence used in this study. The sequence of oligonucleotides used for cloning, genotyping and RT-qPCR are listed.

Target gene	Forward sequence (5'-3')	Reverse sequence (5'-3')	Purpose
	CACCCAATATTTTCATCATATTGAATAT		
<i>OBV</i>	A TCTC	GATTTTTTATATGAGTGAACAATG	cloning/ genotyping
<i>M13</i>	GTAAACGACGGCCAG	CAGGAAACAGCTATGAC	cloning
<i>35S p</i>	CCTCGGATTCCATTGCCCA	----	genotyping
<i>NPTII</i>	GAGGCTATTCGGCTATGACTGG	ATCGGGAGCGGCGATACCGTA	genotyping
<i>OBV</i>	ACTTGTGGTACTAGAGGCCATTCT	CATGTGGTGGGATATGAGTTGTAG T	qPCR
<i>TIP4</i>	GCTGCGTTTCTGGCTTAGG	ATGGAGTTTTTGAGTCTTCTGC	qPCR
<i>EXP</i>	GCTAAGAACGCTGGACCTAATG	TGGGTGTGCCTTTCTGAATG	qPCR
<i>ARF1</i>	TGCTTTAGGAATGTGGAAGTC	GGAACCATTCCTACCATAACC	qPCR
<i>ARF2A</i>	GCAAGGTCAAGAGTTATCGA	CATTGGTTTCTCAGACAAGTC	qPCR
<i>ARF2B</i>	CTGGGTTAAGCGACAAGCTC	CCCCGCATTTGATACAGAG	qPCR
<i>ARF3</i>	GATTGTTTTGCTCCCTTGGA	GTGGCTGACCCCGATAGATA	qPCR
<i>ARF4</i>	TGAAAGCCATCAACTCTCGG	ATCCCATCTGACCATCAAGCATC	qPCR
<i>ARF5</i>	TTCCGAGCCAAGAAAAGAAA	CACTCGCATCAGTTGGAAGA	qPCR
<i>ARF6A</i>	CCAACATATCCCTAGTACTTCAG	GTGCCTGAGATATTAGTTGGT	qPCR
<i>ARF6B</i>	AGCCCATCAGAGTTTGTCTATC	ATTGTGCCCATATACCTACGAA	qPCR
<i>ARF7A</i>	CGCCTCTGGATTACTCAATG	CACCCTGTCGTTAGCAAATG	qPCR
<i>ARF7B</i>	TTCGCATGATGTTTGAGACT	CTTGGAAGTGAAGAATGGACTA	qPCR
<i>ARF8A</i>	AGCCCGTCCAATATGTTTCAG	TTTGATGGTTGCTTCTGCTG	qPCR
<i>ARF8B</i>	CATCTCCTCCGACCACAGT	TGGTGGATCAATTTGTCCTGC	qPCR
<i>ARF9A</i>	CAAAGAGTTCCATCATTCAATCTC	GACCTCATCATTGTCTTCTTCAG	qPCR
<i>ARF9B</i>	AGGTCCATTAGTTGATGTTCCA	CTTGTTGAGCCAGAAGTTGAA	qPCR
<i>ARF10A</i>	ATTCTCTGTGCCTAGATACTG	CTATAAATGTGCCTAAACTTCCA	qPCR
<i>ARF10B</i>	GACATGGGATGAACCAGATTTG	GGAAACTCTTGTGGCAAACAC	qPCR
<i>ARF16A</i>	CGGTATTGACAACTCGGAGAT	TTGCGCTGTTTTTCATGAAG	qPCR
<i>ARF16B</i>	TACCATGATTCCCAAGCCAAGTA	GACCAAACAGTACGAATTGTGT	qPCR
<i>ARF17</i>	GCATCCGATTAGTCAGAGTGAAG	CCTCCTCCATTATTCGCATCTG	qPCR
<i>ARF18</i>	AAGCTTCCTATCTTATCATTGGA	AATCTACACTCGGCATTGTC	qPCR
<i>ARF19</i>	CTGGTGGATGAATCTGTTGTC	TACTTAGACAGCTCTGAACCT	qPCR
<i>ARF24</i>	CTGGTTCATTGTTGGATGTTTC	CCACATGAAGTCTTGAAAGTAG	qPCR
<i>IAA1</i>	TGTTGCCAAGGCACAAATTG	GGTTGGATGATGTTTTTCTGTTAG	qPCR
<i>IAA2</i>	ACAGTTCCATCATCCACAAG	ACCATCCATGCTCACTTTAAC	qPCR
<i>IAA3</i>	GCCACCAGTTCGATCATACA	ATAAGGTGCTCCATCCATGC	qPCR
<i>IAA4</i>	ACTCCACCTGTTGCCAAGAC	AGATAAGGGGCTCCATCCAT	qPCR
<i>IAA7</i>	TGATTCATGCAAGCGTTTACG	TGGCTCTTGGTGCTAGTCCAA	qPCR
<i>IAA8</i>	AAGGATCTATCTTCAGCACTAGA	TCCGCCAATTTGCTTTCTTTA	qPCR
<i>IAA9</i>	CAGAGGGGAAGTTTCTGTGCG	CAACCTGTGCCCTTTGTAGCA	qPCR

