저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

Master's Thesis of Science in Agriculture

**Genetic analysis of the tomato *inquieta* mutant links
the ARP2/3 complex to trichome development**

토마토 *inquieta* 돌연변이에서 모상체 발달과 관련된
ARP2/3 복합체의 유전적 연구

August 2018

Na-Rae Jeong

**Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University**

Abstract

Genetic analysis of the tomato *inquieta* mutant links the ARP2/3 complex to trichome development

Na-Rae Jeong

Department of International Agricultural Technology

Graduate School of International Agricultural Technology

Seoul National University

Trichomes are hair-like structures on the aerial surface of many plant species. Trichomes are well characterized for their roles as physical barriers and chemical defense against herbivore attack. Here, we describe the characterization of a monogenic recessive mutant of tomato (*Solanum lycopersicum*) called *inquieta* (*ini*). All trichome types on *ini* plants showed distinct morphological defects (e.g., swelling) that are known to be associated with defects in the actin cytoskeleton. Genetic mapping experiments positioned the *Ini* locus within a 1.5 cM interval on chromosome 11 that contains the tomato homolog of the Arabidopsis *ARPC2A* gene, which encodes a protein involved in nucleating the polymerization of actin filaments. Use of *ARPC2A* as a molecular marker showed that this gene strictly co-segregates with the target locus in a mapping population of 135 F₂ plants. Reverse transcriptase (RT)-PCR and genomic PCR experiments showed that full-length *ARPC2A* is amplified in wild-type but not in the *ini* mutant. Flanking PCR and Southern blot analysis showed that the *ini* mutation corresponds to a complex ~6-kb insertion in the 5th intron of *ARPC2A*. Expression of a wild-type *ARPC2A* in the *ini* mutant background restored normal trichome development. These results provide

molecular evidence that altered trichome development in the *ini* mutant is caused by a defect in actin cytoskeleton formation.

Keywords

ARP2/3-WAVE complex, *ARPC2A*, *inquieta*, Map-based cloning, trichome

CONTENTS

ABSTRACT	i
CONTENTS	iii
LIST OF FIGURES	V
LIST OF TABLES	Vii
ABBREVIATIONS	Viii
INTRODUCTION	1
MATERIALS AND METHODS	5
1. Plant materials and growth conditions	5
2. Analysis of trichome morphology	5
3. Genetic mapping of <i>Ini</i>	6
4. RT-PCR and genomic DNA PCR	7
5. Amino acid alignment and phylogenetic analysis	8
6. Flanking PCR	8
7. Genomic DNA southern blot analysis	9
8. Vector construct	10
9. Tomato transformation	11
RESULTS	13
1. Effect of <i>ini</i> on trichome development	13

2. Genetic mapping of <i>Ini</i>	14
3. <i>Ini</i> likely encodes the tomato homolog of ARPC2A	15
4. <i>ini</i> plants contain a complex rearrangement in the <i>ARPC2A</i> gene	16
5. Genetic complementation of the <i>ini</i> mutant with wild-type <i>ARPC2A</i> gene restores normal trichome development	18
6. Identification of new distorted trichome mutants in tomato	18
 DISCUSSION	 52
REFERENCES	56
ABSTRACT IN KOREAN	65

LIST OF FIGURES

Figure 1. Light microscopes of trichomes on the leaf, stem, hypocotyl, and floral bud of WT, <i>ini</i> , and <i>hl</i> plants	20
Figure 2. Cryo-SEM microscopes of trichomes on the leaf, stem, hypocotyl, and sepal of WT, <i>ini</i> , and <i>hl</i> plants.....	22
Figure 3. The <i>ini</i> mutation cosegregates with the tomato homologs of ARPC2A	24
Figure 4. Predicted mRNA sequence and amino acid sequence of ARPC2A from WT plants	26
Figure 5. Similarity of ARPC2 between tomato and other species.....	28
Figure 6. PCR amplification of partial length <i>ARPC2A</i> genomic DNA from WT and <i>ini</i> plants.....	30
Figure 7. Flanking PCR to analyze mutated sequence within <i>ARPC2A</i> gene of <i>ini</i> mutant	32
Figure 8. Genomic sequence of the affected region of <i>ARPC2A</i> in WT and <i>ini</i> plants	34
Figure 9. Southern blot analysis with <i>ARPC2A</i> probes on genomic DNA extracted from WT and <i>ini</i> plants.....	36
Figure 10. <i>ARPC2A</i> complements the trichome morphology defect of the <i>ini</i> mutant.....	38

Figure 11. Light micrographs of trichomes in WT and new distorted mutants

.....**40**

LIST OF TABLES

Table 1. Arabidopsis genes encoding subunits of the ARP2/3 and WAVE complexes and their homologs in tomato	42
Table 2. Description of PCR-based mapping markers	44
Table 3. Description of primers for RT-PCR and genomic PCR	46
Table 4. Description of flanking PCR primers	48
Table 5. Description of PCR primers used for probe synthesis	50

LIST OF ABBREVIATIONS

<i>Ini</i>	<i>Inqueita</i>
<i>Hl</i>	<i>Hairless</i>
<i>CaMV35s</i>	<i>Cauliflower mosaic virus 35S promoter</i>
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
gDNA	Genomic deoxyribonucleic acid
cDNA	Complementary DNA
PCR	Polymerase chain reaction
(RT)-PCR	Reverse transcription polymerase chain reaction
LB	Left border
RB	Right border
<i>dis</i>	<i>distorted</i>

INTRODUCTION

Plant trichomes are hair-like outgrowths originated from aerial epidermis of leaf, hypocotyl, stem, and floral organs in many plant species. Trichomes perform diverse biological functions such as protection against biotic stresses, including insect and pathogen attack (Shepherd et al. 2005; Kennedy 2003) and adaption to abiotic stresses such as water loss and UV-B radiation (Karabourniotis et al. 1992; Ehleringer and Mooney 1978). Trichomes can be classified morphologically as being either non-glandular or glandular, and either unicellular or multicellular. As trichomes are readily accessible structures and may not be critical for plant growth, they serve as an excellent model for studying the developmental processes underlying the cell fate determination, including cell cycle control and cell morphogenesis (Chang et al. 2016; Yang and Ye 2013; Qian et al. 2009).

