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A loss-of-function allele of a *TAC1*-like gene (*SlTAC1*) located on tomato chromosome 10 is a candidate for the *Erectoid leaf* (*Erl*) mutation

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SUMMARY

The genetic basis of an erectoid leaf phenotype was investigated in distinct tomato breeding populations, including one derived from *Solanum lycopersicum* 'LT05' (with the erectoid leaf phenotype and uniform ripening, genotype *uu*) × *S. pimpinelifollium* 'TO-937' (with the wild-type leaf phenotype and green fruit shoulder, genotype *UU*). The erectoid leaf phenotype was inherited as a semi-dominant trait and it co-segregated with the *u* allele of gene *SlGLK2* (*Solyc10g008160*). This genomic location coincides with a previously described semi-dominant mutation named as *Erectoid leaf* (*Erl*). The genomes of 'LT05', 'TO-937', and three other unrelated accessions (with the wild-type Erl^+ allele) were resequenced with the aim of identifying candidate genes. Comparative genomic analyses, including the reference genome 'Heinz 1706' (Erl^+ allele), identified an *Erectoid leaf*-specific single nucleotide polymorphism (SNP) in the gene *Solyc10g009320*. This SNP caused a change of a glutamine (CAA) codon (present in all the wild-type genomes) to a TAA (= ochre stop-codon) in the *Erl* allele, resulting in a smaller version of the predicted mutant protein (221 versus 279 amino acids). *Solyc10g009320*, previously annotated as an 'unknown protein', was identified as a *TILLER ANGLE CONTROL1* (*TAC1*)-like gene. Linkage between the *Erl* and *Solyc10g009320* was confirmed via Sanger sequencing of the PCR amplicons of the two variant alleles. No recombinants were detected in 265 F_2 individuals. Contrasting S_7 near-isogenic lines were also homozygous for each of the alternate alleles, reinforcing *Solyc10g009320* as a strong *Erl* candidate gene and opening the possibility for fine-tuning manipulation of tomato architecture in breeding programs.

Key words: *Solanum lycopersicum*; resequencing; comparative genomic analysis; plant architecture; breeding.

INTRODUCTION

Genetic factors are the major determinants of plant architecture, even though the spatial structure of a plant might also be influenced by various environmental stimuli such as light, temperature, humidity, mineral and organic nutrition (Wang et al. 2018). For this reason, breeding programs have placed great emphasis in exploiting the genetic diversity associated with plant architecture as a strategy to develop high-yielding cultivars with greater adaptation to wide array of environmental conditions and cropping systems (Coyne 1980; Huyghe 1998; Jiao et al. 2010).

In tomato (*Solanum lycopersicum* L.), plant growth habit (i.e. determinate, semi-determinate, and indeterminate), foliar insertion angle, leaf size and internode length are important plant architectural traits for breeding, especially because of their impact on the vertical distribution of light through the crop canopy and the consequent effects on the efficiency of light interception (Sarlikioti et al. 2011a, 2011b; Silva et al. 2018). Currently, tomato breeding for indeterminate growth habit is focused on reducing the length of internodes to increase the number of trusses per stem length (Zsögön et al. 2017). However, this can increase self-shading and reduce the efficiency of light absorption (Sarlikioti et al. 2011b). At a given light intensity, the leaf insertion angle has direct implications on the amount of light received per leaf surface unit (Ehleringer & Werk 1986; Ezcurra et al. 1991) whereby a smaller leaf insertion angle (i.e. more erect leaves) can lead to more uniform light intensities vertically through the canopy, reducing light stress at the upper levels and increasing photosynthesis in the lower levels. More erect leaves may also improve the efficacy of contact pesticide applications by improving penetration through the crop canopy and reaching more efficiently abaxial leaf surfaces.

Although the control of lateral growth has been extensively studied at the molecular level in some species, the genetic and physiological mechanisms that define the insertion angle of distinct organs (including leaves) have not been properly characterized in many taxa, including Solanaceae (for general review see Wang and Li 2008; Teichmann and Muhr 2015; Roychoudhry and Kepinski 2015). So far, all physiological models converge to the central role of auxin content and distribution in the response to the continuous growth of shoots and roots (Firml et al. 2003; Roychoudhry and Kepinski 2015). Polar transport mediated by PIN and AUX/LAX proteins is a major mechanism that regulates auxin distribution in plants. These gene products control cellular auxin efflux and influx, respectively, through their subcellular localization at the plasma membrane (Wiśniewska et al. 2006; Vanneste and Friml 2009).

In tomato, characterization of genes that control auxin fluxes and directional growth of organs is still quite limited. Pattison and Catalá (2012) studied the function of the genes in tomato homologous to *PIN* and *AUX*/*LAX* and they verified that *SlPIN4* and *SlPIN3* have specific roles in the regulation of vegetative shoot architecture. Recently, Shi et al. (2017) proposed a model to explain how the auxin polar transport mediated by *PIN1* is critical in tomato leaf polarity formation. In other plant species, a distinct set of genes associated with plant architecture has been identified. In monocotyledons, *TILLER ANGLE CONTROL1* (*TAC1*) (Yu et al. 2007) and *LAZY1*, a gravitropism-related gene (Li et al. 2007), are the main genetic factors identified as being involved in the regulation of shoot angle in rice (*Oryza sativa* L.). In the case of *TAC1*, a mutation that reduces gene expression is responsible for a lower insertion angle of the lateral shoots (i.e. more erect). The *LAZY1* gene has sequence motifs similar to *TAC1* with the addition of an Ethylene-responsive element binding factor–associated Amphiphilic Repression (EAR) domain. *LAZY1* loss-of-function mutants are associated with larger insertion angle of lateral shoots. It has been demonstrated that the recessive *lazy1* mutant increases the polar (apical–basal) auxin transport and decreases lateral transport, generating an abnormal auxin flow/distribution. The associated phenotype is due to the loss of gravitropism, leading to larger leaf insertion angles (Roychoudhry and Kepinski, 2015). In peach [*Prunus persicae* (L.) Batsch], the semi-dominant allele "*broomy*" (*br* – which was later designated as "*pillar*") is responsible for a more vertical growth of the branches (Scorza et al. 1989, 2002). Subsequent genetic/genomic characterization determined that the recessive *br* allele is a non-functional mutation of a homologue of the monocotyledonous gene *TAC1*, which they denominated *PpeTAC1* (Dardick et al. 2013). Plants with the homozygous *br* allele showed higher auxin concentration in shoots when compared with wild-type plants with a horizontal branch pattern (Tworkoski et al. 2006). The auxin content in peach was found to be inversely proportional to the expression of the *TAC1* gene (Tworkoski et al. 2015). The silencing of *PpeTAC1* orthologue gene in *Arabidopsis thaliana* generated a "more erect plant", suggesting that this class of genes works universally in promoting vertical growth. *TAC1* and *LAZY1* belong to a superfamily of genes defined by an IGT (GφL(A/T)GT) domain, which is present in a wide array of plant genomes and is related to the vertical growth of the shoots through the regulation of auxin polar transport as demonstrated in rice, maize, *A. thaliana*, and peach (Dardick et al 2013, Roychoudhry and Kepinski 2015). The understanding of the genetic control of shoot architecture could provide breeding tools for selection of cultivars with improved

utilization of light, more adapted to high planting densities (Testa et al., 2016), and that also increases the efficacy of pesticide applications.

