Comprehensive Profiling of Tubby-Like Protein Expression Uncovers Ripening-Related TLP Genes in Tomato (*Solanum lycopersicum*)

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Abstract: Tubby-like proteins (TLPs), which were firstly identified in obese mice, play important roles in male gametophyte development, biotic stress response, and abiotic stress responses in plants. To date, the role of TLP genes in fruit ripening is largely unknown. Here, through a bioinformatics analysis, we identified 11 TLPs which can be divided into three subgroups in tomato (*Solanum lycopersicum*), a model plant for studying fruit development and ripening. It was shown that all SlTLPs except SlTLP11 contain both the Tub domain and F-box domain. An expression profiling analysis in different tomato tissues and developmental stages showed that 7 TLP genes are mainly expressed in vegetative tissues, flower, and early fruit developmental stages. Interestingly, other 4 TLP members (SlTLP1, SlTLP2, SlTLP4, and SlTLP5) were found to be highly expressed after breaker stage, suggesting a potential role of these genes in fruit ripening. Moreover, the induced expression of SlTLP1 and SlTLP2 by exogenous ethylene treatment and the down expression of the two genes in ripening mutants, further support their putative role in the ripening process. Overall, our study provides a basis for further investigation of the function of TLPs in plant development and fruit ripening.

Keywords: tubby-like proteins; TLPs; family genes; fruit ripening; tomato

1. Introduction

Tubby-like proteins (TLPs), first identified in obese mice, are ubiquitous in eukaryotes varying from single-celled to multicellular organisms [1,2]. TLPs are characterized by a signature of the C-terminal tubby domain that forms a closed β barrel with 12 anti-parallel strands and a central hydrophobic α helix [3]. In plants, most known TLPs contain not only a conserved C-terminal tubby domain but also a highly conserved F-box domain at their N-terminus, which is different from the high divergence of the N-terminal sequence in animals [4–8].

Tubby-like proteins were implicated as transcription factors by structural-based functional analysis and subcellular localization assays [3,9]. In animals, TLPs are known to play important roles in the maintenance and functioning of neuronal cells during post-differentiation and development. Mutation of tubby genes can lead to adult obesity, insulin resistance, retinal degeneration, and neurosensory loss [1,8,10,11]. Compared with the wide range of cellular functions of animal TLPs, our knowledge on the role and mode of action of plant TLPs remains largely incomplete. In arabidopsis (*Arabidopsis thaliana*), the plant research model, 11 TLPs were identified and AtTLP9 was shown to be involved...
in responses to salt and drought stress \[4,12\]. Moreover, redundant functions between \textit{AtTLP3} and \textit{AtTLP9} in plants were found in response to ABA and osmotic stress \[4\]. \textit{AtTLP2} was reported to regulate the biosynthetic process of homogalacturonic acid in the mucus of seed coats \[4\]. In rice (\textit{Oryza sativa}), 14 \textit{OsTLPs} were identified and an expression profiling analysis showed that \textit{OsTLPs} are differentially expressed in different tissues at distinct developmental stages \[2\], suggesting that the \textit{OsTLP} family genes may play an important role in different physiological and developmental processes. More recently, \textit{MdTLP} (Tubby-like proteins in \textit{Malus domestica}) family genes were found to be expressed in multiple organs with high levels in roots, stems, and leaves, but low in flowers of apples. Interestingly, the expression of all \textit{MdTLPs} was up-regulated to some extent under abiotic stress, exogenous ABA and H\textsubscript{2}O\textsubscript{2} treatments in leaves and root, suggesting the role of \textit{MdTLPs} in responses to stress. Indeed, expression of \textit{MdTLP7} was reported to enhance abiotic stress tolerance in \textit{arabidopsis} \[13\]. In addition, overexpression of \textit{CaTLP1} in chickpeas was reported to promote tolerance to salt, drought and oxidative stress \[14\]. These studies suggested that \textit{TLPs} play an important role in stress response in different plant species, but the potential role of \textit{TLPs} in fruit development is largely unknown \[15\].