<i>IAA11</i>	GATGAACTGGGCTTGAATTG	CACAACCTGACTGGCACTTG	qPCR
<i>IAA12</i>	ACAAAGATGGAGACTGGATGCT	CGGTCTTCCACCATTCTTCC	qPCR
<i>IAA13</i>	AAGCGGTGAAAAGGAACAAG	TGCTTCTGAGGGATTCTTGGA	qPCR
<i>IAA14</i>	CCACCAGTGAGATCATTAGGA	AGGTGCACCATCCATGCAAA	qPCR
<i>IAA15</i>	ATCGGAGACAGCCAAATCAG	TTTGCTGGAGGTTTGTTC	qPCR
<i>IAA16</i>	GTGCCAAGCTCTAATGATCCT	GCACCGTCAACACTAATT CTATTCAAAAGGTCCATCAGTTTC	qPCR
<i>IAA17</i>	CAAGAATTATTTGATGCCTTAACCA	C	qPCR
<i>IAA19</i>	TGTCGGCGATGTTCCATG	GTCTCTTGCTCCAAGCCCTAT	qPCR
<i>IAA21</i>	TGTTCAAGCTCAACATAGGTGAAT	CCAACAAGCATCAAATCACCTT	qPCR
<i>IAA22</i>	CTGAGCTGAGATTAGGGTTACC	AGGAGCTGTCCATCTCTGAT	qPCR
<i>IAA23</i>	CACTCAACTCCTTAAAGCAATGGA	TCCTCAGTCTCTTGCATGATGAC	qPCR
<i>IAA26</i>	CCTCCAATTCGTTCTTTAGGA	GGAATACCATCCATGTTGATCTT	qPCR
<i>IAA27</i>	GCAAGAGAAGCTCAGTGA	ACATCTCCCAAGGAACATCG	qPCR
<i>IAA29</i>	GGTTTTGATGATAGCTTCTCCGAT	CGTCTTACGTTCAACTACTCCTT	qPCR
<i>IAA32</i>	AGGAATGACCAATGGAGGATT	GGGAAGAATGCTTCATCTTCC	qPCR
<i>IAA33</i>	CCTTGTTGGTGATCTCAGTTG	CTTCCAGTGCGTGAATTTGG	qPCR
<i>IAA35</i>	AAATGGAAGGAGTTGGAATTGC	ATGGTACATCACCAGCAAGAAG	qPCR
<i>IAA36</i>	TGGAAGGAGTAGCCATTGGA	TGGTACATCTCCAGCAAGCA	qPCR