We have observed in some segregating tomato breeding lines a peculiar erect leaf phenotype, which apparently affects all aerial organs, especially young shoots. A highly endogamic breeding line (named 'LT05') was recovered and it showed a genetically stable erect leaf phenotype. Two apparently similar phenotypes have already been described in tomato: a radiation-induced recessive mutant *erecta* (*er*) (Tomato Genetics Resource Center: http://tgrc.ucdavis.edu/, last accessed 12 March, 2018), present in the accession *S. lycopersicum* LA600 (derived from the 'Codine Red' cultivar) and a spontaneous semi-dominant mutation named *Erectoid leaf* (*Erl*) (Georgiev and Kraptchev 1992; Tomato Genetics Resource Center: http://tgrc.ucdavis.edu/, last accessed 12 March, 2018). Additional observations indicated that the *Erl* mutation co-segregated with the *uniform ripening* (*u*) mutation (Georgiev and Kraptchev 1992). The *u* mutation was identified as a loss-of-function allele of the *GOLDEN2-LIKE* (*GLK2*) gene located on tomato chromosome 10 (Kinzer et al. 1990; Powell et al. 2012). Here, we investigate the genetic basis and chromosomal location of the erect leaf phenotype observed in the inbred line 'LT05'. Based on a further genomic analysis, we report a strong candidate gene at the *Erl* locus containing a loss-of-function mutation. The identification of genetic variability associated with leaf angle in tomato opens the possibility for fine-tuning manipulation of plant architecture in breeding programs.

MATERIALS AND METHODS

Accessions employed as parental lines and development of segregating populations

The genetic basis of the erectoid leaf mutation was investigated using three distinct segregating F_2 populations. The first segregating F_2 population was generated from a cross between the *S. lycopersicum* inbred line 'LT05' (with the erectoid leaf phenotype and uniform ripening, genotype *uu*) and *S. lycopersicum* 'LT17' (an inbred line with the wild-type horizontal leaf phenotype). Analyses were conducted with the contrasting parental lines (13 plants each) and five crossing generations: F_1 (n=13), reciprocal F_1' (n=13); backcross (BC) to 'LT05' (n=32), BC to 'LT17' (n=37), and F_2 (n=138). The second segregating F_2 population (n=274) was obtained from the cross 'LT05' × *S. pimpinelifollium* 'TO-937' (with green fruit shoulder, genotype *UU*) (Powell et al. 2012). A third F_2 population was produced by first generating a pair of near-isogenic lines (NILs) and then crossing them. These NILs were created as follows: from the *S. lycopersicum*

'LT05' × *S. lycopersicum* 'LT17' cross, three putative heterozygous F_2 plants (with intermediate leaf phenotype) were visually selected and selfed to generate three segregating F_3 families of 20 plants each. Individual F_3 plants with intermediate leaf phenotype (i.e. putative heterozygous) were then chosen to continue a consecutive progeny testing-based process of selection and subsequent selfing. This process was repeated until obtaining segregating F_5 families. In this step, three individual F_5:F_6 plants with erect leaf phenotype and three with normal leaf phenotype were visually selected within the same segregating progeny and then selfed. Single F_6 plants able to generate progenies with stability for each of the opposing traits (i.e. the erect leaf versus normal leaf) were chosen as the contrasting near-isogenic lines and named as 'IsoL-EL' (with stable erectoid leaf phenotype) and 'IsoL-WTL' (with stable wild-type leaf phenotype). The NILs were then crossed ('IsoL-EL' × 'IsoL-WTL') and the F_1 plants were selfed to generate an F_2 population (IsolF_2) composed by 127 plants (see the representation of the process in **Online resource 1**). The IsolF_2 population was, therefore, segregating mainly for the erect/normal leaf phenotype, whereas outside this locus the genetic background was predominantly a homozygous non-segregating mosaic of the genomes of the two parental lines 'LT05' and 'LT17'. The IsolF_2 population was also used in candidate gene validation analyses (see section below).

Evaluation of the leaf growth pattern (erectoid versus wild-type)

All evaluated plants were cultivated under greenhouse conditions in 5L pots filled with a mixture of soil and commercial peat. Individual plants were pruned to a single main stem. For inheritance studies, the leaf insertion angle (α) between the leaf petiole and the main stem (**Online Resource 2**) was measured in fully developed leaves and employed as a phenotypic indicator of each individual plant. In the case of the F_2 population derived from the cross *S. lycopersicum* 'LT05' × *S. lycopersicum* 'LT17', two measurements were made at 80 days after sowing. One measurement was done in the lower leaf (immediately below the first floral truss) and other in the first fully developed leaf (counting from the apex). For analyses, both measures were averaged to generate a mean α angle value that was converted to an ordinal scale according to the following criteria: mean α angle < 100° = erectoid leaf, mean α angle between 100-125° = intermediate, mean α angle > 125° = wild-type with standard leaf phenotype. For all segregating F_2 populations used in trait chromosomal location and candidate gene validation studies, the classification of the leaf growth trait was done by directly assessing the general aspect of the plants under

greenhouse conditions. The plants were classified as either erect leaf or non-erect leaf. This last category involved intermediate as well as wild-type leaf phenotypes.

Chromosome mapping of the erectoid leaf trait

The F_2 population from the cross *S. lycopersicum* 'LT05' × *S. pimpinelifollium* 'TO-937' was employed to verify the linkage of the erectoid leaf phenotype with the uniform ripening *SlGLK*2 gene (*u, Solyc10g008160*) (Powell et al. 2012). The green fruit shoulder phenotype (presence of the *U* allele) or its absence (due to the homozygous presence of the *u* allele) was used as a phenotypic marker to evaluate co-segregation with the erectoid leaf trait observed in our populations. Evaluation was carried out visually employing a simple scale of presence/absence for both traits.

Genetic and statistical analyses

For leaf insertion angle (α), the standard error and ANOVA were calculated using the software InfoStat version 2014 (Di Rienzo et al. 2013). For ANOVA, significant differences were claimed for $P < 0.01$ in a Tukey and Dunn's post-hoc test. A chi-squared test was applied to: (a) verify across the F_2 populations the goodness-of-fit of the erectoid vs. wild-type segregation ratios to Mendelian segregation models, and (b) to confirm the linkage between the erectoid leaf trait and the phenotypic marker green fruit shoulder, searching for a statistical difference from a 3:1 segregation ratio (i.e. independence) in a sub-group of erectoid and wild-type F_2 genotypes. In all cases a probability level (*P*-value) is given as the value for the null hypothesis.

Comparative genomic analyses of the chromosome 10 and variant screening

Resequencing information was obtained using genomic DNA from the erectoid leaf line 'LT05' and from four genetically diverse wild-type leaf accessions: 'TO-937' (Powell et al. 2012), 'CNPH498' (data not shown), 'Santa Clara' (Carmo et al., 2017), and 'Viradoro' (Giordano et al. 2000). Genomic DNA was extracted from leaf tissue of these accessions using DNeasy PowerPlant Pro Kit (QIAGEN Hilden, Germany). Whole genome sequencing of individual samples was sequenced on one lane of a HiSeq 2500 (Illumina Inc., San Diego, CA), at the Centro de Biotecnologia Animal (ESALQ/USP). Sequencing was performed with 100 bp paired-end reads. Quality control was done using

FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). Sequencing data from each of the five accessions was assembled separately against the tomato reference genome (version SL2.50) using SeqMan NGen version 14 software with default parameters (Lasergene, DNASTAR, Madison, WI, USA). An in-line Bayesian modeled variant detector based on the MAQ caller (Li, 2008) was used to tabulate SNPs and small indels relative to the reference genome in each accession. Variant calls from the five assemblies were then combined for further analysis in ArrayStar 14 (Lasergene Suite 14). Based on the chromosome mapping studies of the erect leaf trait, we carried out a search for gene variants only within chromosome 10 using variant calls from the five accessions. Since we also found the reference genome, 'Heinz 1706' (Tomato Genome Consortium, 2012), which have a standard, wild-type (non-erect) leaf pattern, we focused on identifying non-synonymous variants that were exclusively found in the erect leaf line 'LT05'. To do so, the following series of filtering criteria was used in ArrayStar: (1) non-synonymous variants occurring in 'LT05' but none of four non-erect leaf accessions (and by definition, not in the reference sequence); (2) variant positions with a minimum depth of coverage of 10; (3) SNP% of 100 and (4) Qcall ≥ 7. Summary variant information was exported for individual accessions and then imported into Microsoft Excel (Office 2016; Microsoft Corp., Redmond, WA) for further filtering and analyses. These final variants were manually verified by inspection of the corresponding sequence assemblies to eliminate artifacts arising from assembly differences. Finally, the best candidate genes were chosen considering: the physical/genetic position respect to *SlGLK2*, the protein annotation (ITAG 2.40; http://solgenomics.net/, last accessed 18 April, 2018), the predicted effects of amino acid substitutions on protein function using the PROVEAN tool (Choi et al. 2012) (http://provean.jcvi.org/index.php, last accessed 18 April, 2018) and the expression pattern according to TomExpress RNAseq database (Zouine et al. 2017) (http://gbf.toulouse.inra.fr/tomexpress/, last accessed 12 March, 2018).

