INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19F–120/2020/23–2–364–372
DOI: 10.17957/IJAB/15.1298
http://www.fspublishers.org

Full Length Article

Auxin Response Factor 14 (SlARF14) Regulates Leaf Morphological Development and Photosynthesis of *Solanum lycopersicum*

Xiaoxi Guan[1,2], Tao Xu[2], Changling Sui[1], Xin Liu[2], Weili Mei[2] and Tianlai Li[2*]
[1]*Zunyi Normal University, Zunyi 563006, Guizhou, People's Republic of China*
[2]*College of Horticulture, Shenyang Agricultural University, Shenyang 110161, Liaoning, People's Republic of China*
[*]For correspondence: 745310508@qq.com
Received 22 June 2019; Accepted 16 September 2019; Published 16 January 2020

Abstract

Auxin response factors (ARFs) are a class of transcription factors that are implicated in auxin-mediated responses. In this study, we determined that SlARF14, which lacks domains III and IV, is differentially expressed in leaf organs. Down-regulation of *SlARF14* using RNA interference indicated that this gene plays an important role in leaf morphological development and photosynthesis. The leaves of *SlARF14*-RNAi tomato (*Solanum lycopersicum* L.) lines were dark green and thick and they exhibited reduced lobes, causing a Potato Leaf Tomato-like appearance. Moreover, we observed a lateral development trend in the transformants. On the other hand, down-regulation of *SlARF14* stimulated photosynthetic activity at the early stage of development, which appears to be related with increased Rubisco enzyme activity. The altered expression levels of *RBCL*, *RBCS* and *CAB* reflect this notion. In addition, the expression analysis of *ARFs* and *Aux/IAAs* supported the independent and specific regulation of SlARF14 in leaf morphological development and photosynthesis.

Keywords: Tomato; Auxin response factor; Leaf morphological; Photosynthesis

Introduction

Auxin plays an important role in numerous plant organ growth and developmental processes. Auxin response factors (ARFs) comprise a class of transcription factors implicated in auxin-mediated responses. ARFs regulate the expression of auxin-related genes through the binding of their DBDs (DNA-binding domains) to specific AuxRE components (TGTCNC) in the promoter regions of auxin-responsive genes (Tiwari *et al*. 2003). ARFs also contain two other domains, the MR (middle region) and CTD (C-terminal domain or domains III and IV). Due to its high homology with AUX/IAA, the CTD structure domain forms a heterologous dimer with AUX/IAA under normal circumstances (Tiwari *et al*. 2003). As the auxin concentration increases, AUX/IAA proteins identified by SCFTIR1 are degraded through the ubiquitin-proteasome pathway. This process leads to the expression of a series of auxin-related genes due to functional recovery of ARF proteins. Among tomato (*Solanum lycopersicum* L.) ARF members, ARF3, ARF6-1, ARF13, ARF13-1, ARF12, ARF14 and ARF17 appear to be CTD-deficient (Wu *et al*. 2011). To date, these members have not been thoroughly studied. In *Arabidopsis*, deletion of MP/ARF5 domains III and IV led to reductions in leaf vascular tissue and hypocotyl deformity (Krogan *et al*. 2012). Most studies of ARF family members have focused on the other structure-integrated factors. The MR domain determines whether an ARF activates or represses target genes (Ulmasov *et al*. 1999a, b; Tiwari *et al*. 2003).

The Aux/IAA family of transcription factors also take part in auxin-mediated transcriptional regulation. *SlIAA3* is said to function at the crossroads of auxin and ethylene signaling during differential growth (Chaabouni *et al*. 2009). *SlIAA9*-inhibited lines exhibit altered compound leaves (Wang *et al*. 2005) and the leaves of *SlIAA15*-downregulated plants are thick and dark green (Deng *et al*. 2012). Many reports have also revealed the functions of ARF proteins. In particular, members of the ARF gene family have been studied in the model eudicot *Arabidopsis thaliana*. Arabidopsis *ARF3/ETTIN* is involved in integument development and polarity determination (Kelley *et al*. 2012). Recent studies have also investigated microRNA-directed regulation of *ARF*. *AtARF6* and *AtARF8* regulate flower and fruit maturation (Nagpal *et al*. 2005; Goetz *et al*. 2007); *AtARF17* is involved in this process (Mallory *et al*. 2005). *OsARF1* is an auxin response gene that is associated with the tropism of coleoptiles (Waller *et al*. 2002). *ARFs* (such as *AtARF2* and *AtARF19*) also participate in the auxin and ethylene signaling pathways (Li *et al*. 2006; Schruff *et al*. 2006). Current studies in tomato ARF genes tend to fruit growth and

development. For example, *SlARF4/DR12* regulates the color of ripening fruit (Jones *et al*. 2002). In addition, silencing of *SlARF7* leads to parthenocarpy, which suggests that this gene encodes a negative regulatory factor of fruit development (Jong *et al*. 2009).

Normally, the tomato leaf is multilobed and has an irregular leaf edge, while the Potato Leaf Tomato, which is found in nature, has a smooth leaf edge or possibly a lightly lobed leaf edge. *Potato leaf* (*C*) was identified to be the closest paralog of the shoot branching regulator *Blind* (*Bl*). Although definitive studies on the molecular mechanism underlying the formation of Potato Leaf Tomato leaves have not been performed, this mechanism is likely to be associated with the above-mentioned genes.