\textit{Tomato} (\textit{Solanum lycopersicum}) is not only one of the most important and popular vegetable plants in the world but also a model for fruit development and ripening research \[16\]. In this study, through genome-wide identification, classification and phylogenetic analysis, we identified 11 \textit{TLP} family genes which can be divided into three subgroups in tomato. An expression profiling analysis by qRT-PCR showed that four \textit{TLP} family genes (\textit{TLP1}, \textit{TLP2}, \textit{TLP4}, and \textit{TLP5}) are specifically expressed during fruit ripening, suggesting a potential role of these genes in fruit ripening. Moreover, the expression of \textit{TLP1} and \textit{TLP2} can be induced by exogenous ethylene treatment and their expression was found to be significantly downregulated in \textit{rin} and \textit{nor} ripening mutants, further supporting their putative role in the tomato ripening process. Overall, our study sheds light on the putative role of \textit{TLPs} in plant development and fruit ripening.

\section{Results}

\subsection{Genome-Wide Identification and Phylogenetic Analysis of TLPs in Tomato}

The \textit{TLPs} in the whole genome of tomato were identified by using the sequences of \textit{arabidopsis} \textit{TLPs} as BLAST queries against the tomato genome (ITAG 2.40). Then we used HMMER to verify whether the identified \textit{TLPs} contain typical Tub domains (PF01167). A total of 11 \textit{TLPs} were identified in tomato by using these methods. The \textit{SitLP} peptides ranged in length from 249 to 427 amino acids, with a gene length between 750 and 1284 bp. The predicted isoelectric point (PI) values of \textit{TLPs} are from 9.16 to 9.63 and protein molecular weight (MW) from 27.74 to 47.80 (kDa). Moreover, subcellular localization prediction suggested that most tomato \textit{TLPs} were located in the nucleus, with exception of \textit{SitLP2} and \textit{SitLP6} which were predicted to be located in chloroplasts and \textit{TLP3} was predicted to be located in mitochondria. These sequence characteristics of \textit{TLPs} are shown in Table 1.

To investigate the phylogenetic relationship of \textit{TLP} proteins in tomato, we constructed a phylogenetic tree using the neighbor-joining (NJ) method based on multiple sequence alignments of 11 \textit{arabidopsis} \textit{TLP} proteins, 14 rice \textit{TLP} proteins and 11 tomato \textit{TLP} proteins (Supplementary Table S1). The phylogenetic distribution showed that \textit{TLP} genes in the three species were all divided into three major clades, A, B and C (Figure 1). Clade A can be further divided into A1 and A2 subgroups. Both subgroup A1 and A2 contained three \textit{TLPs} proteins in tomato. Clade B contained four tomato \textit{TLPs} (\textit{TLP7}, \textit{TLP8}, \textit{TLP9}, \textit{TLP10}) and Clade C only possessed one \textit{TLP} (\textit{TLP11}). Among the three clades, A and B were closer to each other, while C was estranged. In addition, \textit{TLPs} in tomato were found to be more similar to that in \textit{arabidopsis} which is also a dicotyledonous plant.
Table 1. Basic Information of tubby-like proteins (TLPs) in tomato.

| Group | Name     | Locus          | Chr  | Start        | End           | Strand | pI  | Mw (kDa) | Protein (aa) | ORF (bp) | Subcellular Localization |
|-------|----------|----------------|------|--------------|---------------|--------|-----|----------|--------------|----------|--------------------------|
| A1    | TLP1     | Solyc09g074510 | Chr09| 66738841     | 66738900      | +      | 9.33| 44.42    | 396          | 1191     | nucl                     |
|       | TLP2     | Solyc01g067680 | Chr01| 76374360     | 76375015      | -      | 9.33| 27.74    | 249          | 750      | chlo                     |
|       | TLP3     | Solyc07g062390 | Chr07| 65290508     | 65294312      | +      | 9.16| 43.04    | 386          | 1161     | mito                     |
| A2    | TLP4     | Solyc01g104670 | Chr01| 92988825     | 92989308      | -      | 9.35| 47.80    | 427          | 1284     | nucl                     |
|       | TLP5     | Solyc10g046970 | Chr10| 38906011     | 38906513      | -      | 9.62| 47.80    | 426          | 1281     | nucl                     |
|       | TLP6     | Solyc04g071440 | Chr04| 58509459     | 58510657      | +      | 9.54| 47.60    | 426          | 1281     | chlo                     |
| B     | TLP7     | Solyc02g085130 | Chr02| 48750167     | 48750836      | +      | 9.63| 46.20    | 411          | 1236     | nucl                     |
|       | TLP8     | Solyc02g062670 | Chr02| 34946438     | 34947426      | +      | 9.25| 46.25    | 411          | 1236     | nucl                     |
|       | TLP9     | Solyc03g033980 | Chr03| 5712189      | 5713153       | +      | 9.39| 45.52    | 406          | 1221     | nucl                     |
|       | TLP10    | Solyc04g071750 | Chr04| 58798600     | 58798766      | +      | 9.46| 44.80    | 400          | 1203     | nucl                     |
| C     | TLP11    | Solyc03g117730 | Chr03| 68266827     | 68267351      | -      | 9.26| 45.82    | 406          | 1221     | nucl                     |
2.2. Motif and Gene Structure Analysis of TLPs in Tomato