Multiple alignments of the predicted protein sequences from *TAC1*-like genes of tomato and other plant species

An erectoid leaf locus-specific non-synonymous, single nucleotide polymorphism (SNP) was found in the putative tomato *TAC1*-like gene located on the chromosome 10 (XP_004248091 = *Solyc10g009320*). For this reason, multiple alignments of the predicted TAC1-like amino acid sequences from the wild-type and erectoid leaf tomato lines as well as from different plant species were carried out. TAC1-like amino acid

sequences of peach (XP_020413395), soybean (KRH06413), *A. thaliana* (OAP08981.1), maize (NP_001170644), and rice (BAF25656.2) were retrieved from GenBank database and aligned employing the Muscle algorithm with the default parameters on Geneious software v7.1.5.

Validation of *Solyc10g009320* as a candidate gene of the erectoid leaf phenotype

Individuals of two F_2 populations were used: the 127 individuals of the IsolF_2 and 71 erect and 67 non-erect individuals of the ('LT05' × 'TO-937') F_2, comprising a total of 265 F_2 individual plants. Genomic DNA was extracted from leaf samples collected at the apex of each individual plants according to a modified methodology employing 2X CTAB and organic solvents (Boiteux et al. 1999). From the sequence variants of *Solyc10g009320*, a primer pair TAC-Tom (F/R) flanking the identified SNP site was designed using PrimerSelect (Lasergene, DNASTAR, Madison, WI, USA). PCR with this primer pair amplified a 400 bp DNA fragment encompassing the SNP site detected within allelic variants of *Solyc10g009320*. Primer sequences for TAC-Tom (F/R) were: 5′-GAG-TTC-AGT-AAG-TGG-TCA-AGA-3′ / 5′-AAA-GAA-AGG-ATC-ACT-CTA-GCA-GA-3′. PCR reagents were adjusted to a final volume of 12.5 µL using 5.95 µL Milli-Q water, 1.25 µL of 10X Buffer *Taq* polymerase (100 mM Tris- HCl, pH 8.3 and 500 mM KCl), 0.6 µL of $MgCl_2$ (50 mM); 0.5 µL of dNTPs (2.5 mM); 1 µL of each primer, 0.1 U/µl *Taq* DNA polymerase (Invitrogen, Gaithersburg, MD, USA), and 2 µL of DNA template (30 ng μL^{-1}). The amplification conditions were an initial stage of denaturation at 94 °C for 2 minutes, followed by 30 cycles of: denaturation at 94 °C for 30 seconds, annealing at 60 °C for 1 minute and extension at 72 ° C for 1.5 minutes; ending with an extension step at 68 °C for 10 minutes. After purification of PCR products using Wizard kit (Promega, Madison, WI, USA), the amplicons were subjected to Sanger sequencing using an ABI Prism 3130 sequencer of the Genomic Analysis Laboratory (Embrapa Vegetable Crops, Brasília–DF, Brazil) employing the ABI Prism BigDye version 3.1 Kit (Applied Biosystems Division, Foster City, CA, USA) and the primer pair TAC-Tom (F/R). Sequence quality analysis, the removal of low quality fragments, and the identification of consensus sequences were performed using the SeqMan program (Lasergene, Madison, WI, USA). Alignment of multiple protein sequences was performed using the Clustal W method of the MegAlign software package (Lasergene, Madison, WI, USA).

RESULTS

Phenotypic characterization and genetic basis of the erectoid leaf growth trait

The most remarkable effect of erectoid leaf phenotype present in the inbred line 'LT05' (our original source of this mutation) consisted of a significantly more vertical growth of leaves, leaflets, and lateral shoots when compared to the control wild-type phenotype 'LT17' (**Fig. 1**). This trait was also easily identifiable by visual analysis in all segregating F_2 populations. In fact, this mutation expressed its phenotypic effects on all aerial structures (i.e. leaflets, leaves, shoots, and trusses) with more striking manifestation in young shoots. However, due to a former description in the literature limited to the leaf phenotype (Georgiev and Kraptchev 1992; Tomato Genetics Resource Center: http://tgrc.ucdavis.edu/), we decided to keep the nomenclature of this mutation as "*erectoid leaf*". The analysis of the phenotypic results of the cross 'LT05' × 'LT17' population showed a clear contrast between the parental lines ($P < 0.0001$). The mean insertion angle α was 77 ° ± 2 ° for 'LT05' (n=13) and 142 ° ± 1 ° for 'LT17' (n=13). The reciprocal hybrids (obtained using each parent as either male or female) displayed similar results. The reciprocal hybrids displayed an intermediate leaf insertion angle between both contrasting lines ($p < 0.0001$). The average insertion angle α was 120 ° ± 1 ° and 119 ° ± 1 ° for F_1 (n=13) and F_1' (n=13), respectively (**Fig. 2 a**). These results suggested the absence of maternal inheritance effects. For the segregating families the distribution of their individuals in three different categories of leaf insertion angle (erectoid, intermediate, and wild-type) was analyzed (**Fig. 2 b**). The backcross family 'LT05' × F_1 segregated very close to a 1:1 (intermediate : erectoid) ratio. The same was observed with the backcross family 'LT17' × F1 with a 1:1 (intermediate : wild-type) ratio. The F_2 family segregated very closely to the expected 1:2:1 (erectoid : intermediate : wild-type) ratio. Finally, the F_3 families derived from the self-pollinating of F_2 plants with wild-type leaf insertion (**Fig. 2 c right**) displayed 100% individuals with wild-type leaf insertion, while in F_3 families derived from F_2 individuals with erectoid leaf insertion (**Fig. 2 c left**) had 100% of individuals with erectoid leaf insertion. These segregation patterns indicate a strong fit for a single gene model with semi-dominant inheritance of the erectoid leaf phenotype.

Chromosome location of the gene controlling the erectoid leaf phenotype

Each plant of the F_2 family derived from the cross between 'LT05' and 'TO-937' was simultaneously evaluated for the erectoid leaf and the uniform fruit ripening phenotype.

A clear-cut 1:3 segregation (monogenic) was observed for both erectoid leaf growth (75:199) and uniform fruit ripening (68:206) (**Table 1**). Within the subgroup of "erectoid" leaf growth (n=75) and the subgroup of "intermediate or wild-type" leaf growth (n=199), it was possible to observe a strong deviation of the expected ratio 3:1 for green fruit shoulder (dominant) and uniform fruit ripening (recessive), indicating that the two loci were not segregating independently, thus confirming their co-location on the tomato chromosome 10. A frequency of 12% of recombinants (33/274) indicated a relatively close genetic distance between these two loci.