In the model plant *Arabidopsis*, which has only one type of unicellular, non-glandular trichome, a transcriptional regulatory network of trichome initiation and development has been elucidated through extensive genetic and molecular analyses. This work has shown, for example, that trichome development is controlled by a trimeric transcriptional complex consisting of R2R3 MYB proteins, basic helix-loop-helix (bHLH) proteins, and a WD40 repeat (WDR) protein. This MYB-bHLH-WDR complex positively regulates the expression of the homeodomain transcription factor GL2 to regulate trichome initiation (Ramsay and Glover 2005; Zhao et al. 2008; Szymanski et al. 1998). Recently, C2H2 zinc finger proteins (ZFPs) including GLABROUS INFLORESCENCE STEMS (GIS) and ZFP5 were identified as upstream transcriptional regulators to control the expression of *MYB* or *bHLH* genes

(Gan et al. 2006; Yan et al. 2014). After trichome formation is initiated, trichome cells are enlarged and develop branches by arresting cell division and switching to the endoreplication programme, which are genetically controlled by several different genes including *SIAMESE* and *ZWICHEL* (Walker et al. 2000; Oppenheimer et al. 1997; Grebe 2012). Finally, the actin-related protein (ARP)2/3 complex involved in nucleating actin filaments, and the WAVE complex that regulates ARP2/3 activity, are required to maintain polarized stalk and branch growth. The ‘distorted group’ mutants, which are defective in genes encoding components of the ARP2/3 and WAVE complexes, exhibit swollen and twisted trichomes (Szymanski 2005; Basu et al. 2004; Deeks et al. 2004; Uhrig et al. 2007).

Cultivated tomato (*Solanum lycopersicum* L.) and its wild relatives have at least seven distinct types of trichomes that differ with respect to size, cell number, and the presence of glandular secreting cells (Luckwill 1943). Among the non-glandular trichome types (type II, III, and V), type II and type III trichomes are similar in length but differ by the presence of a multicellular and unicellular base, respectively. The shortest type V trichomes have a unicellular base. Among four different glandular trichomes (type I, IV, VI, and VII), type I trichomes have a multicellular base, a long multicellular stalk, and a small glandular tip. Type IV trichomes contain a unicellular base, a short multicellular stalk, and a small secreting glandular tip. Type VI trichomes consist of a four-celled glandular head on a short multicellular stalk, whereas type VII trichomes have a unicellular stalk and an irregularly shaped 4- to 8-celled gland (Luckwill 1943; Kang et al. 2010a). The glandular trichomes have been well characterized as chemical “factories” that produce diverse specialized metabolites

implicated in anti-insect defense. For instance, terpenoids, flavonoids, 2-tridecanone and other methyl ketones synthesized in type VI trichomes of cultivated tomato and its wild relatives exert toxic effects on several arthropod pests, including tobacco hornworm (*Manduca sexta*), aphids (*Macrosiphum euphorbiae* and *Myzus persicae*), and Colorado potato beetle (*Leptinotarsa decemlineata*) (Kennedy 2003; Kang et al. 2010b; Williams et al. 1980). Acyl sugars secreted from type IV trichomes of *S. pennellii* play an important role in the resistance to numerous insects such as whiteflies (*Bemisia argentifolii* and *Trialeurodes vaporariorum*), beet armyworm (*Spodoptera exigua*), tomato fruitworms, and aphids (Goffreda et al. 1990; Rodriguez et al. 1993; Juvik et al. 1994; Kennedy 2003). Given the important roles of these compounds in plant protection, recent research has focused on understanding the underlying biochemical pathways required for their synthesis (Schilmiller et al. 2009; Schilmiller et al. 2012; Sallaud et al. 2009; Bleeker et al. 2012; Kang et al. 2014). However, our understanding of how multicellular trichomes develop is still in its infancy and only a few genes controlling trichome development have been identified. For example, the *Woolly* gene encoding a homeodomain-containing transcription factor regulates type I trichome development (Yang et al. 2011). The *Hairless (HL)* gene encoding the SRA1 subunit of the WAVE regulatory complex is required for proper cell enlargement and cell shape of all trichome types in tomato (Kang et al. 2016).

The *inquieta (ini)* mutant of tomato was first reported a half-century ago as a radiation-induced mutant that is defective in trichome development (Stubbe 1964). Previous studies have noted that the trichome phenotype of the *ini* and *hl* mutants are

similar, with both having severely bent trichomes that impart a granular appearance to aerial tissues (Reeves 1977; Dempsey and Sherif 1987). Subsequent studies showed that *ini* is non-allelic to *hl* and that the *Ini* locus is located on the short arm of chromosome 11 (Zobel et al. 1970; Tanksley et al. 1992). Here, we analyzed the effect of *ini* on trichome morphology and also used a map-based cloning approach to identify candidate genes for *Ini*. Our results show that the *ARPC2A* gene, which encodes a subunit of ARP2/3 complex, strictly cosegregates with *ini* in an F₂ mapping population. Molecular analyses revealed a DNA insertion in the *ARPC2A* gene of *ini* mutant plants, and also showed that this insertion is associated with the loss of detectable expression of *ARPC2A* transcripts. These collective data provide compelling evidence that altered trichome development in the *ini* mutant is caused by a defect in *ARPC2A*.

