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REGULAR PAPER

A single-base deletion mutation in *SlIAA9* gene causes tomato (*Solanum lycopersicum*) *entire* mutant

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Abstract The *entire* (*e*) locus of tomato (*Solanum lycopersicum* L.) controls leaf morphology. Dominant *E* and recessive *e* allele of the locus produce pinnate compound and complex reduced leaves. Previous research had indicated that *SlIAA9*, an *Aux/IAA* gene, was involved in tomato leaf morphology. Down-regulation of *SlIAA9* gene by antisense transgenic method decreased the leaf complex of tomato and converted tomato compound leaves to simple leaves. The leaf morphology of these transgenic lines was similar with leaf morphology of tomato *entire* mutant. In this paper, we report that a single-base deletion mutation in the coding region of *SlIAA9* gene results in tomato *entire* mutant phenotypes.

Keywords *Aux/IAA* · Bin map · *Entire* mutant · *SlIAA9* · Tomato

Introduction

The phytohormone auxin regulates a wide variety of plant developmental and physiological processes. At the cellular level, auxin controls cell division, elongation and differentiation. On the whole-plant level, auxin plays an essential role in many processes, including vascular differentiation and embryogenesis, root elongation, lateral root initiation, stem elongation, fruit set and development, apical dominance, gravitropism and tropisms responses (Friml 2003). This multiplicity of regulatory activities has spurred considerable interests in studying the mechanisms of auxin signalling and response. A major breakthrough in auxin signalling research is the discovery of the F-box protein transport inhibitor response 1 (TIR1), which functions as an auxin receptor (Dharmasiri et al. 2005; Kepinski and Leyser 2005). It has been known that auxin signalling operates by recruiting specific transcription factors, leading to the expression of downstream genes that perform the required responses (Vogler and Kuhlemeier 2003). Three major classes of early auxin response genes have been identified from various plant species: *GH3* family, the *SAUR* family, and the *Aux/IAA* family (Guilfoyle 1999). Auxin induces many of these genes rapidly, specifically and without the requirement of de novo protein synthesis, and these genes are considered primary response genes (Tian et al. 2002).

Aux/IAA genes encode short-lived and nuclear-localised proteins that contain four highly conserved domains (I, II, III and IV) (Abel et al. 1995). Domain I functions as a transcriptional repressor (Tiwari et al. 2004). Domains II and IV contain functional nuclear localisation signals (Abel and Theoligis 1995). In addition, domain II plays a role in destabilising Aux/IAA proteins and may be a target for ubiquitination (Colon-Carmona et al. 2000). Domain III contains a $\beta\alpha\alpha$ motif similar to the DNA binding domain found in prokaryotic repressor of the ArcA family (Abel et al. 1994). Evidence indicates that domains III and IV mediate the homodimerisation and heterodimerisation between Aux/IAA proteins and heterodimerisation between Aux/IAA proteins and auxin response factors (ARFs) (Ouellet et al. 2001).

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Screening *Arabidopsis thaliana* mutants has identified mutations in several different *Aux/IAA* genes, including *IAA1*, *IAA3*, *IAA6*, *IAA7*, *IAA12*, *IAA14*, *IAA17*, *IAA18*, *IAA19* and *IAA28* (Park et al. 2002; Yang et al. 2004; Tian and Reed 1999; Kim et al. 1996; Nagpal et al. 2000; Hamann et al. 2002; Fukaki et al. 2002; Rouse et al. 1998; Reed 2001; Tatematsu et al. 2004; Rogg et al. 2001). Those screened mutants exhibited changes of a variety of auxin-related developmental phenotypes, and all of them were gain-of-function mutations. No obviously visible phenotypes were observed on loss-of-function mutations in most Arabidopsis *Aux/IAA* genes. Down-regulation of an *Aux/IAA* gene from tomato, *SlIAA9*, which has been proved to be involved in tomato fruit development and leaf morphogenesis, was able to convert tomato compound leaves to simple leaves (Wang et al. 2005).

Leaf functions in light capture and photosynthesis, thus it is critical to plant survival. The leaves of higher plants can be divided into two categories: simple leaves and complex leaves. Complex leaves can be pinnate or palmate. The cultivated tomato, *Solanum lycopersicum* L., has a typical unipinnate compound leaf. However, a large number of tomato mutants, including the *entire (e)*, *potato leaf (c)*, *trifoliate (tf)*, *wiry* (*w*), *wiry3* (*w3*), *wiry4* (*w4*), *wiry6* (*w6*), *Lanceolate* (*La*), *clausa* (*clau*), *Mouse ears* (*Me*), *Petroselinum* (*Pts*) and *tripinnate* (*tp*), that either decrease or increase the degree of leaf complexity, are available. Among these mutants, *clau*, *Me*, *Pts* and *tp* mutants increase the complexity of tomato leaves, and the others decrease the complexity. Leaf development in most of the above mutants have been studied extensively at the morphology and histology levels (Kessler et al. 2001), whereas there are very limited reports on these mutants at the molecular level.