Comparative analyses of chromosome 10 in the genomes of contrasting (erectoid vs. wild-type) lines

To identify potential polymorphisms associated with the erectoid leaf trait, a strategy based upon the comparative genome analyses of the original erectoid leaf source (= *S. lycopersicum* 'LT05') with the genomes of genetically unrelated accessions displaying standard (wild-type) leaf angle phenotypes (viz. 'Viradoro', 'TO-937', 'Santa Clara', 'CNPH498' and 'Heinz 1706') was employed. A total of 27,259 variants were found only on chromosome 10. After filtering for variants exclusively present in 'LT05', a total of 1,702 variants were obtained (**Fig. 3 a**). When filtering those variants for non-synonymous variants only five SNPs remained (**Fig. 3 b, Table 2**). One of these variants (corresponding to the alkaloid biosynthesis gene *Solyc10g086620*) was discarded because it was located far from the *SlGLK2* gene (*Solyc10g008160* located at position 2,293,088 in SL2.50), which was not in agreement with our data of 12% recombination (**Table 1**). The PROVEAN tool predicted that from the four remaining gene variants only one (*Solyc10g009320*) contained an amino acid change capable of inducing deleterious effect: an SNP that creates a new stop codon (**Fig. 3 c and d**). This *Erectoid leaf*-specific single nucleotide polymorphism (SNP) was found in the position 3,394,715 of chromosome 10, where a DNA substitution of a cytosine (C) for a thymine (T) (C>T) was observed. This SNP resulted in a change in the CAA codon (=coding for a glutamine) of the wild-type genomes to a TAA (= ochre stop-codon) in the genome of the line 'LT05' (with erectoid leaf trait). *Solyc10g009320* is about 1.1 Mbp from *SlGLK2*, in the euchromatin, consistent with the observed recombination frequency of 12%. The TomExpress RNASeq database reported a similar expression pattern in seeds, meristems, stem, leaves, and fruits for *Solyc10g009320*, but with higher expression in flowers and lower in roots (**Online**

Resource 3). Thus, *Solyc10g009320* became our single candidate gene for controlling the erectoid leaf growth phenotype.

Sequence analysis of *Solyc10g009320* and impact on the predicted proteins

The gene *Solyc10g009320* was found to encode a predicted protein of 279 amino acids. When translated, the *Erl*-specific SNP caused a change of a glutamine (CAA) codon (present in all the wild-type genomes) to a TAA (= ochre stop-codon) at amino acid position 222 in exon 3 (Q222*) in the 'LT05' genome (with erectoid leaf trait), resulting in a smaller version of the predicted mutant protein (221 amino acids) (**Fig. 3 c and d, Online Resource 4**). BLAST analysis (tBLASTx tool) of the predicted protein encoded by *Solyc10g009320* gene indicated 36% amino acid identity with the gene *PpeTAC1*, belonging to the gene family IGT, described to have a major role in determining plant architecture in peach.

Multiple alignments of the predicted protein sequences from *TAC*1-like genes of tomato and other plant species

Multiple alignments of TAC1-like proteins from tomato and from a diverse group of plant species revealed the presence of the conserved domains described by Dardick et al. (2013), and specifically the presence of an IGT (GφL(A/T)GT) domain (**Fig. 4**), which is present in a wide array of plant genomes and is related to the vertical growth of the shoots (Dardick et al. 2013, Roychoudhry and Kepinski 2015). Due to the similarities between *TAC1* and *Solyc10g009320* we named this tomato ortholog as *S. lycopersicum TAC1* (*SlTAC1*) gene.

Sanger sequencing validation of the *SlTAC1*-derived marker

PCR products obtained with the TAC-Tom (F/R) primers (designed to flank the identified SNP causing an early stop codon on *Solyc10g009320*) were Sanger sequenced in individual samples of two F_2 populations and the two contrasting near-isogenic lines. A total of 265 F_2 individuals were sampled from two F_2 populations. Sequence analyses of these individuals indicated the constant presence of the homozygous C>T substitution in all 108 F_2 plants with the erectoid leaf growth phenotype, in the parents, 'LT05' and in the near isogenic line IsoL-EL. On the other hand, the C>T substitution was found to be either absent or in heterozygous condition in all 157 F_2 with either intermediate or wild-

type growth phenotype, in the parental lines 'TO-937' and IsoL-WTL (**Online Resource 5**).

DISCUSSION

The erectoid leaf phenotype is controlled by a semi-dominant locus on chromosome 10

Our results indicated that the erectoid leaf phenotype observed in the line 'LT05' is controlled by a single semi-dominant gene/locus at the top of chromosome 10. This information was experimentally confirmed by the phenotypic analyses across distinct segregating populations and by linkage analysis with the uniform fruit ripening-coding *SlGLK2* gene, which is located on chromosome 10 (Kinzer et al. 1990; Powell et al. 2012). Two mutations with similar phenotypes were previously described in tomatoes: the *Erectoid leaf* (*Erl*) (Georgiev and Kraptchev 1992) and *erecta* (*er*) (LA0600). Given the similar characteristics in terms of location and pattern of inheritance, we assume that the gene that determines the erectoid leaf phenotype in the line 'LT05' is either the same gene or an allelic variant of the wild-type (Erl^{+}) gene as previously described by Georgiev and Kraptchev (1992) (Tomato Genetics Resource Center: http://tgrc.ucdavis.edu/, last accessed 12 March, 2018). Our results indicated that the Erl^{+} allele is associated with the wild-type phenotype, whereas the *Erl* allele is associated with the erectoid leaf trait. Heterozygous (Erl^{+}/*Erl*) plants displayed an intermediate leaf growth phenotype. Genes with similar phenotypic expression have been reported in other dicot and monocot plant species. In rice, the recessive loss-of-function allele of the *OzTAC1* gene (Yu et al. 2007) is one of the determinants of erect tiller growth and it has been used, along with other allelic variants, in several modern cultivars in order to generate more efficient crops (Dong et al., 2016). In peach, the "*broomy*" (*br*) allele of *PpeTAC1* defines the plant architecture by modifying the angle of insertion of the lateral branches and also displays a semi-dominant inheritance (Scorza et al. 1989, 2002). In this case, the possibility of manipulating the degree of branch inclination with distinct doses of the allele *br* (homozygous or heterozygous form) was suggested (Tworkoski and Scorza 2001).

A *TAC1*-like gene is the best candidate related to the erectoid leaf phenotype

Our genomic and genetic analyses allowed us to indicate *Solyc10g009320* (previously annotated as an 'unknown protein') as being the best candidate gene related to erectoid leaf growth observed in the 'LT05' line (zero recombinants in 265 F_2 plants analyzed,

Online Resource 5). The methodology that allowed to identify *Solyc10g009320* as the more likely candidate gene was based on the genomic comparison of a line with phenotypically stable erectoid leaf phenotype ('LT05') with the genomic information obtained from the reference genome 'Heinz 1706' (Tomato Genome Consortium, 2012) and from four accessions with wild-type leaf growth (viz. 'Viradoro', 'TO-937', 'Santa Clara', and 'CNPH498'). The previous confirmation (by mapping) of the chromosomal location of the erectoid leaf phenotype was a key information that allowed us to apply genomic filters directly to chromosome 10, starting with a total number of 27,259 variants and reaching only five candidate genes according to additional genomic and putative gene function analyses (**Table 2**). This filtering allowed a more precise landing on potential candidate genes/loci. The estimated location on the chromosome 10 (close to the *SlGLK2* gene), and the identification of loss-of-function mutation altogether allowed selection of the *Solyc10g009320* as the most likely candidate gene associated with the erectoid leaf growth phenotype. The validation using Sanger sequencing of the F_2 segregating population showed a 100% association of the erectoid leaf phenotype and *Solyc10g009320* mutation.