MATERIALS AND METHODS

1. Plant materials and growth conditions

Tomato (*Solanum lycopersicum*) cv Rheinlands Ruhm (accession number LA0535) and cv Micro-tom (accession number LA3911) were used as the wild-type (WT) for all experiments. Seeds for WT and *inquieta* (*ini*, accession number LA0953) were obtained from C.M. Rick Tomato Genetics Resource Center (University of California, Davis). The EMS mutagenized mutant population was obtained from Gene Research Center (University of Tsukuba, Japan).

Seedlings were grown in Jiffy peat pots (Hummert International, Earth City, MO, USA) in a growth chamber maintained under 16 h of light ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 24°C and 8 h of dark at 18°C and 60% humidity. Three- to four-week-old plants were sampled for trichome morphological analysis.

2. Analysis of trichome morphology

A dissecting microscope (Leica M205A, Wetzlar, Germany) equipped with LED5000 RL light sources (Leica, Wetzlar, Germany) and a Leica MC170 HD Camera (Leica, Wetzlar, Germany) was used to view trichome morphology. The images were analyzed with Leica Application Software (LAS v4.8) and assembled with Photoshop Imaging Suite. To examine trichome morphology in detail, Cryo Scanning Electron Microscopy (CryoSEM) was performed using a Tabletop

Microscope TM3030plus (Hitachi High-Technologies Corporation, Tokyo, Japan) equipped with DEBEN coolstage (Deben, London, UK) for freezing and fixing tissues. The images were captured using 15kV to minimize surface charging of the trichomes. The images were analyzed with TM3030plus application software (ver. 01-05-02) and assembled with Photoshop Imaging Suite. All measurements were performed on WT and *ini* plants grown side-by-side in the same growth chamber.

3. Genetic mapping of *Ini*

Fine mapping of *Ini* was performed with an F₂ population derived from a cross between *ini* mutant (LA0953) and *S. pennellii* introgression line IL11-3 (LA4094), and was facilitated by the tomato genome sequence (TomatoGenomeConsortium 2012). A population of 135 F₂ plants was scored for the distorted trichome phenotype and subsequently genotyped with PCR-based conserved ortholog markers located within the introgressed region of chromosome 11 (Tomato-EXPEN 2000 map; <https://solgenomics.net>). Two plants showing recombination between markers T1460 and C2_At5g60600 were identified. Primer sequences used for mapping are listed in Table. 2. The TG400 and ARPC2A markers co-segregated with the *Ini* target locus. Genomic DNA extraction and PCR conditions were as described previously (Kang et al. 2010b)

4. RT-PCR and genomic DNA PCR

RNA extracted from leaves (TRIzol Reagent, Thermo Fisher Scientific) was used for cDNA synthesis (Thermoscript RT-PCR system, Invitrogen) according to the manufacturer's instructions. Full-length cDNAs corresponding to *SLARPC2A* were amplified by PCR (GeneAmp PCR System 9700, Applied Biosystems), using the ARPC2A-full primer set (Table. 3). A cDNA encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was PCR-amplified using the GAPDH primer set (Table. 3) and used as a loading control. Reverse transcription (RT)-PCR reactions (50 μ L) contained 2 μ L cDNA, 1 μ L 10 μ M solution of each primer, 4 μ L dNTP mixture (2.5 mM each), 5 μ L 10X Ex Taq buffer, and 0.25 μ L TaKaRa Ex Taq polymerase (TaKaRa). The amplification protocol included an initial 30 s denaturation step at 98°C, followed by 30 cycles in which the template was denatured for 10 s at 98°C, annealed for 30 s at 55°C, and extended for 90 s at 72°C. Amplified DNA products were separated on a 1% agarose gel. Full-length and partial genomic DNA fragments corresponding to *SLARPC2A* from WT and *ini* plants were PCR-amplified using the following primer sets (full-length: ARPC2A-full, partial length: ARPC2A-gDNA1 ~ ARPC2A-gDNA9) listed in Table. 3. PCR reactions (50 μ L) contained 2 μ L gDNA template, 1 μ L of a 10 μ M solution of each primer, 4 μ L dNTP mixture (2.5 mM each), 5 μ L of 10X Ex-Taq buffer, and 0.25 μ L of TaKaRa Ex Taq polymerase (TaKaRa). Amplicons were produced by an initial 2 min denaturation step at 98°C, followed by 30 cycles in which the template was denatured for 10 s at 98°C, annealed for 30 s at 53°C or 55°C, and extended for 2~10 min at 72°C, followed by a final incubation for 5 min or 10 min at 72°C. Amplified products were separated on a 1% agarose gel.

Automated nucleotide sequencing was performed at Cosmogenetech (Seoul, Korea).

5. Amino acid alignment and phylogenetic analysis

ARPC2 amino acid sequences from *S. lycopersicum* SLARPC2A (Solyc11g068610), *S. tuberosum* STARPC2A (XP_006362875), *N. tabacum* NTARPC2A (NP_001311940), *O. sativa* OSARPC2A (XP_015612421), *A. thaliana* ATARPC2A (NP_564364), *D. melanogaster* DMARPC2 (NP_610033), *B. Taurus* BTARPC2 (1K8K_D), *C. elegans* CEARPC2 (NP_741088) and *S. cerevisiae* SCARPC2 (NP_014433) were aligned using CLUSTALW. In CLUSTALW, the gap-opening and gap-extension penalties were set at 10 and 0.1, respectively, and the alignment was refined by using color align conservation (<http://www.bioinformatics.org/sms2/index.html>). The phylogenetic tree was constructed with the Maximum Likelihood method based on the JTT matrix-based model in MEGA7.