The tomato *e* mutation has fewer leaf segments and looks superficially less complex than wild type (Fig. 1, Rick and Butler 1956). Down-regulation of *SlIAA9* converted tomato compound leaves to simple leaves and exhibited a similar leaf phenotype of the *entire* mutant. In addition, the transcription level of the *SlIAA9* gene was decreased in the *entire* mutant (Wang et al. 2005). These results suggested that there were some relationships between the *entire* mutant and *SlIAA9* gene. In this paper, we reported that there is a single-base deletion in the coding region of *SlIAA9* from the *entire* mutant. DNA blot analysis indicated that *SlIAA9* was present as a single copy

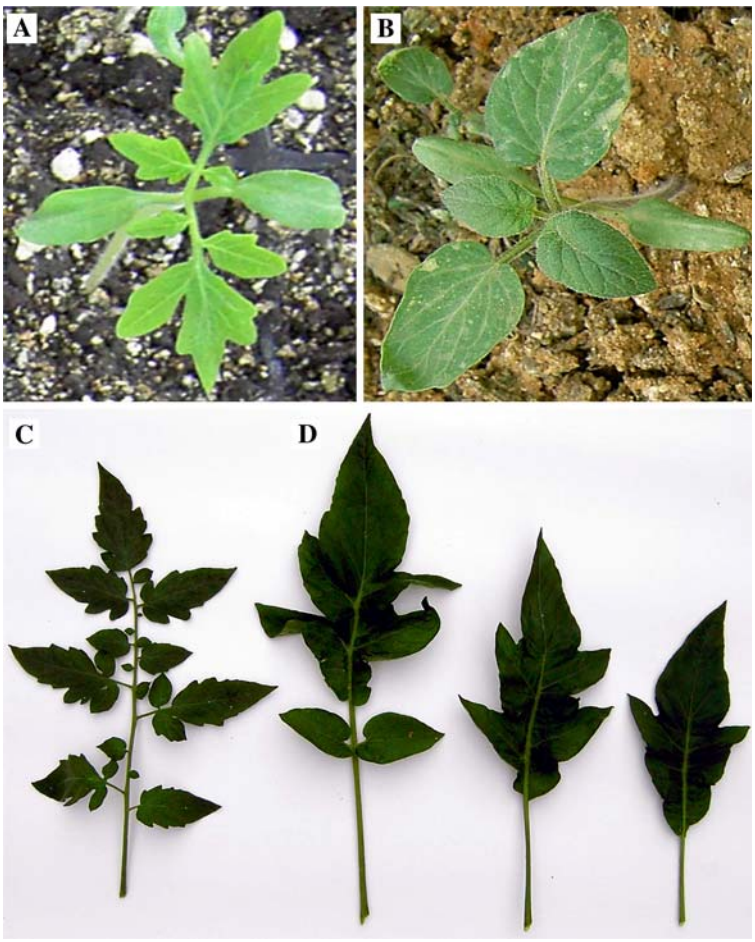

Fig. 1 Leaves from tomato cultivar Ailsa Craig (AC) and *entire* AC mutant. **a** Seedling of tomato cultivar AC; **b** seedling of *entire* AC mutant (LA2922); **c** compound leaf of tomato cultivar AC; **d** complexity-reduced leaves of *entire* AC mutant

in the genome of tomato. Bin-mapping results showed that *SlIAA9* and *e* genes are located at the same region on tomato chromosome 4. Further investigation also showed that another single-base deletion occurred in the coding region of *SlIAA9* from *e0880*, an *entire* allelic mutant generated by ethane methyl sulfonate (EMS) mutagenesis.

Materials and methods

Plant materials and growth conditions

Tomato cultivar LA3475 (M82) and the wild species LA716 (*S. pennellii*) were used for genomic DNA analysis. Seventy-five introgression lines (ILs) generated by Eshed and Zamir (1995) and their parents LA716 and LA3475 were used for genetic mapping of *SlIAA9*. Tomato cultivar Ailsa Craig (AC) and *entire* mutants including LA2922 (*entire* AC), LA0159, LA0885, LA0902, LA0917, LA0990, LA0991, and LA2360 were used for cDNA sequence analysis, as well as tomato cultivar LA3475 (M82) and *entire* allelic mutant *e0880*. The immature leaves of tomato Ailsa Craig and tomato *entire* mutants, LA2922, LA0159, LA0885, LA0902, LA0917, LA0990, LA0991 and LA2360 were used for expression analysis of *SlIAA9*. All plants were grown in a naturally illuminated greenhouse.