Several studies have also provided evidence that *ARF* genes regulate leaf development and senescence. Two Arabidopsis *ARF* members, *AtARF1* and *AtARF2*, participate in senescence, and *AtARF7* and *AtARF19* are also involved in this process (Ellis *et al*. 2005; Okushima *et al*. 2005b; Lim *et al*. 2010). *NPH4/ARF7* and *AtARF19* are involved in leaf blade expansion in Arabidopsis (Okushima *et al*. 2005a; Wilmoth *et al.* 2005; Okushima *et al.* 2007). Another two genes, *AtARF3/ETTIN* and *AtARF4* involved in polarity determination are necessary for specifying abaxial leaf fate (Allen *et al.* 2005; Pekker *et al.* 2005; Iwasaki *et al.* 2013). In another dicotyledonous plant, tomato, a failure to negatively regulate their orthologs *SlARF3* and *SlARF4* causes tomato shoestring leaves. In addition, tomato *SlARF10* has been found to affect leaf morphological development (Hendelman *et al.* 2012). A recent study on *Medicago truncatula*, an important leguminous model plant, has surmised *ARF3* might affect leaf margin development by regulating the establishment of leaf polarity (Zhou *et al.* 2013).

Leaf shape and size are closely associated with photosynthesis, as the leaf is the primary site of photosynthesis. As photosynthesis contributes greatly to growth, development and fruit yield in plants, this process appears to be generally irreplaceable. The products of photosynthesis, in turn, fuel leaf growth. In general, photosynthesis indirectly reflects the level of leaf development. The small subunit of Rubisco is encoded by *Rubisco Small Subunit* (*RBCS*) genes, while its large subunit is encoded by *Rubisco Large Subunit* (*RBCL*) gene. CAB is a Chlorophyll a/b binding protein. These are controlled by multiple factors, such as plant hormones under complex situations. ABA reduces the accumulation of Rubisco in pea embryos (Bartholomew *et al.* 1991). One goal of the current study was to determine if *ARF14*-inhibited plants would exhibit altered photosynthesis.

Previously, we performed a study of *ARF* genes during tomato flower abscission and found that most of these genes participate in floral organ abscission (Guan *et al*. 2014). In addition, we examined several *ARF* mutants to explore the function of ARFs in the development of plant organs, especially leaves and flowers. Here, we describe the functional analysis of *SlARF14* during tomato leaf development. The results show that SlARF14 shares high sequence homology with SlARF10, which has been found to affect leaf morphological development, suggesting that these proteins are functionally related. Down-regulation of this gene affected the color, thickness and formation of lobes in the leaves, causing a Potato Leaf Tomato-like appearance. Moreover, we also observed a lateral development trend in the transformants. On the other hand, down-regulation of *SlARF14* stimulated photosynthetic activity at the early stage of leaf development, as it affected Rubisco enzyme activity. In addition, the expression analysis of *ARFs* and *Aux/IAAs* supported the independent and specific regulation of SlARF14 in leaf morphological development and photosynthesis.

Materials and Methods

Experimental details and treatments

Plant material and growth conditions: Tomato (*S. lycopersicum* cv. Chinese Vegetable NO. 6) plants were grown under greenhouse conditions. The culture conditions were as follows: 14 h day /10 h night cycle, 25°C day / 20°C night temperatures, 80% relative humidity, 250 μmol m^{-2} s^{-1} intense luminosity. Plump seeds used in tissue culture were harvested and selected.

Plant transformation

To generate *SlARF14*-RNAi transgenic plants, the forward 5'-CACCGGGACACCAGAACGTCATCT-3' and reverse 5'-CCAGGGGATTTGCAATGCTG-3' primers, encompassing the B3 DNA-binding domain, which encodes amino acids 120 to 222, were used to amplify a partial SlARF14 clone. The 422 bp fragment was cloned into the Gateway vector pB7GWIWG2(I) which contain a double 35S promoter (http://gateway.psb.ugent.be/vector/show/pB7GWIWG2%28I%29/search/index/silencing/any) *via* pDONR221 using the BP and LR cloning system (Invitrogen, Karlsruhe, Germany) as described in the manufacturer's manual. The constructs were transformed into EHA105 by electroporation. Transgenic plants were generated by transformation of tomato cotyledon explants. The transformed lines were selected *via* BASTA resistance and analyzed by PCR to detect transgenic lines. BAR-F: 5'-AAGCCCTGTGCCTCCA-3' and BAR-R: 5'-GTCGCCTAAGGTCACTATCAG-3' amplified the BASTA resistance gene. Through positive certification, thirty independent lines were gained in this work.

Phylogenetic analysis

Protein sequences were blasted from NCBI (http://www.ncbi.nlm.nih.gov/) and SGN

(http://solgenomics.net/). ARF protein sequences of tomato (21) and *Arabidopsis* (4) were aligned with Clustal X 1.83. An unrooted neighbor-joining phylogenetic tree was constructed.

RNA extraction and quantitative real-time pcr analysis

Leaf samples were frozen in liquid nitrogen and stored at -80°C for RNA isolation. RNA was extracted with a Total RNA Extraction Kit (Tiangen, Beijing, China) according to the manufacturer's protocol and 0.8% agarose gel electrophoresis was used to detect the integrity of the RNA. The RNA samples were reverse transcribed into cDNAs in a volume of 20 μL. Specific primer pairs were designed using Primer 5.0 software (Supplementary Table 1). The relative mRNA levels were quantified with respect to the internal standard, actin. qPCR analysis was performed as described by Jain *et al.* (2006).

Semi-quantitative RT-PCR

The relative expression levels of *SlARF14* were analyzed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). The PCR program for *SlARF14* and *ACTIN* was performed with 30 cycles at 94℃ for 30 s, 58℃ for 30 s and 72℃ for 60 S. Specific primer pairs were designed as follows: *SlARF14*-F: 5'-TCACGTAAACCTCTTTCG-3' and *SlARF14*-R: 5'-AGGAGAAGTCTCGGGTT-3'; ACTIN-F: 5'-ATGTATATTGCAATCCAG-3' and ACTIN-R: 5'-TAGTCTCAAGCTCCTGTTC-3'.