From the Pfam database, we found that the key domain of TLPs in tomato was Tub domain (PF01167) and all TLPs except TLP11 also contain F-box domain (PF00646). To further explore the conservation and diversity of the TLPs, 10 conserved motifs ($E \leq 0.01$) were found by MEME (Figure 2 and Supplementary Table S2). All TLPs were found to contain motif 1 and motif 4. Specifically, besides TLP11, all other TLP members contained motif 2, motif 4, motif 5, motif 6, and motif 8. As shown in Figure 2, all TLP genes contained both exons and introns. Moreover, the conservation of TLP proteins was higher than that in the gene structure (Figure 2).

Figure 2. The Motif, domain, and gene structure of TLPs in tomato.
2.3. Chromosomal Distribution and Selective Pressure Analysis of TLPs in Tomato

To study the distribution of TLP genes on chromosomes, we mapped the chromosomal location of tomato TLP family genes. The results show that the 11 TLPs in tomato were dispersed on seven chromosomes with TLP2 and TLP4 located on chromosome 1, TLP7 and TLP8 on chromosome 2, TLP9 and TLP10 on chromosome 3, TLP6 and TLP10 on chromosome 4, TLP3 on chromosome 7, TLP1 on chromosome 9, and TLP5 on chromosome 10.

To further explore the potential evolutionary mechanism of TLPs in tomato, collinear genes in the tomato genome were identified through Blastp and MCScanX. As shown in Figure 3, two groups of genes were found to have strong collinearity. One group was TLP4 and TLP5 and another group was TLP7, TLP8, and TLP9. We also calculated their Ka/Ks by MCScanX and found that they are all less than 1 (Ka/Ks: TLP4-TLP5, 0.10; TLP7-TLP8, 0.20; TLP7-TLP9, 0.17; TLP8-TLP9, 0.15), which implies that they have strongly purifying selection during evolution.

2.4. Analysis of Promoter Sequences of SlTLPs

To study the putative role of TLPs in tomato, the promoter sequences of tomato TLPs were analyzed (CDS upstream 2000 bp) by PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). The cis elements of all SlTLPs promoters are shown in Figure 4 and Table 2. Noteworthily, among all TLP family genes, TLP3, TLP10, and TLP11 contain a number of different cis elements and TLP6, TLP8, and TLP9 contain fewer cis elements. Specifically, most TLP promoters contained both CGTCA-motif and TGACG-motif which were related to the jasmonate acid response. Moreover, ARE, which was related to anaerobic reaction and ABRE, which was associated to the abscisic acid response, were found in most TLPs’ promoters [2,4,6,15]. These results suggest that TLPs may play an important role in stress response, but this needs further experimental verification.
Table 2. Cis-acting regulatory elements in the promoter sequences of tomato TLP genes.