Cloning and sequence analysis of *SlIAA9*

We had isolated the full-length cDNA of *SlIAA9* from tomato cultivar Zhongshu No. 5 and submitted the sequences to GenBank (accession number: DQ115325). Because the *SlIAA9* cDNA clone was found to correspond to a previously isolated 301-bp partial tomato *IAA4* clone (GenBank accession number AF022015; Nebenfuhr et al. 2000), initially we named this gene *SlIAA4*. In order to comply with the numbering retained for the *Arabidopsis Aux/IAA* genes, Wang et al. (2005) renamed this gene *SlIAA9*. So we use this designation throughout our paper. According to the full-length cDNA sequences, the primers 5′-GTTACTGTCTGCCAAATGGAGGGT-3′ (forward) and 5′- GCACACGCATACTTAATCAAACGACA-3′ (reverse) were used to amplify the cDNA fragment of *SlIAA9* from tomato cultivars Ailsa Craig and LA3475 (M82), *entire* mutants and *entire* allelic mutant. Polymeraise chain reaction (PCR) products were gel purified and cloned into a plasmid vector, pMD18-T (TaKaRa, Japan), and four independent clones were sequenced to determine the nucleotide acid sequences. The sequence comparisons were performed using the Clustal W programme with standard parameters (http://www.ebi.ac.uk/clustalw/).

DNA isolation, DNA gel-blot analysis and bin mapping of *SlIAA9* gene

Tomato cultivars M82 and LA716 were used for genomic DNA analysis. Total genomic DNA was isolated from these two accessions using the method described in Fulton et al (1995). Genomic DNA (15 μg) was digested with *Eco*R I , *Eco*R V, *Dra* I, and *Hae* III, respectively, then fractionated in 1% agarose gel, transferred onto a nylon membrane and hybridised with ^{32}P-labelled *SlIAA9* full-length cDNA. The hybridisation was done at 65°C in a phosphous buffer (0.5 M) containing 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin (BSA) and 1 mM ethylenediaminetetraacetate (EDTA). The membrane was washed with a solution of 2× sodium saline citrate (SSC) and 0.5% SDS at room temperature for 10 min, followed by a solution of 0.2× SSC and 0.1% SDS under 65°C for 5 min.

Seventy-five LA716-derived ILs were used to bin map the *SlIAA9* gene. Each IL differs in a single defined LA716 chromosome fragment introgressed into the M82 background, and collection of 75 ILs provides overlapping coverage of the *entire* LA716 genome (Eshed and Zamir 1995). A DNA gel blot containing genomic DNA from all 75 ILs and their parents (M82 and LA716) digested with *Hae* III was generated and hybridised with *SlIAA9* full-length cDNA under high-stringency conditions as described in genomic DNA analysis.

Semiquantitative RT-PCR analysis

Total RNA was isolated from young leaves with Trizol reagent (Invitrogen, USA) according to the supplier's instruction. Reverse transcription (RT) reactions were carried out as recommended by the manufacturer (Takara, Japan). The products of RT were diluted with diethylpyrocarbonate (DEPC)-treated water. The resulting dilutions were used for gene expression analysis by semiquantitative RT-PCR. To examine the transcription level of *SlIAA9*, PCR reactions were carried out for 26 cycles of 94°C 45 s/57°C 1 min/72°C 1 min in a volume of 20 μl containing cDNA template dilution, 200 μM each dNTP, 4 mM $MgCl_2$, 4 mM forward and reverse primer pairs, 1 U *Taq* polymerase (recombinant, MBI, USA). The primers used for *SlIAA9* were 5′-GTTACTGTCTGCCAAATGGAGGGT-3′ (forward) and 5′-CTCGCCTACTAGAAATGCTGGA-3′ (reverse). *SlUBI3*, a tomato ubiqitin 3 gene (Hoffman et al. 1991), was used as an internal control. The primers used for *SlUBI3* were 5′-CTGGGGATGGTGTCAGCCACAC-3′ (forward) and 5′-CACCGAACTTTCTCTCGGAAGGTG-3′ (reverse). Ten microlitre PCR products were detected by agarose gel electrophoresis. The PCR reactions were repeated three times using independent samples.

Results

A deletion mutation occurs in the tomato *entire* mutant

The *SlIAA9* gene was involved in tomato fruit development and leaf morphogenesis, and down-regulation of *SlIAA9* would convert tomato compound leaves to simple leaves, a phenomenon also observed in the *entire* mutant. Transgenic lines with repressed expression of *SlIAA9*, the same as the *entire* mutant, carried a lower frequency, asymmetrical sepals and a multifusion phenotype affecting leaves, flowers and fruits mutant. Molecular analysis also indicated that the *SlIAA9* transcription level was decreased in the *entire* mutant (Wang et al. 2005). So it was logical to think that there might be some relationships between *SlIAA9* gene and the *entire* mutant. In order to examine these relationships, the complete coding sequence of *SlIAA9* from Ailsa Craig and LA2922 (*entire* AC) were analysed. Sequence analysis revealed that a single-base cytosine deletion of *SlIAA9* mRNA occurs at the nucleotide position 626 in the *entire* AC (Fig. 2). Amino acid sequences of *SlIAA9* were identical between Ailsa Craig and the *entire* AC for the first 208 bases. Then, the deletion changed the subsequent reading frame in the *entire* AC altered the following two amino acids, and the polypeptide was terminated immediately. The truncated polypeptide consisted of only 210 amino acids and lacked domains III and IV (Fig. 3). Previous studies indicated that domains III and IV of Aux/IAA proteins were involved in mediating the homodimerisation and heterodimerisation between Aux/IAA proteins and ARFs (Ouellet et al. 2001). The deletion of domain III and IV regions should make the *SlIAA9* protein functionally defective.