Due to the structural similarities of *Solyc10g009320* with an array of *TAC1*-like genes (see **Fig. 4**), we tentatively named this gene as *S. lycopersicum TAC1* (*SlTAC1*). Our annotation of *Solyc10g009320* as an ortholog of *TAC1* genes is consistent with a recent phylogenetic analysis (Guseman et al. 2017) where this tomato gene was placed into a small cluster along with other *TAC1* genes (e.g. *PpTAC1*, *OsTAC1*, *ZmTAC1*, and *AtTAC1*) within the IGT gene family. This work also identified a second tomato gene (*Solyc01g096260*) within the cluster of *TAC1*-like genes (Guseman et al. 2017). *Solyc01g096260* is located on tomato chromosome 1 and displayed ≈ 40% amino acid identity with *SlTAC1* (**Online Resource 6**). For this reason, we also examined polymorphisms within this gene across our resequenced accessions with contrasting leaf architecture. However, no variants were identified in comparative analyses of the proteins encoded by *Solyc01g096260* in the 'LT05' genome (with erectoid leaf growth phenotype) and the genomes of the four accessions with wild-type leaf phenotype (**Online Resource 7**). These analyses indicated that this evolutionary and functionally-related gene on chromosome 1 has no allelic variation in the coding sequence that could explain the phenotypic impact on the erectoid leaf growth in the germplasm employed in the present study, and indeed the genetic analysis showed that segregation of the locus on chromosome 10 was able to fully explain the occurrence of the erectoid leaf phenotype

without the need to propose the involvement of a second locus. Moreover, the expression patterns reported in the TomExpress RNA Seq database (**Online Resource 8**) show differences to *Solyc01g096260* when compared with *Solyc10g009320*, with a notably greater increase of expression in flowers and fruits and a low or no expression in vegetative parts. Although these data should be confirmed with expression and functional tests, it is likely that these genes, even though belonging to the same family, could be under control of distinct expression mechanisms across distinct plant organs.

It is not yet known how the *TAC1* genes are involved in determining the direction of lateral growth, although some evidence suggests that it would be directly or indirectly implicated in a negative regulation of *LAZY1* (Dardick et al. 2013) and this interaction, which varies depending on the position and aerial organ of the plant, would arise from a gravitropic response. In all cases described in the literature, the loss-of-function of *TAC1* genes (in both monocots and dicots plants) is associated with narrower insertion angles in tillers, leaves, and flowers (i.e. more erect posture) in comparison to the wild-type controls (Yu et al. 2007, Ku et al. 2011, Dardick et al. 2013). This fact is associated with higher auxin content in the affected organs, apparently resulting from a modification in the polar transport of this hormone (Li et al. 2007, Yoshihara and Iino 2007, Yoshihara et al. 2013). Since auxin transport/content is regulating a multiplicity of developmental processes (Reinhardt et al. 2003) mutants involved in this process might have pleotropic effects in several aspects of agronomic interest.

Auxin also controls many aspects of fruit development, including the sequential stages of fruit formation, expansion, ripening, and abscission (Gillaspy et al., 1993; Srivastava and Handa, 2005). In tomato, Pattison and Catalá (2012) showed a coordinated action of PIN and AUX/LAX proteins in the establishment of auxin gradients during fruit development. Artificially increasing the auxin levels in the ovary can bypass fertilization and lead to the development of parthenocarpic fruits (Lipari & Paratore 1988; Ficcadenti et al. 1999). This would have an interesting effect in extreme/hostile environments where fertilization is compromised by pollen viability problems. Additionally, regulation of auxin efflux by *SlPIN1* prevents flower abscission by maintaining a high auxin transport activity in the abscission zone (Shi et al. 2017).

On the other hand, little is known about possible effects of the *TAC1*-like genes at the root system. The pioneering study of Tworkoski and Scorza (2001) in peach provided information that mutations in aerial architecture (including *tac1*) is also associated with changes in root growth pattern. In rice, it was also reported that *DEEPER*

*ROOTING*1 (*DRO1*) controls the depth of the root system via the regulation of the insertion angle of lateral roots (Uga et al. 2013). An increase of the expression of this factor increases the depth of the root system. The deeper roots determined by *DRO1* allow a better performance and higher yield of the rice in water deficit conditions (Uga et al. 2013). Later, Guesan et al. (2017) generated evidence and hypothesize that, since the functions of *LAZY1* and *DRO1* are homologous, in shoots and roots respectively, *TAC1* could be a negative controller of both genes, and this may explain why a reduced function of *TAC1* could influence the vertical growth both of the canopy and of the roots. In this context, it would be also of interest to investigate the potential effects of the *Erl* locus on the root system in tomatoes.

Phenotypic and agronomic effects of the *Erectoid leaf* (*Erl*) mutation

So far, no strategies have been proposed to genetically manipulate the inclination angles of lateral shoots in order to obtain tomato ideotypes with adaptation to a wide range of environmental conditions (Zsögön et al. 2017). The importance of leaf insertion angle in relation to efficiency and distribution of light absorption (and hence the photosynthetic crop capacity) has been established in theoretical models using tree/forest species (Pearcy and Yang 1998, Sinoquet et al. 2005). Most studies are in agreement that larger leaf insertion angles (i.e. more horizontal leaves) intercept a considerable greater amount of light when the sun is placed at high angle in relation to the horizon (e.g. midday, summer season, low latitude areas) while smaller leaf insertion angles (i.e. erectoid leaf) intercept a greater portion of light when the sun is at low angles in respect to the horizon (e.g. during early mornings and late afternoons, winter season, and high latitude areas). Falster and Westoby (2003) reinforced these observations by performing studies with several plant species under high radiation conditions, proposing that the cost of increased light interception of horizontal leaves can involve higher leaf temperatures, higher risk of sunscalds and photo-inhibition. Thus, under high radiation conditions, a smaller leaf insertion angle (i.e. more erect leaves) would allow greater protection against the damages caused by the excess of radiation, including the reduction of the heat levels at the leaf surface, increasing the efficiency of water use, minimizing leaf burn damage (Werner et al. 2001), decreasing photo-inhibition (Ryel et al. 1993, Valladares and Pugnaire 1999, Werner et al. 2001) and improving the water use in relation to the daily carbon gain (Cowan 1982). Our data also indicate that is possible to manage the dosage of the *Erl* allele in tomato hybrids, regulating, to a certain extent, the angle of inclination of leaves

and leaflets in relation to the horizontal plane, which in turn, could result in plants with wider adaptation. Higher levels of environmental adaptation would result not only in the increase of the light interception and temperature control, but also by improving the rates of ventilation renewal in the plant canopy. Better ventilation in the plant canopy can influence the CO_2 content as well as the relative humidity of the surrounding air. Another positive crop management consequence of the erectoid leaf trait is to facilitate the distribution of pesticides, especially those targeting the abaxial leaf surface. This leaf surface is the major site of oviposition of important pests such as whiteflies (Silva et al 2014), being also the place of sporulation of a wide array of fungal pathogens. In fact, the erectoid leaf trait may also provide micro environmental conditions that prevent fungal spore germination by maintaining lower humidity levels under the canopy, thus potentially reducing the frequency of fungicide applications. This possible increase in efficiency in pesticide application might reduce both the frequency of sprayings as well as the production costs.

In summary, we found that the genetic factor controlling a peculiar tomato erectoid leaf phenotype (that affects all the aerial organs, especially the young leaf shoots) is located at chromosome 10. We also found that this semi-dominant allele *Erl* co-segregated with a loss-of-function mutation of the gene *Solyc10g009320*. This gene is a strong candidate for the genetic identity of *Erl*. In fact, the protein coded by *Solyc10g009320* has structural features of a TAC1-like proteins of the IGT gene family, which are distributed across a wide array of plant genomes, having a crucial role in the control the vertical growth of the shoots (Dardick et al 2013, Roychoudhry and Kepinski 2015). *TAC*1-like genes regulate auxin polar transport and content in shoots of several species, although it is not possible to discard that *TAC1*-like genes also control auxin transport/content in fruits and roots. From the breeding standpoint, this characterization of the mutant *Erl* will open the possibility for fine-tuning manipulation of tomato architecture and will allow development of more practical co-dominant molecular markers for employment in marker-assistance selection systems of this important trait. In addition, the genetic characteristics (loss-of-function mutation) of *Solyc10g009320* make it a potential target for gene editing strategies (Belhaj et al. 2015; Zsögön et al. 2017).