6. Flanking PCR

To characterize sequence changes within *ARPC2A* gene of *ini* plants, flanking PCR was performed as described previously with slight modification (Thole et al. 2009). Briefly, genomic DNA of WT and *ini* plants was digested with *Bfa*I or *Hpy*CH4VI. For *Bfa*I adapter preparation, ADP2 and ADP3 primers (Table. 4), and for *Hpy*CH4VI adapter preparation, ADP2 and ADP4 primers (Table. 4) were used. After ligation between the *Bfa*I-digested genomic DNA and the *Bfa*I adapter, the first

PCR reaction was carried out using the forward primer *ini* fAP1-F1 or the reverse primer *ini* fAP1-R1 in the intron 5 region of *ARPC2A* and the AP1 universal primer in the adapter (Table. 4). After the first PCR amplification, a second PCR amplification was performed with the primer closer to the mutation region (*ini* fAP1-F2 or *ini* fAP1-R2) and the AP2 universal primer in the adapter (Table. 4). For *HpyCH4VI*-digested genomic DNA ligated with *HpyCH4VI* adapter, the first PCR reaction was carried out using the forward primer *ini* fAP1-F3 or the reverse primer *ini* fAP1-R3 in the intron 5 region of *ARPC2A* gene and the AP1 universal primer in the adapter (Table. 4). The second PCR amplification was performed with *ini* fAP1-F4 or *ini* fAP1-R4 primer, and the AP2 universal primer in the adapter (Table. 4). Flanking PCR condition included an initial 3 min denaturation step at 95 °C, followed by 30 cycles in which the template was denatured for 20 sec at 95 °C, annealed for 40 sec at 58 °C, and extended for 2min at 72 °C, followed by a final incubation for 5 min at 72 °C. Amplified PCR products were gel-purified using a Gel and PCR clean up kit (Cosmogenetech, Seoul, Korea) and sequenced to identify the inserted sequence in *ARPC2A* of *ini* mutant.

7. Genomic DNA Southern blot analysis

Ten µg genomic DNA from WT and *ini* plants was digested with *HindIII*, electrophoresed on a 1 % agarose gel, and blotted onto nylon membrane (Amersham Hybond-N+, GE Healthcare Life Science, Chicago, USA). To prepare template DNA for probes of *ARPC2A*, genomic DNA of WT plants was amplified with *ARPC2A*-

gDNA6-F and ARPC2A-gDNA3-R primers (Table. 5) spanning exon 5 to intron 7 region. The amplified PCR products were gel-purified using a Gel and PCR clean up kit (Cosmogenetech, Seoul, Korea). Five ng of the gel-purified template DNA was re-amplified with ini-probeT-F1 and ARPC2ACG-R1 primers (Table. 5) for probe1 (229 bp), or ini-probeB-F1 and ini-probeB-R1 primers (Table. 5) for probe2 (322 bp), using PCR DIG Probe synthesis Kit (Roche Applied Science, Mannheim, Germany). The reamplified PCR products were gel-purified using a Gel and PCR clean up kit (Cosmogenetech, Seoul, Korea). All other steps of Southern blot procedure were performed as described previously (Lee et al. 2016).

8. Vector construct

RNA was extracted from Rheinlands Ruhm (LA0535) tomato leaf using TRIZOL (Sigma-aldrich, 15596018, St.Louis, USA). The full length of ARPC2A fragment was amplified from cDNA using EF-Taq polymerase (Solgent, SEF16-R250, Daejeon, Korea) with 5'-TCGATGATATTACTGCAATCTCCCTCTAG-3' and 5'-GTTCGCGAAGTTGGAAGTAGAA-3' primer set. PCR product was purified using a gel extraction kit (Cosmogenetech, CMA0112, Seoul, Korea). Purified PCR products were cloned into pGEM-T easy vector (Promega, A1360, Medison, USA) and digested with *Bam*HI-HF (NEB, #R3136S, Ipswich, USA) and *Xho*I (NEB, #R0146S, Ipswich, USA) restriction enzyme. The digested *ARPC2A* DNA fragments were inserted into the pENTR-3C vector (Invitrogen, A10464, Carlsbad, USA) before being combined into an appropriate destination vector using the Gateway LR reaction

(Invitrogen, 11791-020, Carlsbad, USA). pKCo-DC-myc was used for destination vector to generate *35S::ARPC2A* overexpression construct.