SlIAA9 gene expressed at a lower level in *entire* mutants

Besides LA2922 (*entire* AC), the *e* gene had been interlined into tomato materials with different backgrounds by

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AC-SlIAA9    ATGTCTCCGCCGCTCCTTGGTGTTGGGGAGGAGGAGGGCCAGAGTAATGTAACTCTACTG 60
eAC-SlIAA9   ATGTCTCCGCCGCTCCTTGGTGTTGGGGAGGAGGAGGGCCAGAGTAATGTAACTCTACTG 60
             ************************************************************
AC-SlIAA9    GCTTCTTCAACTTCCTTAGGAAGCATATGCATAAAAGGATCAGCTCTTAAAGAGCGAAAC 120
eAC-SlIAA9   GCTTCTTCAACTTCCTTAGGAAGCATATGCATAAAAGGATCAGCTCTTAAAGAGCGAAAC 120
             ************************************************************
AC-SlIAA9    TATATGGGTCTATCTGATTGTTCGTCGGTGGACAGCTGTAATATTTCCACCTCATCAGAG 180
eAC-SlIAA9   TATATGGGTCTATCTGATTGTTCGTCGGTGGACAGCTGTAATATTTCCACCTCATCAGAG 180
             ************************************************************
AC-SlIAA9    GACAATAATGGGTGTGGATTAAATCTCAAGGCAACGGAGCTCAGGCTCGGTCTACCTGGA 240
eAC-SlIAA9   GACAATAATGGGTGTGGATTAAATCTCAAGGCAACGGAGCTCAGGCTCGGTCTACCTGGA 240
             ************************************************************
AC-SlIAA9    TCTCAGTCTCCCGAAAGAGGTGAGGAGACTTGCCCTGTGATTTCGACAAAGGTTGATGAG 300
eAC-SlIAA9   TCTCAGTCTCCCGAAAGAGGTGAGGAGACTTGCCCTGTGATTTCGACAAAGGTTGATGAG 300
             ************************************************************
AC-SlIAA9    AAGCTGCTCTTCCCCTTGCACCCTTCCAAAGATACTGCTTTCTCGGTATCGCAGAAAACA 360
eAC-SlIAA9   AAGCTGCTCTTCCCCTTGCACCCTTCCAAAGATACTGCTTTCTCGGTATCGCAGAAAACA 360
             ************************************************************
AC-SlIAA9    GTAGTTAGTGGCAACAAACGAGGATTTTCAGACGCTATGGATGGATTCTCAGAGGGGAAG 420
eAC-SlIAA9   GTAGTTAGTGGCAACAAACGAGGATTTTCAGACGCTATGGATGGATTCTCAGAGGGGAAG 420
             ************************************************************
AC-SlIAA9    TTTCTGTCGAATTCCGGTGTGAAAGCAGGTGATACAAAGGAGACCTCACGTGTGCAACCA 480
eAC-SlIAA9   TTTCTGTCGAATTCCGGTGTGAAAGCAGGTGATACAAAGGAGACCTCACGTGTGCAACCA 480
             ************************************************************
AC-SlIAA9    CCTAAAATGAAAGATGCTAATACTCAGAGTACAGTTCCAGAGAGGCCTTCTGCTGTGAAT 540
eAC-SlIAA9   CCTAAAATGAAAGATGCTAATACTCAGAGTACAGTTCCAGAGAGGCCTTCTGCTGTGAAT 540
             ************************************************************
AC-SlIAA9    GATGCCTCAAACCGTGCGGGCAGTGGTGCCCCTGCTACAAAGGCACAGGTTGTTGGTTGG 600
eAC-SlIAA9   GATGCCTCAAACCGTGCGGGCAGTGGTGCCCCTGCTACAAAGGCACAGGTTGTTGGTTGG 600
             ************************************************************
                                      ⬇
AC-SlIAA9    CCACCCATTCGATCTTTTAGAAAGAACACTCTAGCCTCTGCCTCGAAGAATAACGAAGAG 660
eAC-SlIAA9   CCACCCATTCGATCTTTTAGAAAGAA-ACTCTAGCCTCTGCCTCGAAGAATAACGAAGAG 659
             ********************************* ********************************
AC-SlIAA9    GTTGACGGAAAAGCTGGCTCACCAGCTCTTTTTATTAAGGTCAGCATGGATGGTGCTCCC 720
eAC-SlIAA9   GTTGACGGAAAAGCTGGCTCACCAGCTCTTTTTATTAAGGTCAGCATGGATGGTGCTCCC 719
             ************************************************************
AC-SlIAA9    TATTTGAGGAAAGTGGACCTCAGAACCTGTTCTGCATACCAGGAGCTATCTTCTGCTCTT 780
eAC-SlIAA9   TATTTGAGGAAAGTGGACCTCAGAACCTGTTCTGCATACCAGGAGCTATCTTCTGCTCTT 779
             ************************************************************
AC-SlIAA9    GAAAAAATGTTCAGCTGTTTTACAATAGGTCAATATGGATCTCATGGAGCTCCTGGGAAG 840
eAC-SlIAA9   GAAAAAATGTTCAGCTGTTTTACAATAGGTCAATATGGATCTCATGGAGCTCCTGGGAAG 839
             ************************************************************
AC-SlIAA9    GATATGTTAAGTGAGAGCAAATTGAAGGATTTGCTTCATGGATCTGAGTATGTCCTCACT 900
eAC-SlIAA9   GATATGTTAAGTGAGAGCAAATTGAAGGATTTGCTTCATGGATCTGAGTATGTCCTCACT 899
             ************************************************************
AC-SlIAA9    TACGAAGATAAGGATGGGGACTGGATGCTTGTCGGTGATGTCCCCTGGGAGATGTTTATC 960
eAC-SlIAA9   TACGAAGATAAGGATGGGGACTGGATGCTTGTCGGTGATGTCCCCTGGGAGATGTTTATC 959
             ************************************************************
AC-SlIAA9    GATACTTGCAAAAGGTTGAGGATCATGAAAGGTTCAGATGCCATTGGCCTGGCCCCAAGG 1020
eAC-SlIAA9   GATACTTGCAAAAGGTTGAGGATCATGAAAGGTTCAGATGCCATTGGCCTGGCCCCAAGG 1019
             ************************************************************
AC-SlIAA9    GCTATGGAAAAGTGTCGGAGCAGAAATTAG 1050
eAC-SlIAA9   GCTATGGAAAAGTGTCGGAGCAGAAATTAG 1049
             ********************************
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Fig. 2 A single-base cytosine deletion of *SlIAA9* mRNA occurs in the *entire* AC mutant. AC-SlIAA9:*SlIAA9* from tomato cultivar Ailsa Craig; eAC-SlIAA9:*SlIAA9* from the *entire* AC mutant. *Arrow* indicates the position of cytosine deletion of eAC-SlIAA9