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Conflict of Interest: The authors declare that they have no conflict of interest.

TABLES AND FIGURES

Table 1 Phenotypic evaluation of an F_2 population (n=274) derived from the cross between 'LT05' (erectoid leaf growth; uniform fruit ripening) and 'TO-937' with standard (wild-type) leaf growth and green fruit shoulder trait.

	Erectoid leaf growth	**No-erectoid leaf growth (intermediate or standard)**
Uniform ripening	20	186
Green shoulder	55	13
Fit for expected 3:1 ratio	χ^2>20 (P<0.0001)	χ^2>20 (P<0.0001)

Table 2 Candidate genes located in the genomic region associated with the erectoid leaf growth trait (located at chromosome 10), containing *Solanum lycopersicum* 'LT05'-specific non-synonymous, single nucleotide polymorphisms (SNPs).

Gene identity	**Nucleotide position (SL2.50)**	**Protein annotation (ITAG 2.40)**	**SNP position (SL2.50)**	**Non-conservative amino acid substitution**	**PROVEAN Prediction Score[1]**
Solyc10g005230	184314-188942	Unknown protein	184523	V43M	Neutral (-1.068)
Solyc10g006890	1323124-1333653	WD-repeat protein	1323966	R183C	Neutral (-1.514)
Solyc10g007100	1487998-1492135	Protein detoxification 18	1491582	C56S	Neutral (-0.057)
Solyc10g009320	3390388-3395779	Unknown protein	3394715	Q222*	Deleterious (-61.263)
Solyc10g086620	65405159-65407893	Tropinone reductase homolog At5g06060 isoform X1	65392038	G170*	Deleterious (-248.543)

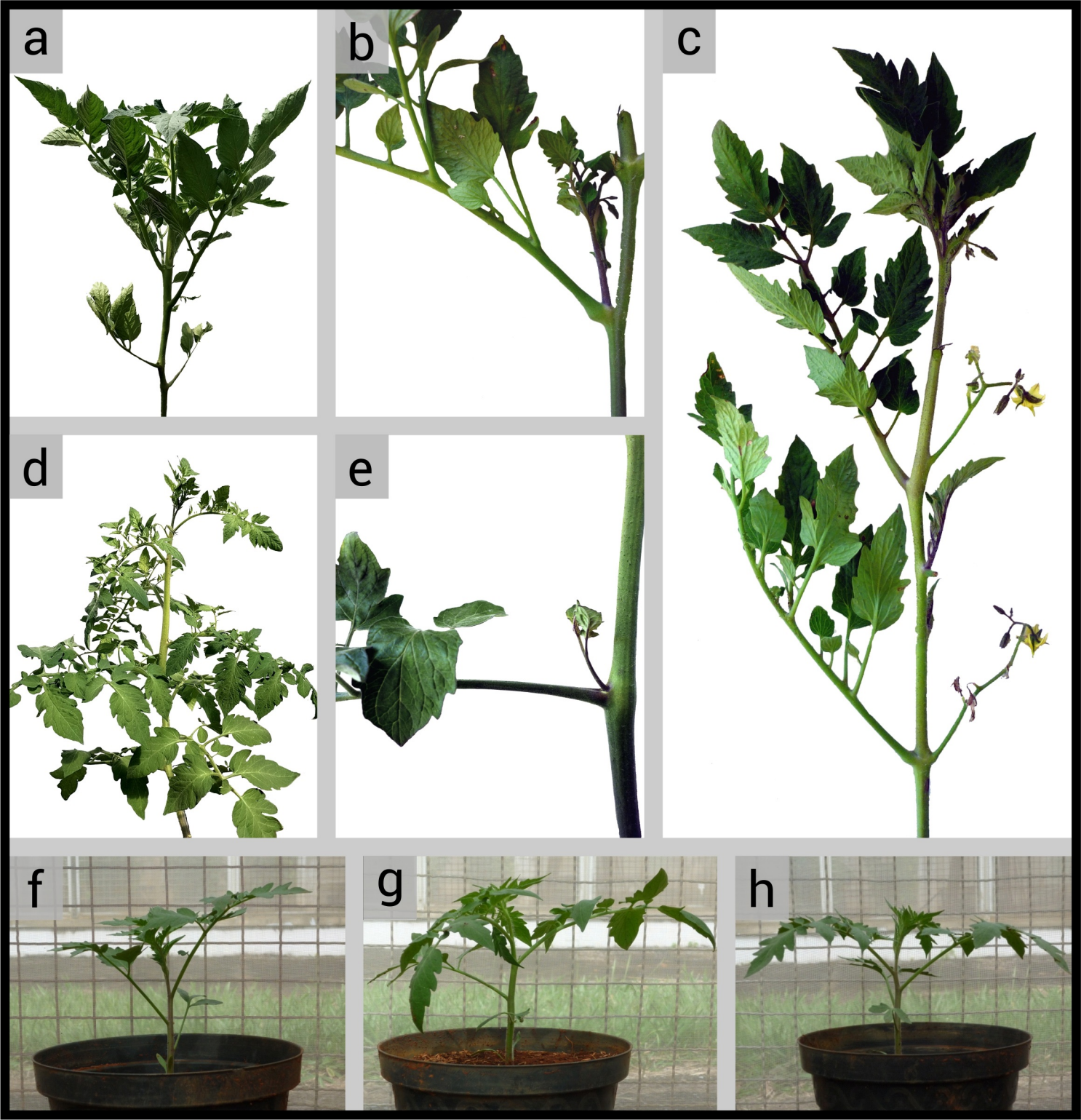
[1]PROVEAN (Protein Variation Effect Analyzer) is a tool which predicts impact of an amino acid substitution or indel on the biological function of a protein. Variants with a score equal to or below –2.6 are considered 'deleterious' and variants with a score above –2.6 are considered 'neutral'.

Fig. 1 Vegetative effects of the contrasting erectoid leaf alleles (*Erl*$^{+}$ versus *Erl*) in distinct developmental stages of tomato (*Solanum lycopersicum*) plants. (**a)** Plant of the inbred line 'LT05', the original homozygous (*Erl*/*Erl*) source of the erectoid leaf phenotype; (**b)** Detail of the foliar insertion angle with respect to the main stem in the 'LT05' line. (**c)** Detail of an apical section of a main stem of 'LT05' line, showing the effects of the erectoid leaf mutation on the growth of leaflets, leaves, shoots, and trusses. (**d)** Plant of the inbred line 'LT17', used as a reference for the standard wild-type phenotype (E*rl*$^{+}$/*Erl*$^{+}$). (**e)** Detail of the foliar insertion angle with respect to the main stem in 'LT17' line. (**f)** Plant of 'IsoL-EL' isoline (E*rl*/*Erl*). (**g)** Plant of 'IsoL-WTL' isoline (*Erl*$^{+}$/*Erl*$^{+}$). (**h)** Heterozygous (*Erl*$^{+}$/*Erl*) F_1 plant derived from the cross of 'IsoL-EL' × 'IsoL-WTL' with intermediate leaf angle phenotype.

Fig. 2 Leaf insertion angles in 'LT05' × 'LT17' in segregating populations. (**a)** Value of the α angle in 'LT05' (erectoid) and 'LT17' (wild-type with standard leaf growth) and the reciprocal hybrids generated in both crossing directions F_1 ('LT05' × 'LT17') and F_1' ('LT17' × 'LT05'). Values shown as mean ± standard error (n=13). Treatments with different letters in the bars are significantly different (P<0.0001). (**b)** Phenotypic frequency (mean α angle of erectoid < 100°, mean α angle of intermediate between 100-125° and mean α angle of wild-type >125°) in three segregating families: ('LT05' × F_1), ('LT17' × F_1) and F_2 (F_1 × F_1). The Chi-squared test for Mendelian segregation models is shown. (**c**) **Left**: F_3 families derived from self-pollinating of F_2 plants with erectoid leaf growth phenotype. **Right**: F_3 families derived from self-pollinating of F_2 plants with the standard, wild-type (=non-erectoid) leaf growth phenotype.