| Name  | MeJA | Anaerobic | Light | ABA | SAL | Zein | Metabolism | Defense and Stress | Cold | Meristem | Cell Cycle | Gibberellin | Auxin | Circadian Control | Total |
|-------|------|-----------|-------|-----|-----|------|------------|-------------------|------|----------|------------|-------------|-------|------------------|-------|
|       | CGTCA | TGACG     | ARE   | ACE | G-Box | ABRE | TCA | O2-Site | TC-Rich Repeats | LTR | CAT-Box | MSA-Like | TATC-Box | AuxRR-Core | Circadian |       |
| TLP1  | 3     | 3         | 1     | 0   | 0    | 0    | 1   | 0      | 2                 | 0   | 0        | 0         | 0           | 0     | 0                | 10    |
| TLP2  | 2     | 2         | 2     | 1   | 2    | 0    | 0   | 0      | 0                 | 0   | 0        | 0         | 0           | 0     | 0                | 12    |
| TLP3  | 1     | 1         | 2     | 0   | 7    | 7    | 2   | 1      | 0                 | 0   | 1        | 0         | 0           | 0     | 1                | 11    |
| TLP4  | 1     | 1         | 6     | 0   | 1    | 1    | 1   | 1      | 0                 | 0   | 0        | 0         | 0           | 0     | 0                | 11    |
| TLP5  | 0     | 0         | 1     | 0   | 1    | 2    | 1   | 1      | 1                 | 1   | 1        | 0         | 0           | 0     | 1                | 9     |
| TLP6  | 0     | 0         | 3     | 0   | 0    | 1    | 0   | 0      | 1                 | 0   | 0        | 0         | 1           | 0     | 0                | 5     |
| TLP7  | 0     | 0         | 2     | 0   | 0    | 0    | 0   | 2      | 0                 | 2   | 2        | 0         | 1           | 0     | 0                | 9     |
| TLP8  | 2     | 2         | 0     | 0   | 0    | 0    | 0   | 2      | 0                 | 1   | 0        | 0         | 0           | 0     | 0                | 7     |
| TLP9  | 0     | 0         | 2     | 0   | 1    | 1    | 0   | 1      | 1                 | 1   | 0        | 0         | 0           | 0     | 0                | 6     |
| TLP10 | 1     | 1         | 1     | 0   | 4    | 3    | 2   | 0      | 0                 | 0   | 0        | 0         | 1           | 0     | 0                | 13    |
| TLP11 | 2     | 2         | 0     | 1   | 2    | 1    | 1   | 1      | 1                 | 0   | 2        | 2         | 0           | 0     | 1                | 15    |
| Total | 12    | 12        | 20    | 3   | 17   | 17   | 10  | 8      | 6                 | 4   | 4        | 2         | 2           | 1     | 1                | 119   |
2.5. Expression Profiling of Tomato TLP Family Genes

To explore the putative function of TLPs in tomato, we examined the expression of the 11 TLPs in various tissues and different development stages, including the fruit development and ripening process. As shown in Figure 5, based on the expression pattern, the 11 TLPs were divided into two subgroups. The TLPs in subgroup I (TLP3, TLP6, TLP7, TLP8, TLP9, TLP10, and TLP11) are mainly expressed in roots, stems, buds, and flower and young fruit which suggests a role of these genes in both vegetative and reproductive development. Interestingly, members of subgroup II (TLP1, TLP2, TLP4, and TLP5) are highly expressed during the fruit ripening and softening process. More particularly, TLP1 and TLP2, being specifically accumulated from Br (Breaker) to Br+10 (Breaker post 10 days) stages and TLP4 and TLP5 are specifically expressed after the Br+10 stage. The specific expression during fruit ripening and softening suggested that SlTPL1 and SlTLP2 may play an important role in fruit ripening and SlTLP4 and SlTLP5 may be involved in fruit softening.
Figure 5. Expression of TLPs in different tissues of tomato (20DPA: Fruit at 20 days after anthesis; IMG: Immature green fruit; MG: Mature green fruit; Br: Breaker stage fruit; Br+3: 3 days post-breaker; Br+5: 5 days post-breaker; Br+7: 7 days post-breaker; Br+10: 10-day post-breaker; Br+15: 15 days post-breaker).

2.6. Expression of TLPs in Fruit Ripening Mutants

The role of TLPs in stress resistance has been extensively studied in other plants, while the role of TLPs in fruit ripening remains largely unknown. To further investigate the function of the ripening-related TLPs (TLP1, TLP2, TLP4, and TLP5) in tomato fruit ripening, we examined the expression levels of TLP1, TLP2, TLP4, and TLP5 in ripening-inhibitor (rin) and non-ripening (nor), two key ripening mutants [17,18]. The results show that TLP1 is significantly downregulated in rin at MG stage, and in nor at the Br stage (Figure 6). It is noteworthy that the expression levels of TLP2 were significantly decreased in both rin and nor mutants at the MG and Br stages (Figure 6). However, TLP4 showed no different expression in ripening mutants compared with WT. Interestingly, TLP5 displayed an upregulation in rin at the Br stage. The downregulation of TLP1 and TLP2 in ripening mutants further supports the specific role of the two genes in fruit ripening.
2.7. Expression of TLPs Under Exogenous Ethylene Treatment

To further investigate the role of TLPs in fruit ripening, we investigated the expression of ripening-related TLPs (TLP1, TLP2, TLP4, and TLP5) under exogenous ethylene treatment at MG (mature green) stage fruits (Figure 7). In line with the potential role of TLP1 and TLP2 in fruit ripening, we found that the expression of TLP1 and TLP2 was significantly induced with ethylene treatment. In contrast, the expression of TLP4 and TLP5 showed no significant change. These results suggest that TLP1 and TLP2 may be involved in ethylene-dependent fruit ripening.