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ATGTCTCCGC CGCTCCTTGG TGTTGGGGAG GAGGAGGGCC AGAGTAATGT AACTCTACTG 60
 M  S  P   P  L  L  G   V  G  E   E  E  G   Q  S  N  V   T  L  L
GCTTCTTCAA CTTCCTTAGG AAGCATATGC ATAAAAGGAT CAGCTCTTAA AGAGCGAAAC 120
 A  S  S   T  S  L  G   S  I  C   I  K  G   S  A  L  K   E  R  N
TATATGGGTC TATCTGATTG TTCGTCGGTG GACAGCTGTA ATATTTCCAC CTCATCAGAG 180
 Y  M  G   L  S  D  C   S  S  V   D  S  C   N  I  S  T   S  S  E
GACAATAATG GGTGTGGATT AAATCTCAAG GCAACGGAGC TCAGGCTCGG TCTACCTGGA 240
 D  N  N   G  C  G  L   N  L  K   A  T  E   L  R  L  G   L  P  G
TCTCAGTCTC CCGAAAGAGG TGAGGAGACT TGCCCTGTGA TTTCGACAAA GGTTGATGAG 300
 S  Q  S   P  E  R  G   E  E  T   C  P  V   I  S  T  K   V  D  E
AAGCTGCTCT TCCCCTTGCA CCCTTCCAAA GATACTGCTT TCTCGGTATC GCAGAAAACA 360
 K  L  L   F  P  L  H   P  S  K   D  T  A   F  S  V  S   Q  K  T
GTAGTTAGTG GCAACAAACG AGGATTTTCA GACGCTATGG ATGGATTCTC AGAGGGGAAG 420
 V  V  S   G  N  K  R   G  F  S   D  A  M   D  G  F  S   E  G  K
TTTCTGTCGA ATTCCGGTGT GAAAGCAGGT GATACAAAGG AGACCTCACG TGTGCAACCA 480
 F  L  S   N  S  G  V   K  A  G   D  T  K   E  T  S  R   V  Q  P
CCTAAAATGA AAGATGCTAA TACTCAGAGT ACAGTTCCAG AGAGGCCTTC TGCTGTGAAT 540
 P  K  M   K  D  A  N   T  Q  S   T  V  P   E  R  P  S   A  V  N
GATGCCTCAA ACCGTGCGGG CAGTGGTGCC CCTGCTACAA AGGCACAGGT TGTTGGTTGG 600
 D  A  S   N  R  A  G   S  G  A   P  A  T   K  A  Q  V   V  G  W
                        ↓
CCACCCATTC GATCTTTTAG AAAGAAACTC TAGCCTCTGC CTCGAAGAAT AACGAAGAGG 660
 P  P  I   R  S  F  R   K  K  L   *
TTGACGGAAA AGCTGGCTCA CCAGCTCTTT TTATTAAGGT CAGCATGGAT GGTGCTCCCT 720