***OBV* Sequence selected for the post-transcriptional gene silencing by interference RNA (RNAi)**

GTACTCCAGATCCCGATGCACAAGTTGTATATCTTACAGCTGAGATGTTAATGGAATCTGATCGTTACGTT
TGTGAAATCTGCAACCTTAGCTTTCAAAGAGAGCAAAATCTACAAATGCATCGTCGTCGCCATAAGGTTCC
ATGGAAGTTGAAGAAGAAGGAAGAAGAGAAAAATGAGATGGATCAAGTTATTAAGAAGAGAGTATATGT
GTGTCCAGAGCC

Table S6. Growth parameters measured in Micro-Tom (MT), *obv* mutant and overexpressing lines. Measurements were performed at 50 days after germination. Data are means $\pm$ se (n = 12 plants). Different letters indicate statistically significant differences (Tukey's test, P < 0.05).

Parameters	MT				<i>obv</i>	
	MT	<i>obv</i>	<i>OBV</i> ^{OE} #3	<i>OBV</i> ^{OE} #5	<i>OBV</i> ^{OE} #10	<i>OBV</i> ^{OE} #12
Plant height (cm)	15.89 $\pm$ 0.43a	15.30 $\pm$ 0.30a	10.32 $\pm$ 0.20b	8.43 $\pm$ 0.19c	10.99 $\pm$ 0.27b	10.04 $\pm$ 0.20b
Internode length (4 th)	1.30 $\pm$ 0.07a	1.19 $\pm$ 0.05a	0.77 $\pm$ 0.05b	0.52 $\pm$ 0.02c	0.81 $\pm$ 0.05b	0.70 $\pm$ 0.03bc
Internode length (5 th)	1.52 $\pm$ 0.05a	1.57 $\pm$ 0.09a	0.97 $\pm$ 0.04b	0.70 $\pm$ 0.03c	1.01 $\pm$ 0.05b	0.88 $\pm$ 0.03bc
Internode length (6 th)	1.52 $\pm$ 0.09a	1.59 $\pm$ 0.08a	1.03 $\pm$ 0.04b	0.77 $\pm$ 0.03c	1.08 $\pm$ 0.04b	1.04 $\pm$ 0.04b
Internode diameter (4 th)	0.64 $\pm$ 0.02b	0.70 $\pm$ 0.01a	0.60 $\pm$ 0.01b	0.47 $\pm$ 0.01c	0.66 $\pm$ 0.02ab	0.63 $\pm$ 0.01b
Internode diameter (5 th)	0.68 $\pm$ 0.02ab	0.74 $\pm$ 0.01a	0.62 $\pm$ 0.01b	0.48 $\pm$ 0.01c	0.66 $\pm$ 0.02b	0.64 $\pm$ 0.01b
Internode diameter (6 th)	0.72 $\pm$ 0.02a	0.73 $\pm$ 0.01a	0.63 $\pm$ 0.01b	0.49 $\pm$ 0.01c	0.64 $\pm$ 0.02b	0.61 $\pm$ 0.01b
Stem diameter (cm)	0.57 $\pm$ 0.01ab	0.58 $\pm$ 0.02a	0.51 $\pm$ 0.01b	0.43 $\pm$ 0.01c	0.55 $\pm$ 0.02ab	0.54 $\pm$ 0.02ab