Photosynthesis-related measurements

Photosynthesis-related measurements were performed every other week during functional leaf development. The seedlings were grown under natural light in a greenhouse. The temperature was in the range of 25 ± 3℃ during the day and 15 ± 3℃ during the night and the relative humidity was approximately 55–60%. The photosynthetic rate was obtained simultaneously using GFS-3000 and DUAL-PAM-100 measuring systems (Heinz Walz, Effeltrich, Germany) as described by Yamori *et al.* (2011). A plant RuBP ELISA Kit (produced by Shanghai Wonderful Research Biological Technology Co., Ltd., China) was utilized for the Rubisco carboxylase activity assay. A simple method was used to determinate chlorophyll levels. Leaves were cut into filaments and soaked in 80% (v/v) acetone until the color changed to white. The supernatant of the extract was subjected to light absorption measurements according to Arnon (1949).

Leaf morphological measurements

To measure the rapidly expanding leaf blades, the leaves were measured every other week from leaf buds (8 mm) to mature leaves. A Vernier caliper was used to measure the length (from leaf blade base to cusp) and width (maximum transverse diameter) at various stages. a leaf area meter (LI-3000) was used to measure leaf area. Specific leaf weight (SLW) and specific leaf area was estimated every four days after leaf emergence. Anatomical characteristics of leaves used toluidine blue staining.

Results

Protein structure and homology analysis

SlARF14 contains the B3 DNA binding domain and middle region, but lacks domains III and IV, which are required for interaction with IAA proteins (Fig. 1a). It is presumed inaccessibility of SlARF14 to negative regulated by Aux/IAA proteins. AtARF1, AtARF2, AtNPH4/ARF7 and AtARF19 have been shown to influence leaf development and senescence and AtARF7 and AtARF19 are involved in leaf blade expansion. Here, phylogenetic tree analysis indicated that SlARF7 and SlARF19 share high homology with these factors (Fig. 1b). These two ARFs generally act as transcriptional activators, while SlARF14 is likely acting a repressor and is therefore thought to have the opposite function to that of SlARF7 and SlARF19. Phylogenetic analysis showed that SlARF10, SlARF14, SlARF16 and SlARF17 exist in the same cluster, which suggests their close correlation. Another two genes, SlARF3 and SlARF4, involved the Wiry Leaf Syndrome, exist in a single cluster.

SlARF14 plays a role in leaf morphology

To examine the function of SlARF14, we obtained transgenic *SlARF14*-RNAi lines. The expression of *SlARF14* gene in the lines was detected by semi-quantitative RT-PCR. As described in Fig. 2a, we categorized the expression level of *SlARF14* in two sets: L8 and L12 exhibited weaker expression and were considered as low-level expression lines; On the other hand, L3 and L6 slightly lowered genes expression. Some distinct phenotypes between the two different sets reflected that *SlARF14* expression was down-regulated (Fig. 2b). The leaf of wild type is multilobed and has an irregular leaf edge. L3 showed mildly deeper green leaves and a lightly lobed leaf edge, whereas L12 had a smooth leaf edge so that the leaf margin twisted to some degree. Moreover, L12 also had partially fused lateral leaves and folioles. In order to further quantify the extent of gene down-regulation, qRT-PCR was performed among these typical lines (Fig. 2c). Compared with WT and vector plants, L3 and L6 have brought down gene regulation by up to 57% and 55%, respectively. Transgenic lines L8 and L12 cut down their expression level by 80% and 84%, respectively. Based on the gene expression level and distinct phenotypes, lines L8 and L12 were chosen mainly for further analysis.

Compared with the wild type, the leaf blade of L3 and

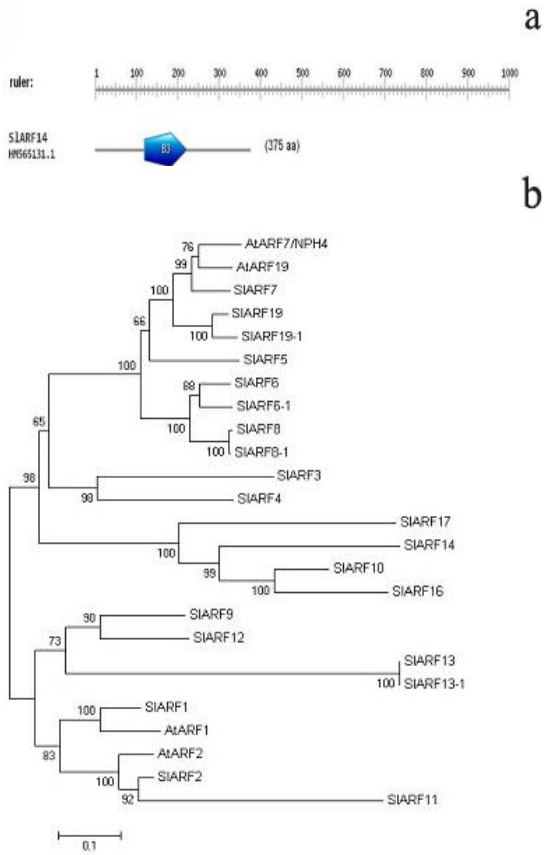

Fig. 1: (**a**) Analysis of protein structure was performed by PROSITE (http://us.expasy.org/prosite/) online. (**b**) Phylogenetic relationships among 21 SlARF and four AtARF proteins. The unrooted tree was generated using MEGA4.1 by the neighbor-joining method. Bootstrap values (above 50%) from 1,000 replicates are indicated at each branch