9. Tomato transformation

Cotyledons were used for transformation when there were no or minimal true leaves present on seedlings. Cotyledons were cut into four pieces and tips and bases were discarded. Middle parts of cotyledons were placed upside up on MS medium containing 1 mg/L zeatin (MS-Z) with a sterile filter paper for 2 days in a growth chamber at 25 °C, under 16h of light ($50\mu\text{mol m}^{-2}\text{s}^{-1}$), 8h of dark and 60% humidity. The *A. tumefaciens* strain LBA4404 with vector construct culture was grown in 15ml LB media with no selection antibiotic in a 250ml flask at 28 °C for 2days. Cultured media were spun down for 5 min at 3000 rpm, and the pellet was washed with MSO liquid medium each time. The washed pellet was resuspended with 10ml of MSO liquid media and then diluted to O.D.₆₀₀ value of 0.6. 0.074M acetosyringone was added to the final concentration of 375uM. 5ml of acetosyringone treated *Agrobacterium* culture was pipetted over the pre-incubated cotyledons and incubated for 10 min. The explants placed upside down on new MS-Z plate with sterile filter paper and co-cultivated for 2days in a 25 °C growth chamber. After two days of co-cultivation, the explants were rinsed in sterile water with 100mg/L carbenicillin. The steriled explants were transferred onto MS media containing zeatin (2mg/L), Carbenicillin (500mg/L), Kanamycin (100mg/L). The plates were incubated in the 25 °C growth chamber for 10days. On day 10, cotyledons were transferred to new media plates. Thereafter explants were transferred at intervals of tree weeks. Grown shoots

were cut off from explants and placed in MS media including 1mg/L of IBA, 50mg/L Kanamycin, and 500mg/L of Carbenicillin.

RESULTS

1. Effect of *ini* on trichome development

We used light microscopy to compare the morphology of trichomes on leaves, stems, hypocotyls, and floral organs of the *ini* mutant to its wild-type parent (cv Rheinlands Ruhm). Compared to wild-type in which type I trichomes were aligned perpendicular to the epidermal surface, *ini* showed highly twisted and swollen trichomes (Fig. 1). The identity of the distorted structures of *ini* as type I trichomes was confirmed by low temperature scanning electron microscopy (Cryo-SEM). This analysis showed that type I trichomes on the *ini* mutant contain highly swollen cells that fail to orient perpendicular to the epidermal surface, resulting in highly distorted and twisted structures (Fig. 2). Other trichome types on the *ini* mutant also showed swollen and distorted structures. Especially, the type VI trichome, which contains a short neck cell that connects the four-celled glandular head to the stem, showed irregular patterns of cell division on the *ini* mutant. The neck cell of type VI trichomes on the *ini* mutant also protruded from the side of the stem (Fig. 2). Previously we reported that aerial tissues of the tomato *hl* mutant have swollen and distorted trichomes (Kang et al. 2010a). Comparison of the *ini* and *hl* mutants showed that their distorted trichome phenotypes are strikingly similar (Fig. 1 and 2), suggesting that the *ini* and *hl* mutations affect similar developmental processes.

2. Genetic mapping of *Ini*

F₁ plants derived from a cross between *ini* and its wild-type parent showed normal trichome phenotypes, indicating that the mutation is recessive. An F₂ population obtained by selfing the F₁ plants was scored at the seedling stage (3-week-old plants) for the trichome distortion phenotype. Among 116 F₂ plants, 27 plants exhibited trichome distortion, whereas the remaining F₂ plants appeared normal. This ratio (3.3:1) is in good agreement with that predicted for a single recessive mutation ($\chi^2 = 0.18$; $P = 0.67$). Owing to the similar trichome phenotype between the *ini* and *hl* mutants, we generated F₁ plants derived from a cross between *ini* and *hl* plants to test for genetic complementation between *ini* and *hl*. All F₁ plants obtained from six independent crosses showed normal trichome development, indicating that *Ini* and *Hl* are different genes.

Ini was previously mapped to the short arm of chromosome 11 near the RFLP marker TG36 (Tanksley et al. 1992). Among the introgression lines (ILs) derived from a cross between *S. lycopersicum* cv. M82 (LA3475) and *S. pennellii* (LA0716), the IL11-3 (LA4094) line contains TG36 (Eshed and Zamir 1995). We used a population of 135 F₂ plants derived from a cross between *ini* (LA0953) and IL11-3 to refine the map position of *Ini* using additional markers (Table. 2) located in the IL11-3 region. The results indicated that markers T1460 and C2_At5g60660 are most closely linked to *Ini*, with one recombination event identified between *Ini* and each of these markers (Fig. 3A). The ~22,000 kb region defined by T1460 and C2_At5g60660 is predicted to contain approximately 790 hypothetical genes (ITAG2.4 gene models; <https://solgenomics.net/>) (Fig. 3A).

3. *Ini* likely encodes the tomato homolog of ARPC2A

Recently, we demonstrated that *Hl* encodes the tomato homolog of SRA1, which is a subunit of the highly conserved WAVE regulatory complex involved in actin filament nucleation in eukaryotic cells (Kang et al. 2016). Studies with *Arabidopsis* have shown that mutations affecting the ARP2/3 and WAVE complexes, which control the formation of the actin cytoskeleton, also cause abnormally distorted trichomes (Basu et al. 2004; El-Assal et al. 2004; Szymanski 2005). To test the hypothesis that *Ini* may encode a component of the ARP2/3-WAVE complex, we identified putative tomato homologs of the known subunits of the *Arabidopsis* ARP2/3 and WAVE complexes (Szymanski 2005) (Table 1). Only one gene (Solyc11g068610) was located in the region between markers T1460 and C2_At5g60600. Solyc11g068610 is predicted to encode the ARPC2A subunit of the ARP2/3 complex. To further test the genetic relationship between Solyc11g068610 and *Ini*, we developed a CAPS marker for *SlARPC2A* (ARPC2A, Table. 2) and tested recombination events in the F₂ mapping population. The results showed that the ARPC2A marker strictly co-segregated with *Ini* in the population of 135 F₂ plants (Fig. 3A), suggesting that *ARPC2A* and *Ini* are the same gene.