Leaves to 1 st inflorescence	11 $\pm$ 0.18a	10 $\pm$ 0.15b	11 $\pm$ 0.13a	11 $\pm$ 0.12a	11 $\pm$ 0.23a	11 $\pm$ 0.19a
Leaf angle (5 th)	72 $\pm$ 2.24a	63 $\pm$ 2.47b	63 $\pm$ 1.26b	57 $\pm$ 1.04b	61 $\pm$ 1.38b	57 $\pm$ 1.72b
Leaf angle (6 th)	75 $\pm$ 2.22a	64 $\pm$ 2.62b	46 $\pm$ 1.82c	45 $\pm$ 1.87c	48 $\pm$ 1.98c	45 $\pm$ 1.09c
Leaf angle (7 th)	72 $\pm$ 2.78a	59 $\pm$ 2.94b	43 $\pm$ 1.37c	39 $\pm$ 0.97c	42 $\pm$ 1.72c	42 $\pm$ 1.25c
Leaflets number (5 th)	5 $\pm$ 0.0a	5 $\pm$ 0.11a	5 $\pm$ 0.18a	5 $\pm$ 0.19a	5 $\pm$ 0.0a	5 $\pm$ 0.0a
Leaflets number (6 th)	6 $\pm$ 0.22a	6 $\pm$ 0.23a	6 $\pm$ 0.19a	7 $\pm$ 0.29a	7 $\pm$ 0.26a	7 $\pm$ 0.34a
Leaflets number (7 th)	7 $\pm$ 0.40a	7 $\pm$ 0.41a	7 $\pm$ 0.29a	8 $\pm$ 0.25a	8 $\pm$ 0.24a	8 $\pm$ 0.30a
Perimeter/area (cm ⁻¹)	1.65 $\pm$ 0.08c	1.23 $\pm$ 0.03c	2.63 $\pm$ 0.15ab	2.93 $\pm$ 0.10a	2.26 $\pm$ 0.09b	2.66 $\pm$ 0.13ab
Perimeter ² /area	34.97 $\pm$ 2.38cd	24.01 $\pm$ 0.81d	58.29 $\pm$ 4.53ab	66.60 $\pm$ 3.95a	47.31 $\pm$ 3.03bc	68.17 $\pm$ 4.70a
Leaflets area (cm ²)	13.50 $\pm$ 0.83b	16.32 $\pm$ 0.97a	8.76 $\pm$ 0.49c	7.65 $\pm$ 0.25c	9.38 $\pm$ 0.43c	9.79 $\pm$ 0.39c
Total leaf area (cm ²)	339.2 $\pm$ 24.8a	365.4 $\pm$ 19.1a	321.5 $\pm$ 14.0a	279.4 $\pm$ 12.2a	361.8 $\pm$ 24.8a	356.9 $\pm$ 29.3a
Circularity	0.39 $\pm$ 0.02b	0.53 $\pm$ 0.01a	0.25 $\pm$ 0.02cd	0.21 $\pm$ 0.01cd	0.29 $\pm$ 0.02c	0.20 $\pm$ 0.01d
Serration number	11.52 $\pm$ 0.49b	8.28 $\pm$ 0.34c	13.57 $\pm$ 0.38a	14.90 $\pm$ 0.36a	13.28 $\pm$ 0.52ab	13.90 $\pm$ 0.55a
SLA (cm ² g ⁻¹)	312.3 $\pm$ 5.40	341.2 $\pm$ 12.37	366.6 $\pm$ 5.92	408.4 $\pm$ 7.69	389.8 $\pm$ 2.75	375.8 $\pm$ 5.41