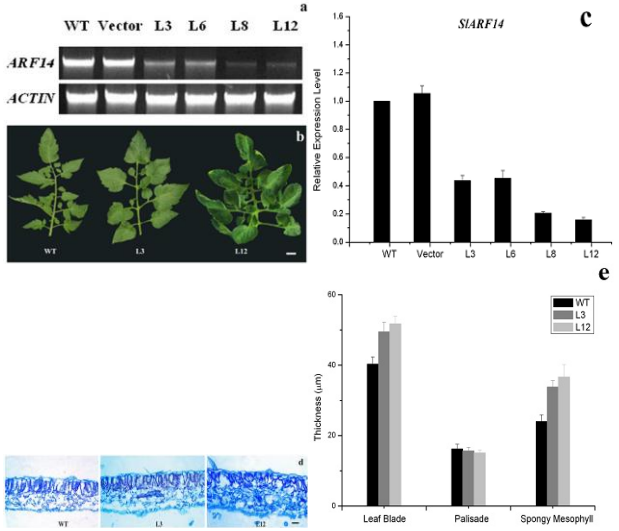

Fig. 2: Morphological phenotypes of *SlARF14*-RNAi plants. (**a**) *SlARF14* expression analysis in tomato transgenic lines using RT-PCR. (**b**) A single compound leaf of wild type and lines L3 and L12. (**c**) Relative expression level of *SlARF14* in different lines. (**d**) Transverse sections through the leaves of wild type and *SlARF14*-RNAi lines. Semi-thin sections were stained with toluidine blue and viewed with a light microscope. (**e**) Thickness of leaf blades, palisade and spongy mesophyll. The data correspond to mean values of three replicates ± standard error. Bars = 2 cm in (b) and 10 μm in (d)

L12 were both thickened resulted from an increase in the number of spongy mesophyll cells and larger intercellular space. Palisade mesophyll cells in L12 appeared irregularly cylindrical so that the contrast between spongy and palisade mesophyll cells was weaker in these lines (Fig. 2d and e). This would be explained that excessive down-regulation of *SlARF14* leads to the abnormal leaf blade surface.

The leaves of *SlARF14*-RNAi lines were dark green and thick with reduced lobes (Fig. 3a and b), which resembles the leaf morphology observed in Potato Leaf Tomato. L3 and L12 did not make significant difference over time in the analysis. However, the transformant lines tended to exhibit increased lateral growth (Fig. 3c). Due to the differences in width between the two lines, there were subtle differences in leaf size between control and *SlARF14*-RNAi plants.

To monitor the growth and development of control and *SlARF14*-RNAi plants, we measured several indices (Fig. 4a and b). SLW, an important physiological index of photosynthesis, reflects the amount of organic substances produced by photosynthesis and their distribution during different periods of growth. Consistent with the WT leaves, the SLW of L12 first increased sharply, followed by a steady increase. In addition, the SLW values in the L12 were markedly and irreversibly higher than those of the control. The SLA represents the ratio of leaf area to dry weight, which reflects blade thickness. As shown in Fig. 5, the SLA consistently decreased before becoming somewhat steady during leaf development. These results demonstrate that the blade thickened constantly at the early stage of leaf development and completed its expansion as the leaf mesophyll cells swelled.

Leaf development can be divided into two aspects: blade expansion and substance accumulation. During blade expansion, the key period that was altered in L12 was from 12 to 20 d, during which expansion was rapid compared with the control. The regulatory function of ARF14 was evident during the middle and later periods of leaf development, which largely occurred 16 days after the period of substance accumulation.

Role of SlARF14 in regulating photosynthetic activity

To assess the utilization efficiency of solar energy in transgenic leaves, the function of SlARF14 in regulating photosynthetic activity, photosynthetic rate and chlorophyll fluorescence were measured every 7 days after leaf emergence. In both sets of plants, the photosynthetic rate tended to increase slowly during early development followed by a slight decrease (to similar

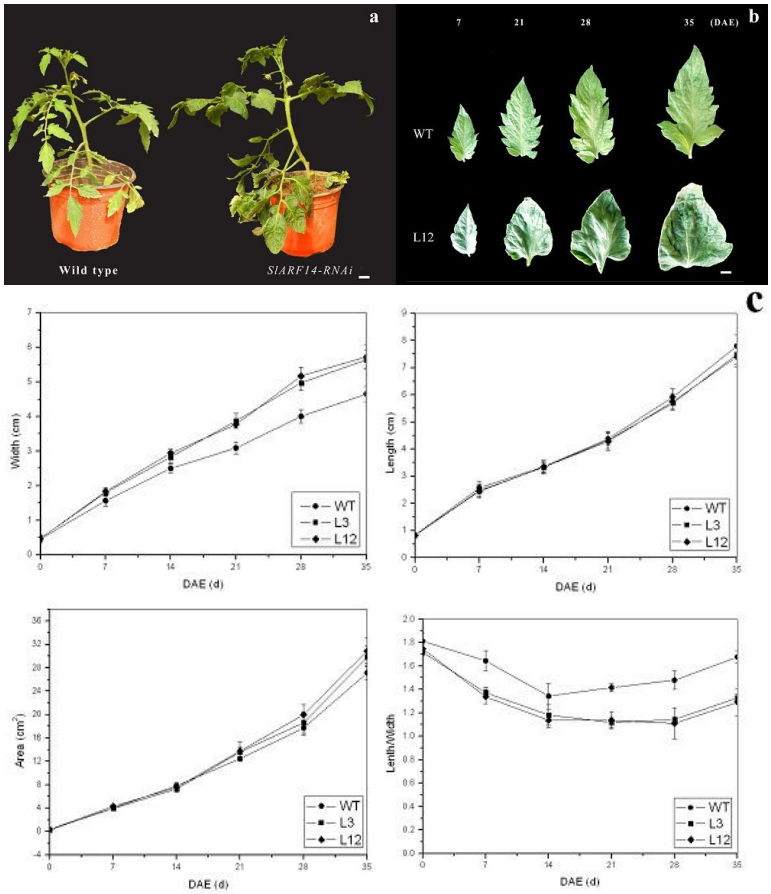

Fig. 3: Morphological investigation of wild type and *SlARF14*-RNAi transgenic lines. (**a**) Wild type and *SlARF14*-RNAi transgenic plants were cultivated under normal growth conditions. (**b**) Morphological changes in leaves were observed in the two lines over time until they were fully grown. The photographs show representative leaves at each time point. (**c**) Changes in leaf blade size at 0, 7, 14, 21, 28, and 35 days after emergence (DAE). Error bars indicate standard deviation (SD, n = 6). Bars = 2 cm in (a) and 5 mm in (b)