Fig. 3 (a) Variant density plot of the tomato (*Solanum lycopersicum* L.) chromosome 10. The figure depicts synonymous and non-synonymous variants that were exclusive for the genome of 'LT05' (an inbred line with the erectoid leaf growth) in comparison with the genomes of four accessions with standard (wild-type) leaf (viz. 'Viradoro', 'TO-937', 'Santa Clara', and 'CNPH498') as well as the reference genome 'Heinz 1706'; (**b)** Positioning of the single nucleotide polymorphisms – SNPs (black circles) that were selected after applying a filter for non-synonymous variants. The black arrow indicates the position of the linked *SlGLK*2 gene (controlling uniform fruit ripening) that was used as phenotypic marker to confirm the chromosome location of the erectoid leaf growth trait; (**c)** Structural features of the *Solyc10g009320* gene, identified as a strong candidate for control of the erectoid growth trait in the tomato line 'LT05'; (**d)** Genomic sequences of the accessions with standard (= wild-type) and with the erectoid leaf growth. The asterisk marks the position 3,394,715 of chromosome 10, where the 'LT05' genome (with the erectoid leaf growth trait) displayed a substitution of a cytosine (C) for a thymine (T) (C>T). This SNP resulted in a change in the CAA codon (=coding for a glutamine) of the wild-type leaf growth to a TAA (= ochre stop-codon) in the 'LT05' genome and in F_2 individuals carrying the erectoid leaf growth trait.