SlARPC2A consists of 10 exons and 9 introns, and its genomic and coding DNA sequence are 7,582 and 987 bp in length, respectively (Fig. 3B and Fig. 4A). We used a reverse transcription (RT)-PCR assay to test whether *ini* plants are affected in the expression of *SlARPC2A*. The result showed that *ARPC2A* transcripts were expressed in wild-type leaves but not in *ini* leaves (Fig. 3C). Moreover, genomic PCR analysis showed that full-length genomic DNA of *ARPC2A* was amplified in wild-

type but not in *ini* plants (Fig. 3D). These results provide compelling molecular evidence that *Ini* corresponds to *ARPC2A*, which is consistent with the established role of this ARP2/3 complex subunit in regulating actin cytoskeleton formation (Deeks et al. 2004; El-Assal et al. 2004).

SlARPC2A is predicted to encode for a 328-amino-acid protein having a molecular mass of ~34 kDa (Fig. 4B). Sequence alignment of *SlARPC2A* with homologs from diverse species indicated that the tomato protein is very similar (>70% identity) to other plant homologs and less similar (21~24%) to *ARPC2As* from metazoan species (Fig. 5A). Based on the crystal structure of the bovine ARP2/3 complex, the C-terminal domain of vertebrate *ARPC2* forms an extended alpha-helix that makes multiple contacts with *ARPC4* (Robinson et al. 2001). This C-terminal domain is well conserved among the different species, including tomato. Phylogenetic analysis showed that plant *ARPC2As* cluster as a distinct phylogenetic clade relative to *ARPC2As* in yeast and animals (Fig. 5B).

4. *ini* plants contain a complex rearrangement in the *ARPC2A* gene

The inability to PCR amplify the full-length *ARPC2A* mRNA or genomic DNA from the *ini* mutant, suggested that the structure of this gene is altered in the *ini* mutant. To test this hypothesis, we attempted to amplify four regions (1 – 4) that span the gene (Fig. 6A). Whereas PCR products corresponding to regions 1, 2, and 4 were amplified from genomic DNA isolated from both WT and *ini* plants, region 3 was not amplified from *ini* genomic DNA (Fig. 6B). We employed a similar PCR-based

strategy to identify the subregion (i.e., region 7) within region 3 that harbored the putative DNA polymorphism (Fig. 6A and B). Further PCR experiments delimited the polymorphism, as determined by lack of PCR amplification, to a 360 bp fragment (i.e., region 8) within region 7. Repeated attempts to amplify the region 8 from *ini* genomic DNA were unsuccessful, suggesting that intron 5 of *ARPC2A* in the *ini* mutant may harbor a large insertion.

We used flanking PCR analysis (Thole et al. 2009) to further explore the nature of the polymorphism in intron 5 of the *ini ARPC2A* gene (Fig. 7A). DNA sequencing of PCR fragments that flank intron 5 from *ini* plants revealed that a part of zinc finger protein-related gene (Solyc11g045230) was incorporated into the intron 5 region of *ARPC2A*. The left and right flanking regions contained a ~600 bp DNA fragment (3' intergenic region of Solyc11g045230) and a ~340 bp DNA fragment (part of exon 5, intron 5, exon 6, and 3' UTR region of Solyc11g045230), respectively (Fig. 7B and Fig. 8). DNA sequence alignments showed that these two fragments do not overlap, which implies that an additional DNA fragment is inserted into the mutant form of *ARPC2A*. We used two different DNA probes (probes 1 and 2) in Southern blot experiments to confirm the insertion-deletion (indel) polymorphism in this region (Fig. 9). DNA hybridizations performed with probes 1 and 2 clearly revealed ~4.9 kb and ~1.5 kb size polymorphisms, respectively, in genomic DNA from *ini* plants (Fig. 9B). These data demonstrate that the *ini* mutant harbors a complex indel mutation in intron 5 of *ARPC2A*, resulting in lack of expression of a functional *ARPC2A* transcript.

5. Genetic complementation of the *ini* mutant with wild-type *ARPC2A* gene restores normal trichome development

To determine whether the mutation in *ARPC2A* causes the distorted trichome phenotype of *ini* plants, we used *Agrobacterium*-mediated transformation to complement the mutant with WT *ARPC2A* gene, under the control of the 35S promoter of *Cauliflower Mosaic Virus* (CaMV) (Fig. 10A). Visual observations indicated that all primary (T₀) transgenic plants exhibited normal trichome development on all tissues including leaves and stems. Light microscopy showed that the distorted trichome morphology on leaves and stems of the *ini* mutant was complemented by the 35S::*ARPC2A* transgene in the *Ini*-A and *Ini*-B lines which were indistinguishable from WT plants at this level of morphological analysis (Fig. 10B). This result indicates that the distorted trichome morphology in the *ini* mutant was caused by the defective *ARPC2A* gene.

6. Identification of new distorted trichome mutants in tomato

To identify more genes involved in trichome structure in tomato and to study their physiological and functional roles, we screened EMS-mutagenized tomato (cv Micro-tom) population which were obtained from the Gene Research Center (GRC, University of Tsukuba, Japan). Based on the description of mutant phenotypes in the GRC, fourteen EMS-mutagenized lines were grown and examined to observe morphology of trichomes on leaves and stems using light microscopy. Among the fourteen lines, we found that four lines had defects on trichome morphology [hereafter

referred as *distorted1* (*dis1*), *dis6*, *dis10*, and *dis13*]. These mutant lines showed a similar trichome phenotype such as highly twisted and swollen trichomes compared to wild-type (Fig. 11). The morphology of new distorted mutants are very similar to *ini* and *hl* mutants, suggesting that the new distorted mutants are also defective in actin polymerization. We are planning to do map-based cloning to identify causal genes of the new distorted mutants.