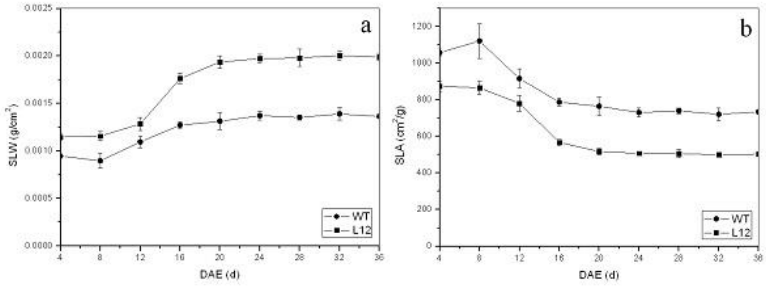

Fig. 4: Measurements of specific leaf weight (SLW) (**a**) and specific leaf area (SLA) (**b**) at 4, 8, 12, 16, 20, 24, 28, 32, and 36 days after emergence. Error bars indicate standard deviation (SD, n = 6)

levels) during leaf development. L12 displayed higher levels than the control up to 28 days. The maximum photosynthetic rate of L12 appeared at approximately 21 days old, which was earlier than that of the control (Fig. 5a). It improves early photosynthetic efficiency, providing more efficient substance support for blade expansion. Down-regulation of *ARF14* in tomato plants also resulted in increased chlorophyll content (per unit leaf area); we examined the total chlorophyll content in WT and L12. There was a steady increase in total chlorophyll over time, and the total chlorophyll content in fully expanded leaves was higher in the line L12 than in WT (Fig. 5b).

Rubisco carboxylase activity also varied throughout leaf blade development in both sets of plants (Fig. 6a). The maximum activity in L12 appeared at approximately 14 days old, which was much earlier than that observed in the

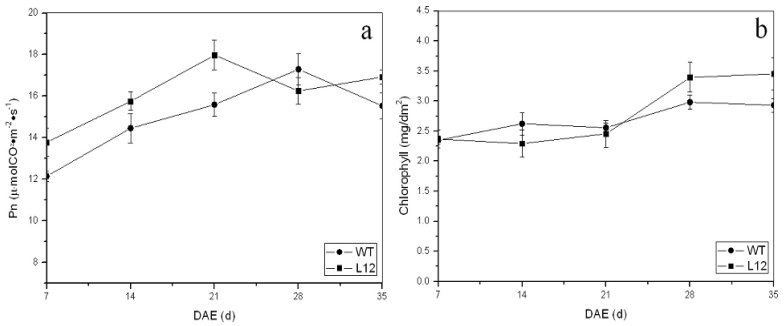

Fig. 5: Analysis of the role of SlARF14 in the regulation of photosynthetic activity at 7, 14, 21, 28, and 35 days after emergence. (**a**) Photosynthetic rate (Pn). (**b**) Chlorophyll contents. The data correspond to mean values of three replicates ± standard error

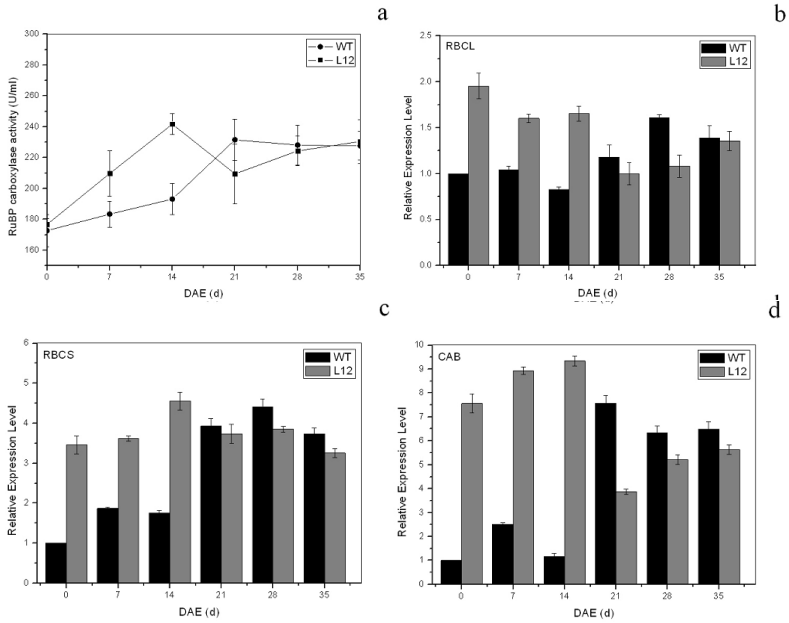

Fig. 6: Changes in Rubisco carboxylase activity and gene expression levels at 0, 7, 14, 21, 28, and 35 days after emergence. (**a**) Rubisco carboxylase activity. (**b**, **c**, **d**) Relative expression levels of *RBCL*, *RBCS* and *CAB*. Primers used are listed in Supplementary Table 1. The data correspond to mean values of three replicates ± standard error

control (approximately 21 days old). The expression levels of two genes, *RBCL* and *RBCS*, followed nearly the same pattern (Fig. 6b and c). Interestingly, this change occurred prior to the change in photosynthetic rate. This observation can most likely be attributed to the notion that the photosynthetic rate increased rapidly after maximum Rubisco carboxylase activity was attained. As CAB is a Chlorophyll a/b binding protein, *CAB* mRNA levels displayed a sharp increase 21 days later in the control, but the opposite trend was observed in L12 plants (Fig. 6d). Based on these results, we conclude that down-regulation of *SlARF14* improves early Rubisco carboxylase activity, which enhances the process of photosynthesis.