ATTTGAGGAA AGTGGACCTC AGAACCTGTT CTGCATACCA GGAGCTATCT TCTGCTCTTG 780

AAAAAATGTT CAGCTGTTTT ACAATAGGTC AATATGGATC TCATGGAGCT CCTGGGAAGG 840

ATATGTTAAG TGAGAGCAAA TTGAAGGATT TGCTTCATGG ATCTGAGTAT GTCCTCACTT 900

ACGAAGATAA GGATGGGGAC TGGATGCTTG TCGGTGATGT CCCCTGGGAG ATGTTTATCG 960

ATACTTGCAA AAGGTTGAGG ATCATGAAAG GTTCAGATGC CATTGGCCTG GCCCCAAGGG 1020

CTATGGAAAA GTGTCGGAGC AGAAATTAG
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Fig. 3 A single-base deletion of *SlIAA9* from the *entire* AC mutant changed the subsequent reading frame and prematurely terminated the translation. Conserved domains I and II of Aux/IAA proteins are *framed*, the cytosine deletion site is shown by the *arrow* and the stop codon is indicated by *asterisks*

hybridising and self crossing, including 3-616, LA0159, LA0281, LA0648, LA0774, LA0781, LA0784, LA0885, LA0886, LA0888, LA0902, LA0917, LA0920, LA0982, LA0990, LA0991, LA1038, LA1075, LA2360 and LA2492. We collected some of these *entire* mutants from the Tomato Genetics Resource Center (TGRC), including LA0159, LA0885, LA0902, LA0917, LA0990, LA0991 and LA2360. The transcription levels and the complete coding sequences of *SlIAA9* from these collected materials were investigated. RT-PCR analysis indicated that the transcription of *SlIAA9* in young leaves was significantly lower in these *entire* mutants, as well as *entire* AC, when compared to that in wild-type cultivar Ailsa Craig (Fig. 4). Sequence analysis indicated that the coding sequences of *SlIAA9* gene from all the investigated *entire* mutants were the same as that of *entire* AC, with a single-base cytosine deletion at nucleotide position 626.

SlIAA9 and *e* gene locate on the same region of tomato chromosome 4

Genetically, the *e* gene had been mapped on tomato chromosome 4 (Tanksley et al. 1992). In order to define

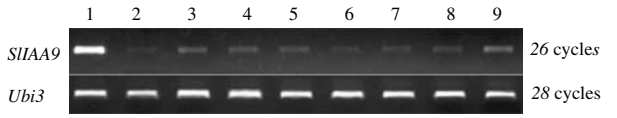

Fig. 4 Transcript accumulation of *SlIAA9* in tomato leaves. Reverse transcription polymerase chain reaction (RT-PCR) was performed using RNA extracted from young leaves of tomato cultivar Ailsa Craig (*lane 1*) and different tomato *entire* mutants LA2922 (*entire* AC, *lane 2*), LA0159 (*lane 3*), LA0885 (*lane 4*), LA0902 (*lane 5*), LA0917 (*lane 6*), LA0990 (*lane 7*), LA0991 (*lane 8*) and LA2360 (*lane 9*). PCR reactions were performed for 26 cycles for *SlIAA9* and 28 cycles for an internal control *ubi3*

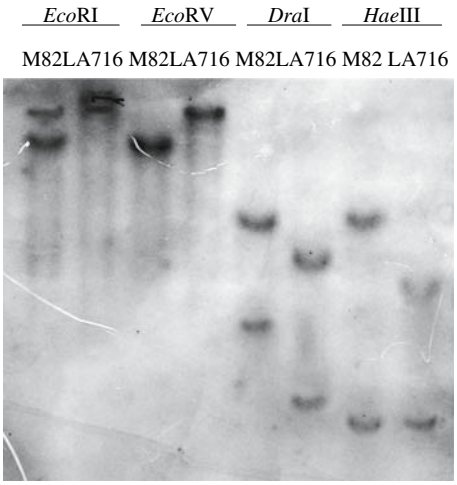

Fig. 5 Southern-blot analysis of *SlIAA9* in tomato genome. Each lane contains 15 μg genomic DNA extracted from leaves of tomato M82 and LA716 following digestion with the indicated enzymes. Genomic DNA was size fractionated on an agarose gel, blotted and hybridised with the ^{32}P-labelled *SlIAA9* full-length cDNA

SlIAA9 gene linkage to the *e* gene, the *SlIAA9* gene was bin mapped in an effort to check whether it also located on the same region of tomato chromosome 4. As plant *Aux/IAA* genes exist as a multigene family, DNA gel blot was first performed to investigate the copy number of *SlIAA9* in M82 and the wild relative LA716. The hybridisation pattern is shown in Fig. 5. One prominent band was revealed in the DNA sample digested with *Eco*RV. Two prominent bands were detected in the DNA samples digested with *Eco*RI, *Dra* I or *Hae*III. The *SlIAA9* cDNA sequence used to probe the blots has restriction sites for *EcoR* I (one site), *Dra* I (two sites) and *Hae*III (five sites), whereas no site was identified for *Eco*RV. Except that some small fragments could not be detected and there might be other sites in intron sequence for these restriction enzymes, the number of hybridising fragments detected in each case suggested that the gene corresponding to *SlIAA9* should be present as a single copy in the genome of M82 and the wild relative LA716. The result also indicated that the DNA