Root dry weight (g)	0.35 ± 0.03ab	0.36 ± 0.01a	0.19 ± 0.01cd	0.11 ± 0.001d	0.26 ± 0.03bc	0.23 ± 0.01c
Stem dry weight (g)	1.33 ± 0.12a	1.57 ± 0.08a	0.67 ± 0.06bc	0.35 ± 0.02c	0.86 ± 0.11b	0.73 ± 0.04b
Leaf dry weight (g)	1.15 ± 0.09a	1.11 ± 0.06a	0.90 ± 0.04ab	0.70 ± 0.03b	1.00 ± 0.09ab	1.00 ± 0.08ab
Total dry weight (g)	2.84 ± 0.24a	3.05 ± 0.13a	1.77 ± 0.12bc	1.17 ± 0.06c	2.13 ± 0.23b	1.97 ± 0.12b

Table S7. Gas exchange and chlorophyll fluorescence parameters determined in fully-expanded leaves of *Solanum lycopersicum* cv M82. Measurements were performed at 45 days after germination. Data are means $\pm$ se (n = 6 plants). Different letters indicate statistically significant differences (Tukey's test, $P < 0.05$).

Parameter	M82	M82-OBV	obv-OBV ^{OE}	p_value
A ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	18 ± 0.78	$21 \pm 0.85^*$	$20.6 \pm 0.49^*$	0.04
g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	0.36 ± 0.03	0.38 ± 0.03	0.35 ± 0.02	0.60
E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	$5.37 \pm 0.32 \text{ a}$	$4.9 \pm 0.16 \text{ ab}$	$4.4 \pm 0.12 \text{ b}$	0.00
WUE_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	51 ± 3.15	55 ± 3.6	59 ± 3.1	0.10
R_{dark} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	1.22 ± 0.12	1.24 ± 0.08	1.44 ± 0.11	0.40
g_m ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	0.21 ± 0.02	0.20 ± 0.02	0.17 ± 0.01	0.14
g_m/g_s ($\text{mol CO}_2 \text{ mol}^{-1} \text{ CO}_2$)	1.07 ± 0.14	0.96 ± 0.15	0.87 ± 0.11	0.46
C_c ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)	$202 \pm 8.5 \text{ a}$	$171 \pm 9.86 \text{ ab}$	$154 \pm 8.91 \text{ b}$	0.01
C_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$)	292 ± 5.39	283 ± 5.57	278 ± 4.90	0.06
C_i/C_c ($\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ CO}_2$)	90 ± 10.05	112 ± 14.21	124 ± 11.23	0.06
ETR	$133 \pm 6.48 \text{ b}$	$164 \pm 9.63 \text{ ab}$	$173 \pm 7.85 \text{ a}$	0.01
ϕ PSII	$0.29 \pm 0.01 \text{ b}$	$0.36 \pm 0.02 \text{ ab}$	$0.38 \pm 0.02 \text{ a}$	0.01
qP	$0.54 \pm 0.02 \text{ b}$	$0.63 \pm 0.03 \text{ ab}$	$0.67 \pm 0.02 \text{ a}$	0.01
$V_{\text{cmax } C_c}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	$97 \pm 7.8 \text{ b}$	$115 \pm 9.44 \text{ a}$	$119 \pm 5.4 \text{ a}$	0.007
$J_{\text{max } C_c}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	$157 \pm 7.1 \text{ b}$	$182 \pm 9.1 \text{ a}$	$182 \pm 5.3 \text{ a}$	0.008
TPU_{C_c} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	8.1 ± 0.3	8.8 ± 0.41	8.8 ± 0.21	0.13
$V_{\text{cmax } C_i}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	71.7 ± 3.63	78.7 ± 3	81 ± 2.43	0.19
$J_{\text{max } C_i}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	$122 \pm 3.57 \text{ b}$	$132 \pm 5.9 \text{ ab}$	$136 \pm 3.4 \text{ a}$	0.03
TPU_{C_i} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	$8.5 \pm 0.31 \text{ b}$	$9.5 \pm 0.43 \text{ a}$	$9.4 \pm 0.25 \text{ ab}$	0.03
Ls	$0.25 \pm 0.02 \text{ b}$	$0.28 \pm 0.02 \text{ ab}$	$0.30 \pm 0.02 \text{ a}$	0.02
Lm	0.27 ± 0.03	0.35 ± 0.05	0.39 ± 0.04	0.05
Lb	$0.46 \pm 0.03 \text{ a}$	$0.35 \pm 0.03 \text{ ab}$	$0.29 \pm 0.03 \text{ b}$	0.006

* A : photosynthetic CO_2 assimilation rate; g_s : stomatal conductance; E : transpiration; WUE_i : water use efficiency; R_{dark} : dark respiration; g_m : mesophilic conductance; C_c : CO_2 concentration in the chloroplast; C_i : internal concentration of CO_2 ; ETR: electron transport rate; ϕ PSII: effective quantum yield of photosystem II; qP: photochemical quenching; V_{cmax} : maximum carboxylation velocity; J_{max} : maximum electron transport rate; TPU : triose phosphate utilization; V_{cmax} , J_{max} and TPU were expressed in C_c and C_i basis. Ls: stomatal limitation; Lm: mesophilic limitation; Lb: biochemical limitation.

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