Down-regulation of *SlARF14* specifically affects the expression of specific *Aux/IAAs* and *ARFs*

To examine the mechanism underlying how reduced *SlARF14* expression led to the observed leaf phenotypes, we performed expression analysis of auxin transcription factor genes, including 12 Aux/IAA and 10 ARF members (Fig. 7a and b). Aux/IAAs interact with ARFs and show rapid response to auxin. Most *Aux/IAA* genes showed no obvious changes in expression in the transgenic lines except *IAA7* that was significantly up-regulated. None of *Aux/IAA* genes was down-regulated, which suggested the failure of negative regulation by Aux/IAA. Consistent with the preconceived assumption, the deletion of domains III and IV probably release ARF from Aux/IAA control. The homology analysis predicted that *ARF14* RNAi would primarily target *ARF10* (58.92%), *ARF16* (54.23%) and *ARF17* (47.44%). Taking a hard look at these relative genes, there was no significant down-regulation by RNAi strategy. While most of the genes showed similar expression in WT, *ARF19* levels increased dramatically, followed by *ARF5* and *ARF6*.

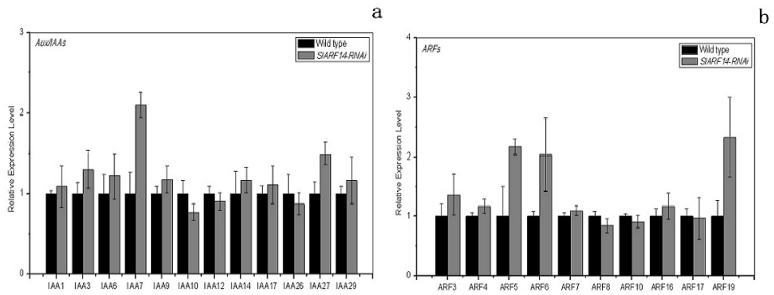

Fig. 7: Impact of down-regulation of *SlARF14* on the expression of auxin-response *ARF* (**a**) and *Aux/IAA* (**b**) genes. Primers used are listed in Supplementary Table 1. The data correspond to mean values of three replicates ± standard error

Discussion

To explore auxin-regulated physiological activity, it is necessary to explore the underlying genetic mechanisms. To date, an increasing number of molecular biology studies have focused on this issue. It is well known that ARFs perform pleiotropic functions during plant growth and organ development. Since the leaf is the main vegetative organ and represents the primary location for photosynthesis, leaves are an important focus of research. However, the mechanism underlying the regulation of leaf morphogenesis by ARFs is not fully understood. Furthermore, to date, studies of tomato *ARF* genes have tended to focus on fruit growth and development. Here, we report the function of *ARF14* in tomato leaf development and photosynthesis.

Gonzalez *et al.* (2012) described five key phases of leaf growth. In the current study, the entire process was considered to encompass the time from the formation of a leaf primordium to that of a mature leaf. The overexpression of *SlARF10* inhibits leaf development in tomato, while Wilmoth *et al.* (2005) found that leaf cell expansion in Arabidopsis requires the activation of *NPH4/ARF7* and *ARF19*. Here, we found that the RNAi plants dramatically strengthened early leaf development. The leaves of *SlARF14*-RNAi lines were dark green and thick, with reduced lobes. This phenotype has some similarities to the phenotype of the leaves of *SlIAA15*-inhibited plants observed in a previous study. Moreover, the morphology of Potato Leaf Tomato leaves was previously thought to merely represent an interesting visual anomaly.

As the leaf is the primary location for photosynthesis, its shape and size are both closely associated with photosynthesis. Cai and Rodermel (1995) suggested that the photosynthetic capacity declines in older, fully expanded leaves after attaining a maximum level in younger, fully expanded leaves. In the current study, we found that the leaf developmental stage plays a vital role in chlorophyll content, photosynthetic rate and RuBP carboxylase activity. The maximum photosynthetic rate of *SlARF14*-RNAi lines occurred in the second week, which was much earlier than that of the control. This result is not consistent with the previous notion of leaf development. On the other hand, the change in Rubisco carboxylase activity occurred prior to the change in photosynthetic rate. This change, as well as changes in the performance of related genes, reveals a potential molecular mechanism of the *ARF14* gene.

Specific responses to auxin are generated from the interaction between ARFs and Aux/IAA proteins. Many studies have tried to uncover the potential mechanism have found the role of ARF8 and Aux/IAA9 proteins in the control of Arabidopsis fruit growth (Goetz *et al.* 2007). Currently, analysis of gene expression was made to detect the specific impact on related genes in transgenic research. At transcript levels no significant changes were observed for *SlIAA1* and *SlIAA2* in *35S:mSlARF10* transgenic lines (Hendelman *et al.* 2012). It suggests structure-integrated ARFs may show different interactions with Aux/IAAs in a complex network. Due to the unique and overlapping functions, some ARFs would be regulated by multiple Aux/IAAs, whereas some other ARFs may be influenced by fewer ones. On the other hand, there is a possibility that one ARF interacts with different Aux/IAAs in different periods or organs, resulting from the specificity of temporal and spatial development. By contrast, the deletion of domains III and IV is predicted to simply release the ARF from Aux/IAA control, as would normally act in response to auxin (Leyser 2006). Gained truncated CTD of OsARF11 which lacks 35 amino acids in its C-terminus. In contrast to the full-length protein, the truncated protein did not interact with any OsIAA through both yeast two-hybrid assays and α-galactosidase quantitative assays (Shen *et al.* 2010). In our study, we found a similar pattern of results as previous researches. The structure of ARF14 suggests that this protein probably functions independently of AUX/IAA proteins. The down-regulation of *SlARF14* has not affected most of IAAs, suggesting that it probably functions by itself transcriptional regulation.