Figure 6. Expression of TLP1, TLP2, TLP4, and TLP5 in WT, rin, and nor (MG: mature green fruit; Br: Breaker stage fruit; WT: Wild type; rin: ripening inhibitor mutant; nor: non-ripening mutant. The values represent the means of three biological replicates. *, p < 0.05 (Student’s t-test)).

Figure 7. Expression of TLP1, TLP2, TLP4, and TLP5 under ethylene treatment (MOCK: mature green stage fruit without ethylene treatment; Ethylene treatment: mature green fruit treated with ethylene for 30 min. The values represent the means of three biological replicates. *, p < 0.05 (Student’s t-test)).
3. Discussion

Tubby-like proteins (TLPs) have been identified in both animals and plants [15]. In several plant species, TLP family genes were identified and mainly shown to be involved in stress response [2,4,6,7]. However, to date, the TLP family in tomato, one of the most important model plants for fruit ripening research, had not been identified. In this study, to investigate the potential role of TLPs in fruit ripening, we identified 11 TLPs in tomato and showed that two TLP genes, TLP1 and TLP2, may act as ripening regulators based on their specific expression pattern during fruit ripening and their downregulation in ripening mutants.

Based on the analysis of the typical domains and gene structure of TLPs, we found that all TLPs expect TLP11 contain both the Tub domain and F-box domain, which is consistent with previous reports that most plant TLPs contain the F-box domain [2,4,6,7]. Moreover, we found that the motifs in TLP2 are different from other TLPs (Figure 2). To further investigate the difference of structures between TLP2 and other tomato TLP proteins, we built 3D models for Tub domains of TLP1, TLP2, TLP4, TLP8, and TLP11 (figure 8). From these 3D models, we found that the Tub domain of TLP2 is not complete and it lacks the important part which was thought to be essential for the typical tubby domain (Figure 8). The different structure of TLP2 may suggest a specific role of this gene compared with other TLP genes in tomato. Indeed, the specific expression during fruit ripening and downregulation in ripening mutants of TLPs further supports this hypothesis.

![Figure 8. Three-dimensional model of TLPs in tomato.](image)

The promoter sequence analysis suggested that most TLPs, especially TLP3, TLP11, and TLP10 in tomato may be related to response to drought and other biotic stresses which were consistent with the function of most TLPs identified in different plant species. Based on the collinear analysis, we found that the TLP4 and TLP5 are paralogs. Moreover, both TLP4 and TLP5 are specific expressed in the late ripening stages. This suggests that paralogs may play similar functions during plant development. Gene expression analysis of tomato TLP genes in different tissues and developmental stages showed that seven genes are mainly expressed in root, stem, flower and young fruit. Interestingly, two genes, TLP1 and TLP2, are found to be highly expressed during fruit ripening, suggesting an important role of the two genes in fruit ripening. Moreover, the downregulation of TLP1 and TLP2 in ripening mutants further supporting the putative role of the two genes in fruit ripening. Overall, our study provides...
new insight into the role of TLP family genes in fruit ripening and more studies are required to reveal the role and mode of action of TLP genes in fruit ripening.

4. Materials and Methods

4.1. Data Collection and Identification

Genome, protein, cDNA sequence, and gene annotation files of tomato were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov/) and Solanaceae Genomics Network (https://solgenomics.net/) [19]. The HMM of the TLP domain (PF01167) was downloaded from Pfam (http://pfam.xfam.org/), and Hmmsearch (3.2.1) was used to identify all possible protein sequences in the whole genome of tomato [20,21]. We used MEME (5.05) [22] (http://meme-suite.org/tools/meme) and Pfam (32.0) to identify the sequences of each presumed protein sequences of TLPs in tomato. We identified proteins based on the best hit proteins in NCBI-Blastp. The isoelectric point (PI) and molecular weight (MW) of TLPs in tomato were analyzed using Expasy [23] (http://web.expasy.org/compute_pi/). The subcellular localization prediction of TLPs in tomato was based on WoLF PSORT [24] (https://wolfpsort.hgc.jp/).