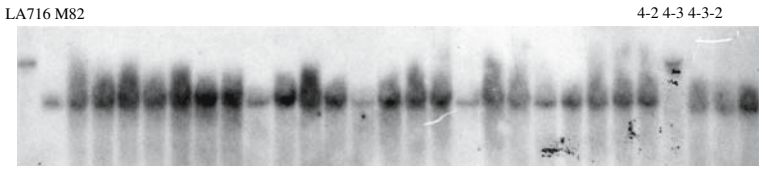

Fig. 6 Bin mapping of *SlIAA9* on tomato genome. Genomic DNA from 75 LA716-derived introgression lines (ILs) and their parents, M82 and LA716, were digested with *Eco*R V, size-fractionated on an agarose gel, blotted and hybridised with the ^{32}P-labelled *SlIAA9* cDNA. *Lanes 4-2*, *4-3* and *4-3-2* represent IL4-2, IL4-3 and IL4-3-2, respectively

sequence of *SlIAA9* was considerably diverged from other members of the tomato *Aux/IAA* family.

Figure 5 also shows the restriction fragment length polymorphisms (RFLPs) for *SlIAA9* between M82 and LA716 when the genomic DNA was digested with the four enzymes. The RFLPs of digestion with *Eco*RV were employed to map the *SlIAA9* gene using the population of 75 ILs whose genomic DNA was also hybridised with the probe of *SlIAA9* (full-length cDNA). IL 4-3 displayed the LA716 allele (Fig. 6), whereas IL 4-3-2 and IL 4-4 did not, indicating that chromosome segment from LA716 in IL 4-3 contained the *SlIAA9* locus, but that chromosome segments from LA716 in IL 4-3-2 and IL 4-4 did not. In accordance with these findings, the *SlIAA9* was mapped to the region designated as 4-G (Fig. 7). Interestingly, the *e* gene was mapped in the same region with the *SlIAA9* gene on the tomato chromosome 4 (Tanksley et al. 1992).

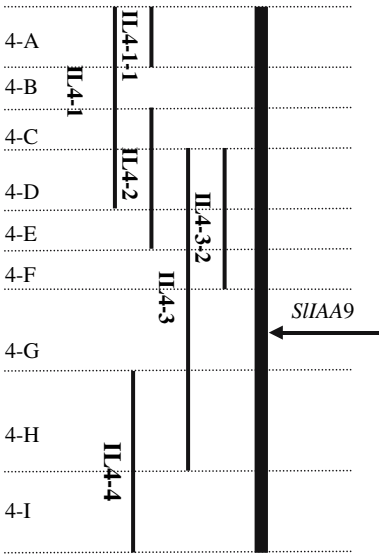

Fig. 7 The position of *SlIAA9* on the tomato genome. The chromosome is depicted as *wide bar*, and the LA716 introgressed segments appear as *thin bars* to the left of the chromosome. *Arrow* indicates the bin location of *SlIAA9* on the tomato genome

A single-base deletion of *SlIAA9* occurs in the mutant with a recessive *entire* allele

A mutant population of 13,000 tomato M2 families of the M82 variety had been generated using EMS and fast-neutron mutagenesis by Zamir lab at the Hebrew University in Jerusalem, Israel (Menda et al. 2004). The plants from this population were visually phenotyped in the field, and some of them had been cataloged according to the previously described phenotypes from the monogenic mutant collection at TGRC (http://www.tgrc.ucdavis.edu/). Several *entire* allelic mutants from this population, including *e0444*, *e0880*, *e2978*, *e2986*, *e3335* and *n0741*, have been determined by crossing to a known *entire* mutant and testing the progeny for the phenotype. The corresponding information of these mutants is available and can be searched on the Web site http://www.zamir.sgn.cornell.edu/mutants/. In an effort to confirm that the *SlIAA9* gene was the *e* gene, the complete coding region of the *SlIAA9* gene from *e0880*, a mutant generated using EMS mutagenesis, was sequenced. Sequence analysis revealed that a single-base guanine deletion of *SlIAA9* occurs at the nucleotide position 583 in *e0880* (Fig. 8a). Amino acid sequences were identical between M82 and *e0880* for the first 194 bases. Then, the deletion changed the subsequent reading frame of *SlIAA9* in *e0880* by altering the following 16 amino acids and terminating the polypeptide thereafter (Fig. 8b). As with the *SlIAA9*, in the *entire* mutant, the truncated polypeptide also consisted of only 210 amino acids. However, besides domain III and IV, this truncated polypeptide also lacked domain II, which had been confirmed to play a role in destabilising Aux/IAA proteins.