The results of phylogenetic tree analysis indicated that SlARF7 and SlARF19 share high homology. The two proteins generally act as transcriptional activators, while SlARF14 is a repressor, suggesting that the function of SlARF14 is opposite that of the two other proteins. Wu *et*

al. (2011) proposed that *SlARF14* gene was the product of an mRNA inserted into the tomato genome. This mRNA was surmised coming from *SlARF10* mRNA, *SlARF16* mRNA, or both. Phylogenetic analysis showed that SlARF10, SlARF14, SlARF16 and SlARF17 exist in the same cluster, which suggests their close correlation.

The sequence homology analysis predicts that *SlARF14* RNAi would primarily target *ARF10* (58.92%), *ARF16* (54.23%), and *ARF17* (47.44%) among all family members. However, none of them exhibited obvious change in transcript level in RNAi lines, which supports the hypothesis that the observed phenotypes resulted from the down-regulation of *SlARF14* gene directly.

Conclusion

Down-regulation of *SlARF14* using RNA interference indicated that this gene plays an important role in leaf morphological development and photosynthesis. In addition, the expression analysis of *ARFs* and *Aux/IAAs* supported the independent and specific regulation of SlARF14 in leaf morphological development and photosynthesis.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (31672197), Key Project of Guizhou Provincial Science and Technology Project (Qian Ke He Zhicheng [2018] 20104), Youth Talent Development Project of Guizhou Provincial Education Department (Qian Jiao He KY [2016] 261), Zunyi City Joint Science and Technology Research and Development Fund Project (Zun Shi Ke He Ji [2018] 10), Cultivation Project for Innovative Exploration of Academic New Seedlings in Guizhou Province (Qian Ke He Pingtai Rencai [2017] 5727-18), Zunyi Science and Technology Innovation Platform Construction Project (Zun Shi Ke He (2017) No. 12), Zunyi Innovation Talent Team Training Project (Zun Shi Ke Ren Cai [2018] No. 1) and Doctoral Foundation of Zunyi Normal College (BS [2015] 14).

References

Allen E, ZX Xie, AM Gustafson, JC Carrington (2005). microRNA-directed phasing during trans-acting siRNA biogenesis in plants. *Cell* 121:207–221

Arnon DI (1949). Copper enzymes in chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24:1–15

Bartholomew DM, GE Bartley, PA Scolnik (1991). Abscisic acid control of rbcS and cab transcription in tomato leaves. *Plant Physiol* 96:291–296

Cai ZJ, SR Rodermel (1995). Regulation of photosynthesis during leaf development in RBCS antisense DNA mutants of tobacco. *Plant Physiol* 107:215–224

Chaabouni S, B Jones, C Delalande, H Wang, Z Li, I Mila, P Frasse, A Latché, JC Pech, M Bouzayen (2009). Sl-IAA3, a tomato Aux/IAA at the crossroads of auxin and ethylene signaling involved in differential growth. *J Exp Bot* 60:1349–1362

Deng W, YW Yang, ZX Ren, C Audran, I Mila, XY Wang, HL Song, YH Hu, M Bouzayen, ZG Li (2012). The tomato *SlIAA15* is involved in trichome formation and axillary shoot development. *New Phytol* 194:379–390

Ellis CM, P Nagpal, JC Young, G Hagen, TJ Guilfoyle, JW Reed (2005). *AUXIN RESPONSE FACTOR1* and *AUXIN RESPONSE FACTOR2* regulate senescence and floral organ abscission in *Arabidopsis thaliana*. *Development* 132:4563–4574

Goetz M, LC Hooper, SD Johnson, JCM Rodrigues, A Vivian-Smith, AM Koltunow (2007). Expression of aberrant forms of *AUXIN RESPONSE FACTOR8* stimulates parthenocarpy in *Arabidopsis* and tomato. *Plant Physiol* 145:351–366

Gonzalez N, H Vanhaeren, D Inzé (2012). Leaf size control: Complex coordination of cell division and expansion. *Trends Plant Sci* 17:332–340

Guan XX, T Xu, S Gao, MF Qi, YL Wang, X Liu, TL Li (2014). Temporal and spatial distribution of auxin response factor genes during tomato flower abscission. *J Plant Growth Regul* 33:317–327

Hendelman A, K Buxdorf, R Stav, M Kravchik, T Arazi (2012). Inhibition of lamina outgrowth following *Solanum lycopersicum* AUXIN RESPONSE FACTOR 10 (*SlARF10*) derepression. *Plant Mol Biol* 78:561–576

Iwasaki M, H Takahashi, H Iwakawa, A Nakagawa, T Ishikawa, H Tanaka, Y Matsumura, I Pekker, Y Eshed, S Vial-Pradel, T Ito, Y Watanabe, Y Ueno, H Fukazawa, S Kojima, Y Machida, C Machida (2013). Dual regulation of ETTIN (ARF3) gene expression by AS1-AS2, which maintains the DNA methylation level, is involved in stabilization of leaf adaxial-abaxial partitioning in *Arabidopsis*. *Development* 140:1958–1969

Jain M, N Kaur, K Tyagi, JP Khurana (2006). The auxin responsive GH3 gene family in rice (*Oryza sativa*). *Funct Integr Genomics* 6:36–46