Fig. 1 Light micrographs of trichomes on the leaf, stem, hypocotyl, and floral bud of WT, *ini*, and *hl* plants

Photographs show the adaxial leaf surface (first row), stem (second row), hypocotyl (third row), and floral bud (fourth row) of each genotype. The Arrows indicate representative type I trichomes. All scale bars represent 2 mm.

Fig. 2 Cryo-SEM micrographs of trichomes on the leaf, stem, hypocotyl, and sepal of WT, *ini*, and *hl* plants

Photographs show the adaxial leaf surface (first row), stem (second row), hypocotyl (third row), and sepal (fourth row) of each genotype. Scale bars represent 500 μm in the leaves, 50 μm in the insets, and 1 mm in the stems, hypocotyls, and sepals. Type I and VI trichomes are indicated by arrows and bold capital characters.

Fig. 3 The *ini* mutation cosegregates with the tomato homolog of ARPC2A

(A) Genetic mapping of *Ini* delimited the target gene to an interval between marker T1460 and C2-At5g60600 on tomato chromosome 11. Numbers in parentheses indicate the number of recombination events identified between markers and the target gene.

(B) Structure of *SlARPC2A*. Vertical black lines depict exons and horizontal lines indicate intervening introns or intergenic regions. The mutation identified in the *ini* mutant affects the length of the fifth intron, denoted with a triangle.

(C) RT-PCR for full length *ARPC2A* from WT and *ini* leaves. *Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* mRNA was used as a loading control.

(D) PCR for full length *ARPC2A* genomic DNA from WT and *ini* plants.

A **B** **C** **D**

**Fig. 4 Predicted mRNA sequence and amino acid sequence of ARPC2A
from WT plants**

A : ARPC2A CDS

```

ATGATATTACTGCAATCTCCCTCTAGATATCTTCTTCAGATCTTGTACAATCGCGTCCAG 60
AGTCTTGAGAAAGGGGTTGAACTGGACAGCCAATGGGTTGAATTTGATGATATCCGTTAC 120
CATATTGAGGGTTCAGTGAAAAATCCAAATGTCTTGCTTCTTTCTGTATCATTGCCATA 180
CCACCTCCAGAAACCGTCTCTTTGGTGGACTTCCACTTGGAGCGCTAGAAGCCATAAAA 240
GCAGCATATGGTGCAGTTGCACAAATCTTGATCCTCCAAGAGATGGGTTTAATCTCACA 300
CTTAAACTAAACTTGTCAAACTTCCCCCAGATGAAGAGCAAAAACATGCACTCCTGACA 360
AAGATTGCATCCGTGAGGGAGGTAGTAATGGGAGCCCCACTGAGGGCAGTTTGAACAA 420
CTTGTTACAAGGACAGTTCCCTCCGATCTAGGCAAGCCCGTTGCCCTGGTTCATCGTCCT 480
AATGAGTCTTTTTTCCCTGTACCTCAGGCAGACAAGGTGACCGTCATATTTCCCTATGAGA 540
TTCAATGATTCCATAGATACTGTTCTAGCAACATCTTTCTTACAGGAATTTGTTGAGGCA 600
AGACGAACTGCTGGACTTAATAATGCCCTCCTTGTTTGTGGTCTCCTTCTCCTCCTCAA 660
GAACTGACAGAAGCTTTTACAGAAGCATTATCTGCTAATGCCGATTTGTTAGTTTGTGTA 720
ATTTTTTCTCGCCACGTGGAAGGCAGAAAGCTGGACAGAACTGTCTGGAACCTATCAACC 780
TTTCATGCTTATGTAAATTATCATGTTAAGTGTTCTGAATGTTTCATGCATACTCGGATG 840
AGGCGTCAGGTGGAGTCATTGATACAGGCACCTTGACCGTGCCAAACCAGATCCCGAGAAG 900
GCCAAGAAAAATTCACCTAACAGATCATTTAAACGAATGAGCCTCAAGGATGGCAATAAC 960
AGTTTGGGTTTCGCGAAGTTGGAAGTAG 987

```

B : ARPC2A amino acid sequence

```

MILLQSPSRYLLQILYNRVQSLEKGVELDSQWVEFDDIRYHIQGSVKNPVLLLSVSLPI 60
PPPETVLFGGPLGALAEIKAAYGAVAQILDPPRDGFNLTLKLNLSKLPDDEEQKHALLT 120
KIASVREVVMGAPLRAVLKQLVTRTVPSDLGKPVALVHRPNESFFLVPQADKVTVIFPMR 180
FNDSIDTVLATSFLQEFVEARRTAGLNNAPCLWSPSPQELTEAFTEALSANAGFVSFV 240
IFSRHVEGRKLDRTVWNLSTFHAYVNYHVKSECFMHTRMRRQVESLIQALDRAKPDPEK 300
AKKNSPNRSFKRMSLKDGNNSLGSRSWK 328

```

Fig. 5 Similarity of ARPC2 between tomato and other species

(A) Comparison of the predicted amino acid sequence of ARPC2A in tomato and other species. Amino acid residues in black indicate identity, and those in gray indicate conserved substitutions. The C-terminal alpha-helix of 43 residues of BTARPC2 that associates with APRC4 (p20) is underlined. *S. lycopersicum* SlARPC2A (Solyc11g068610), *S. tuberosum* StARPC2A (XP_006362875), *N. tabacum* NtARPC2A (NP_001311940), *O. sativa* OsARPC2A (XP_015612421), *A. thaliana* AtARPC2A (NP_564364), *D. melanogaster* DmARPC2 (NP_610033), *B. taurus* BtARPC2 (1K8K_D), *C. elegans* CeARPC2 (NP_741088) and *S. cerevisiae* ScARPC2 (NP_014433).