Discussion

SlIAA9 was involved in fruit development and leaf morphogenesis. Transgenic lines with decreased level of *SlIAA9* showed nearly identical phenotypes as the tomato *entire* mutant in various aspects, including converting compound leaves to entire leaves, carrying a lower frequency, asymmetrical sepals and a multifusion phenotype affecting leaves, flowers and fruits (Wang et al. 2005). *SlIAA9* located on the same region as the *e* gene. One single-base deletion in the coding region of *SlIAA9* was

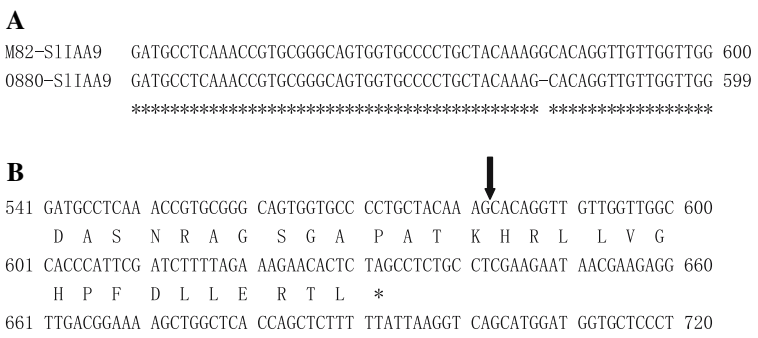

Fig. 8 A single-base guanine deletion of *SlIAA9* occurs in the *entire* allelic mutant *e0880*. **a** M82-SlIAA9 represents the *SlIAA9* coding sequence (CDS) from tomato cultivar M82. The *0880-SlIAA9* represents the *SlIAA9* gene fragment from the *entire* allelic mutant *e0880*. The guanine deletion of the *0880-SlIAA9* gene is indicated by the *arrow*. **b** Deletion mutation altered the subsequent reading frame and prematurely terminated the translation of the *SlIAA9* gene from the *entire* allelic mutant *e0880*. The cytosine deletion site is indicated by an *arrow*. and the stop codon is indicated by *asterisks*

observed in both *entire* and *entire* allelic mutants. All of these results illustrate that the *entire* mutant was produced by the deletion mutation of the *SlIAA9* gene.

The tomato is a powerful system in which to study complex leaf morphogenesis due to the fact that many leaf morphology mutants are available (Kessler et al. 2001). The tomato mutants, including *entire* (*e*), *potato leaf* (*c*), *trifoliate* (*tf*), *wiry* (*w*), *wiry3* (*w3*), *wiry4* (*w4*) and *wiry6* (*w6*), reduce the compound nature of tomato leaves. Semidominant mutant *Lanceolate* (*La*) even converts its leaves to a fully simple architecture (Hareven et al. 1996). Most genes controlling these traits have been mapped. The *w*, *w4* and *e* genes were all mapped on chromosome 4. The *tf* gene was mapped on chromosome 5. The *c* gene was mapped on chromosome 6. The *La* gene was mapped on chromosome 7. The *w6* was mapped on chromosome 10 (Tanksley et al. 1992; Kim et al. 2003). However, to date none of these genes has been cloned. The *e* gene is the first to be cloned and analysed. Further analysis of the *SlIAA9* gene and *entire* mutant will illustrate the mechanism of a compound leaf formation.

The function of several different Arabidopsis *Aux/IAA* genes had been characterised by screening various different mutants. It is striking that all of these mutants were caused by gain-of-function mutations and the mutations were all found in the highly conserved domain II of the canonical Aux/IAA proteins, which are responsible for protein degradation (Kim et al. 1996; Rouse et al. 1998; Tian and Reed 1999; Nagpal et al. 2000; Rogg et al. 2001; Fukaki et al. 2002; Hamann et al. 2002). The insertion mutants of 12 Arabidopsis *Aux/IAA* genes, including the *AtIAA9* gene, showed no visible phenotype alterations, indicating the functional redundancy among the various members of the *Aux/IAA* family (Overvoorde et al. 2005). However, the transgenic plants with a decreased level of *SlIAA9* and mutants with defective mutation of *SlIAA9* all exhibited dramatic phenotype alterations, indicating that there was no obvious functional redundancy of the *SlIAA9* gene in tomato. Furthermore, Wang et al. (2005) revealed that down-regulation of several other tomato *Aux/IAA* genes, including *SlIAA1*, *SlIAA3* and *SlIAA8*, also displayed specific, reproducible phenotypes. Our group recently generated several independent RNAi transgenic lines of *SlIAA14*, which showed a decreased level of the target gene and dramatic auxin-related phenotype alternations (J. Zhang and Z. Ye, unpublished data). All these findings suggest that functional redundancy of *Aux/IAA* genes, which was common in *Arabidopsis thaliana*, was not obvious in tomato. These observations sustain the idea that evolution endowed the unique functions to at least several tomato *Aux/IAA* genes.

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