4.2. Analysis of Gene Structure, Chromosome Localization, Conserved Motif, and 3D Model

We used Tbtools [25] to draw the gene structure of TLPs in tomato which based on the tomato genome and used the MEME to identify the motif of TLPs in tomato. Full length amino acid sequences of TLPs in tomato were used by the MEME tool [22] (http://meme-suite.org/tools/meme) to identify conserved motifs (Parameter setting: output motifs: 10; minimum motif width: 6; maximum motif width: 200). Based on the tomato genome, we draw the chromosome localization of TLPs in tomato by Circos [26]. SWISS-MODEL [27–29] (https://www.swissmodel.expasy.org/) was used for building TLP1, TLP2, TLP4, TLP8, and TLP11 homologous protein model (At least 186 models for each protein were generated using “building model” engine and the best model was selected based on the best global model quality estimation).

4.3. Analysis of Collinearity and Selection Pressure

MCScanX [30] was used for collinearity analysis based on the Blast results file which was obtained by Blastp (E < 1e-5) to self-compare the tomato protein. Meanwhile, we used MCScanX to calculate the ka/ks value of the corresponding TLPs.

4.4. Multiple Sequence Alignment and Phylogenetic Tree Construction

The TLPs in tomato, arabidopsis, and rice were aligncompared by Clustal Omega [31,32] (https://www.ebi.ac.uk/Tools/msa/clustalo/). Neighbor-Joining (NJ) and Maximum likelihood (ML) trees were constructed using MEGA X (10.0.5) [33] with the aligned protein sequences (Bootstrap = 1000 replicates) [34].

4.5. Analysis of the Promoter Cis-Regulating Elements

PlantCare [35] (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to analysis the promoter sequences. 2000 bp of genomic DNA sequence upstream of the transcriptional start sites was obtained from the tomato genome.

4.6. Analysis of Gene Expression

The RNA-Seq data of root, stem, leaf, bud, flower, 20DPA, IMG, MG, Br, Br+3, Br+7, Br+10, Br+15 in tomato were downloaded from the TomExpress database [36] (http://tomexpress.toulouse.inra.fr/). The expression data represent normalized counts per base and mean values of multiple cultivars for different tissues and developmental stages and were used to generate heat map representations with R.
software (https://www.r-project.org). A correlation distance (Spearman) was used to cluster together genes with similar expression profiles.

4.7. Analysis of Gene Expression in Fruit Ripening Mutants and Ethylene Treatment Fruits

We used qRT-PCR to examine the expression of TLPs in WT, rin, and nor, and also the response of TLPs to exogenous ethylene treatment. cDNA was obtained by reverse transcription according to PrimeScript™RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara biomedical technology (Beijing) co., LTD., Beijing, China). Real-time quantitative (RT) PCR was performed as described by Pirrello et al., 2006 [37]. Primers for amplification were designed in software PerlPrimer v1.1.21 [38] (Supplementary Table S3). The values represent the means of three biological replicates. *, p < 0.05 (Student’s t-test).

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/1422-0067/21/3/1000/s1.

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Abbreviations

- TLPs: Tubby-like proteins
- WT: Wild-type Ailsa Craig
- rin: Ripening inhibitor mutant
- nor: Non-ripening mutant
- SlTLPs: Tubby-like proteins in Solanum lycopersicum (Tomato)
- AtTLPs: Tubby-like proteins in Arabidopsis thaliana (Arabidopsis)
- OsTLPs: Tubby-like proteins in Oryza sativa (Rice)
- MdTLPs: Tubby-like proteins in Malus domestica (Apple)
- CaTLPs: Tubby-like proteins in Cicer arietinum (Chickpeas)
- 20DPA: Tomato fruit 20 days after anthesis
- IMG: Immature green fruit
- MG: Mature green fruit
- Br: Breaker stage fruit
- Br+3: 3 d post-breaker
- Br+5: 5 d post-breaker
- Br+7: 7 d post-breaker
- Br+10: 10 d post-breaker
- Br+15: 15 d post-breaker

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