Jones B, P Frasse, E Olmos, H Zegzouti, ZG Li, A Latche, JC Pech, M Bouzayen (2002). Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *Plant J* 32:603–613

Jong MD, M Wolters-Arts, R Feron, C Mariani, WH Vriezen (2009). The *Solanum lycopersicum* auxin response factor 7 (*SlARF7*) regulates auxin signaling during tomato fruit set and development. *Plant J* 57:160–170

Kelley DR, A Arreola, TL Gallagher, CS Gasser (2012). ETTIN (ARF3) physically interacts with KANADI proteins to form a functional complex essential for integument development and polarity determination in *Arabidopsis*. *Development* 139:1105–1109

Krogan NT, W Ckurshumova, D Marcos, AE Caragea, T Berleth (2012). Deletion of MP/ARF5 domains III and IV reveals a requirement for Aux/IAA regulation in Arabidopsis leaf vascular patterning. *New Phytol* 194:391–401

Leyser O (2006). Dynamic integration of auxin transport and signaling. *Curr Biol* 6:424–433

Li JS, XH Dai, YD Zhao (2006). A role for auxin response factor 19 in auxin and ethylene signaling in *Arabidopsis*. *Plant Physiol* 140:899–908

Lim PO, IC Lee, J Kim, HJ Kim, JS Ryu, HR Woo, HG Nam (2010). Auxin response factor 2 (ARF2) plays a major role in regulating auxin-mediated leaf longevity. *J Exp Bot* 61:1419–1430

Mallory AC, DP Bartel, B Bartelc (2005). MicroRNA-directed regulation of *Arabidopsis AUXIN RESPONSE FACTOR17* is essential for proper development and modulates expression of early auxin response genes. *Plant Cell* 17:1360–1375

Nagpal P, CM Ellis, H Weber, SE Ploense, LS Barkawi, TJ Guilfoyle, G Hagen, JM Alonso, JD Cohen, EE Farmer, JR Ecker, JW Reed (2005). Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. *Development* 132:4107–4118

Okushima Y, H Fukaki, M Onoda, A Theologis, M Tasaka (2007). ARF7 and ARF19 regulate lateral root formation *via* direct activation of *LBD/ASL* genes in *Arabidopsis*. *Plant Cell* 19:118–130

Okushima Y, PJ Overvoorde, K Arima, JM Alonso, A Chan, C Chang, JR Ecker, B Hughes, A Lui, D Nguyen, C Onodera, H Quach, A Smith, G Yu, A Theologis (2005a). Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: unique and overlapping functions of *ARF7* and *ARF19*. *Plant Cell* 17:444–463

Okushima Y, I Mitina, HL Quach, A Theologis (2005b). AUXIN RESPONSE FACTOR 2 (ARF2): A pleiotropic developmental regulator. *Plant J* 43:29–46

Pekker I, JP Alvarez, Y Eshed (2005). Auxin response factors mediate Arabidopsis organ asymmetry *via* modulation of KANADI activity. *Plant Cell* 17:2899–2910

Schruff MC, M Spielman, S Tiwari, S Adams, N Fenby, RJ Scott (2006). The *AUXIN RESPONSE FACTOR 2* gene of *Arabidopsis* links auxin signaling, cell division and the size of seeds and other organs. *Development* 133:251–261

Shen CJ, SK Wang, YH Bai, YR Wu, SN Zhang, M Chen, TJ Guilfoyle, P Wu, YH Qi (2010). Functional analysis of the structural domain of ARF proteins in rice (*Oryza sativa* L.). *J Exp Bot* 61:3971–3981

Tiwari SB, G Hagen, T Guilfoyle (2003). The roles of auxin response factor domains in auxin-responsive transcription. *Plant Cell* 15:533–543

Ulmasov T, G Hagen, TJ Guilfoyle (1999a). Dimerization and DNA binding of auxin response factors. *Plant J* 19:309–319

Ulmasov T, G Hagen, TJ Guilfoyle (1999b). Activation and repression of transcription by auxin-response factors. *Proc Natl Acad Sci USA* 96:5844–5849

Waller F, M Furuya, P Nick (2002). OsARF1, an auxin response factor from rice, is auxin-regulated and classifies as a primary auxin responsive gene. *Plant Mol Biol* 50:415–425

Wang H, B Jones, Z Li, P Frasse, C Delalande, F Regad, S Chaabouni, A Latche, JC Pech, M Bouzayena (2005). The tomato *Aux/IAA* transcription factor *IAA9* is involved in fruit development and leaf morphogenesis. *Plant Cell* 17:2676–2692

Wilmoth JC, S Wang, SB Tiwari, AD Joshi, G Hagen, TJ Guilfoyle, JM Alonso, JR Ecker, JW Reed (2005). NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *Plant J* 43:118–130

Wu J, FY Kong, L Cheng, FL Kong, Z Peng, SY Liu, XL Yu, L Gang (2011). Identification, isolation and expression analysis of auxin response factor (ARF) genes in *Solanum lycopersicum*. *Plant Cell Rep* 30:2059–2073

Yamori W, N Sakata, Y Suzuki, T Shikanai, A Makino (2011). Cyclic electron flow around photosystem I *via* chloroplast NAD(P)H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. *Plant J* 68:966–976

Zhou C, L Han, C Fu, J Wen, X Xiao, J Nakashim, J Ma, Y Tang, Y Tan, M Tadege, KS Mysore, G Xia, ZY Wang (2013). The trans-acting short interfering RNA3 pathway and no apical meristem antagonistically regulate leaf margin development and lateral organ separation, as revealed by analysis of an *argonaute7/lobed leaflet1* mutant in *Medicago truncatula*. *Plant Cell* 25:4845–4862