Fig. 4 Multiple alignments of the predicted protein sequences from *TAC1*-like genes of tomato (*Solyc10g009320*), peach (*PpTAC1*), soybean (KRH06413); *Arabidopsis thaliana* (*AtTAC1*), maize (*ZmTAC1*), and rice (*OsTAC1*). The plant species corresponding to each *TAC1* homolog are indicated in left column. Highly conserved residues are highlighted in black, including the conserved GφL(A/T)GT domain (at the position 70), which is characteristic of the IGT gene family. The gene *Solyc10g009320* was found to encode a predicted protein of 279 amino acids. The black asterisk highlights the position of the Q222* on the tomato homolog sequence (corresponding to the position 280 of the consensus alignment) where the stop-codon mutation was fond in the *Erectoid leaf* (*Erl*) allele.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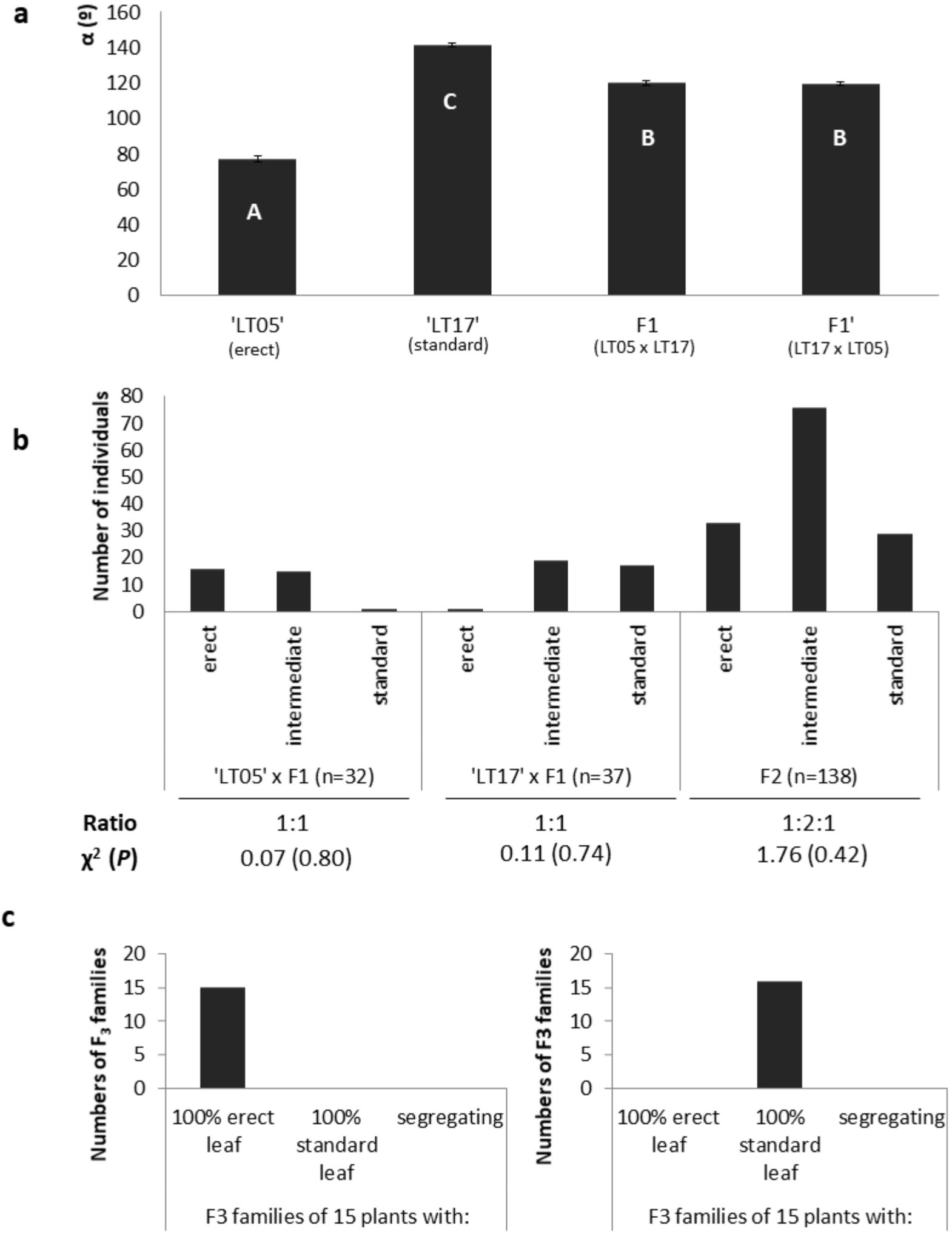
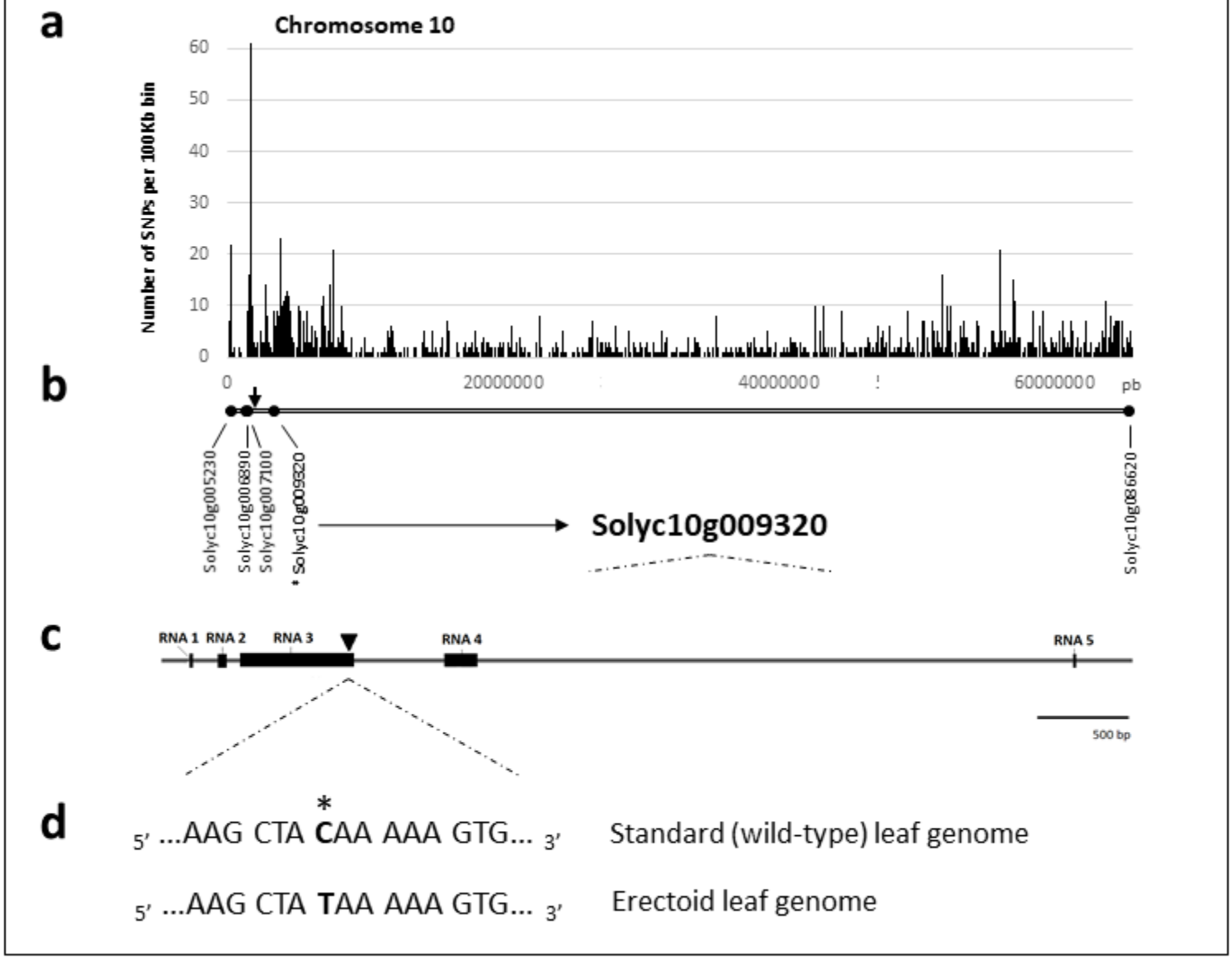
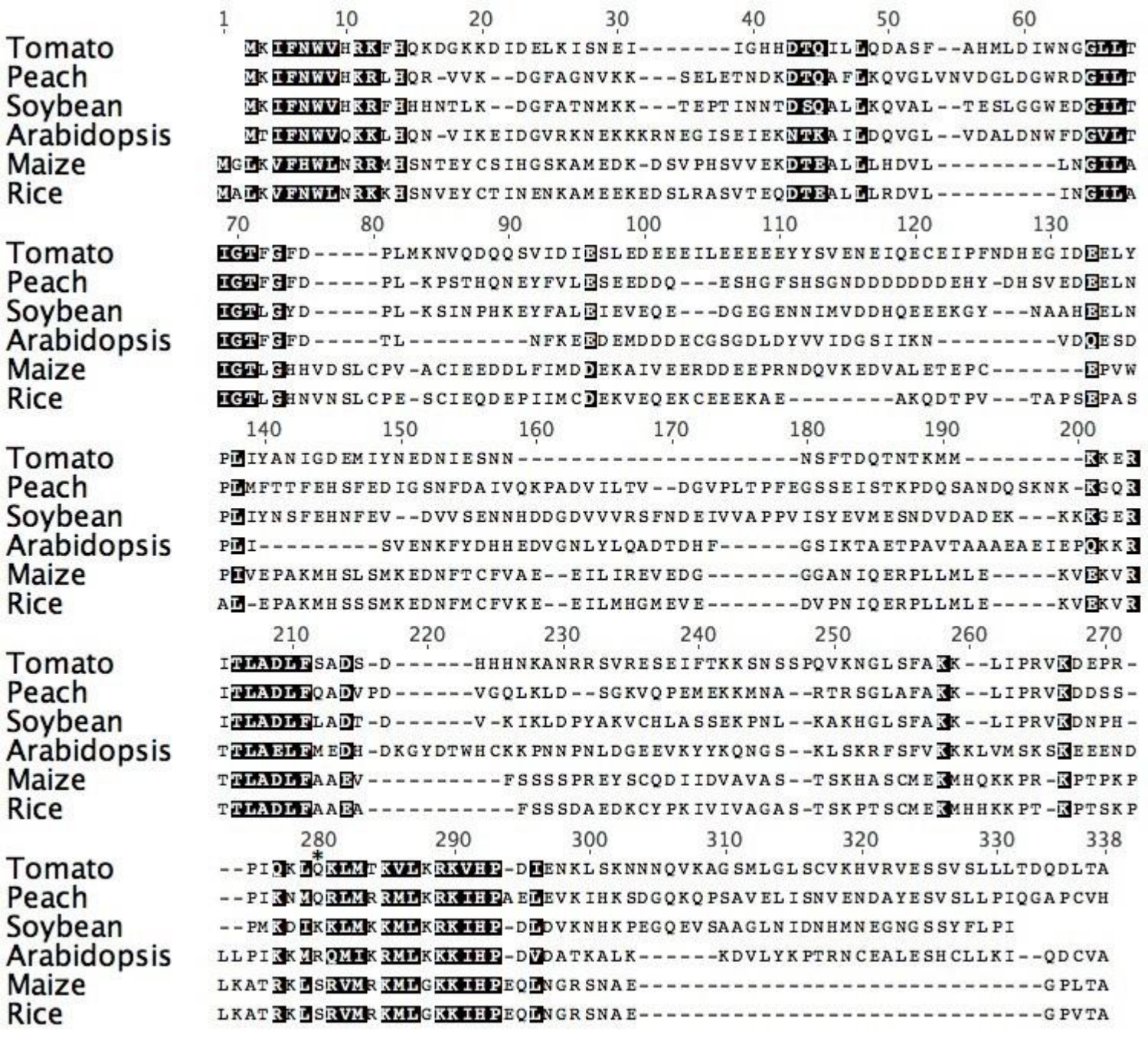

a
α (º)
160
140
120
100
80
60
40
20
0
A
C
B
B
'LT05'
(erect)
'LT17'
(standard)
F1
(LT05 x LT17)
F1'
(LT17 x LT05)
b
Number of individuals
80
70
60
50
40
30
20
10
0
erect
intermediate
standard
erect
intermediate
standard
erect
intermediate
standard
'LT05' x F1 (n=32)
'LT17' x F1 (n=37)
F2 (n=138)
Ratio
1:1
1:1
1:2:1
χ² (P)
0.07 (0.80)
0.11 (0.74)
1.76 (0.42)
c
Numbers of F3 families
20
15
10
5
0
100% erect leaf
100% standard leaf
segregating
F3 families of 15 plants with:
Numbers of F3 families
20
15
10
5
0
100% erect leaf
100% standard leaf
segregating
F3 families of 15 plants with:


a
Chromosome 10
Number of SNPs per 100Kb bin
60
50
40
30
20
10
0
0
20000000
40000000
60000000
pb
b
Solyc10g005230
Solyc10g008890
Solyc10g007100
* Solyc10g009320
Solyc10g009320
Solyc10g086620
c
RNA 1
RNA 2
RNA 3
RNA 4
RNA 5
500 bp
d
*
5′ ...AAG CTA CAA AAA GTG... 3′
Standard (wild-type) leaf genome
5′ ...AAG CTA TAA AAA GTG... 3′
Erectoid leaf genome


Tomato
Peach
Soybean
Arabidopsis
Maize
Rice
1 10 20 30 40 50 60
70 80 90 100 110 120 130
140 150 160 170 180 190 200
210 220 230 240 250 260 270
280 290 300 310 320 330 338