(B) Phylogenic tree of ARPC2 in tomato and other species. The unrooted phylogenetic tree was constructed by the Maximum Likelihood method using MEGA7 with amino acid sequences of ARPC2 from nine different species. *S. lycopersicum* SlARPC2A (Solyc11g068610), *S. tuberosum* StARPC2A (XP_006362875), *N. tabacum* NtARPC2A (NP_001311940), *A. thaliana* AtARPC2A (NP_564364), *O. sativa* OsARPC2A (XP_015612421), *S. cerevisiae* ScARPC2 (NP_014433), *C. elegans* CeARPC2 (NP_741088), *D. melanogaster* DmARPC2 (NP_610033) and *B. taurus* BtARPC2 (1K8K_D)

A

```

SLARPC2A  MLLQSPSRMYLLCILLNRRVQNI---EKGVELDCQWVEFDDIRYHIQGSVKNNPVLLLSVSLPIPPPETVLFGG--IFLGA 75
STARPC2A  MLLQSPSRMYLLCILLNRRVQNI---EKGVELDCQWVEFDDIRYHIQGSVKNNPVLLLSVSLPIPPPETVLFGG--IFLGA 75
NTARPC2A  MLLQSPSRMYLLCILLNRRVQNI---EKGVELDCQWVEFDDIRYHIQGSVKNNPVLLLSVSLPIPPPETVLFGG--IFLGA 75
ATARPC2A  MLLQSHSRFLLCILLTRAQNI---DKAVELDYQWIEFDDIRYHVQVIMKNNPVLLLSVSLPNPPPEAMSFDDG--IFLGA 75
OSARPC2A  MLLQSPSRFLLCILLDRVLSG---DKGVLDICHTVEFDDIRYHIQFSMRNPKVMVLSVALPLPPPEAMLYDG--IFLGA 75
DMARPC2  MLLLEINNRIIEETILLVKYRNAQAGLKPEESTDIRIADFTGVLYHISNVNGKTKVRISSISIKFYKQLQEHGAD----- 73
BTARPC2  MLLLEVNRIIEETILLALKFENAAAGNKPEAVETVADFTGVLYHISNPNGLTKVMVSSISIKFYKELQAHGAD----- 73
CEARPC2  MLLLEQNNRIIEVILLQKPEANAKEGGKPESEVNVTFADFTGVLYHISNPDGRTKILISLSIKFYTELQAHGAD----- 73
SCARPC2  MHLQEPQNLILQKTLINAEALARKGS-FITMDRIVSDFTYTYHISNTAEKKSILLLSVTKAWVSVECCQDGSFTLLK 79

SLARPC2A  LBAIKAAAYCAWACILDPFRDGFNLTILKLNLSKLPDEDEQKHA-----LLTKIASVREVVMGAPLRVIRKQLVTRT 145
STARPC2A  LBAIKAAAYGVVACILDPFRDGFNLTILKLNLSKLPDEDEQKHA-----LLTKIASVREVVMGAPLRVIRKQLVTRT 145
NTARPC2A  LBAIKAAAYGVVACILDPFRDGFNLTILKLNLSKLPDEDEKHA-----LLTKIASVREVVMGAPLRVIRKQLVTRT 145
ATARPC2A  IBAIKATTYGTGFCILDPFRDGFSLTILKLNFSKVRDEE-----LLTKIASVREVVMGAPLRVIRKQLVTRT 141
OSARPC2A  IBAIKAAAYGVVACILDPFRDGFDTMKINLTILKLPDEDEQRNA-----VLTQIASVREVVLGAPLRVIRKQLVTRT 145
DMARPC2  ---ELIKREYK-SILLDTDEGVNVSILNIEEHPED-----CEQIAKRRGLIKRNCFAVFEKVFYDQK 133
BTARPC2  ---ELIKREYK-SYLVNPESEGVNVSILYDDENLPA-----KDSIHQAGMKRNCFAVFEKVFYDQK 133
CEARPC2  ---DLIRRVYGGHMRSTPEGFNVTLIEYNLADLPAD-----TTDLQAAIAKRRNCFAVFEKVFYDQK 134
SCARPC2  FIAIDHYSSIGGVNIPSEVEFGVLYLCTILAEIVQESILQLSVLKTIIILSFFFLAISKFIELSQQCAPVVEAETIGEV 159

SLARPC2A  VPSDLCKREVALVHRPNESEFFVPCADKVTVIPEMRFNESHITVLATSFLOEFVEAR-RTAGLNNAFPCQLWSP-SPECEIT 223
STARPC2A  VPSDLCKREVALVHRPNESEFFVPCADKVTVIPEMRFNESHITVLATSFLOEFVEAR-RTAGLNNAFPCQLWSP-SPECEIT 223
NTARPC2A  VPSDLCKREVALVHRPNESEFFVPCADKVTVIPEMRFNESHITVLATSFLOEFVEAR-RTAGLNNAFPCQLWSP-SPECEIT 223
OSARPC2A  VAEEDDRVAIMRRPNESEFFVPCADKVTVAPEMRFEKSDTILATSFLEFVEAR-RAAALNTAPSCSWP-TACEIDE 219
DMARPC2  VAEENVKREVALVHRPNESEFFVPCADKVTVVPEMRFEKSDTILATSFLOEFVEAR-RTSALNNAFPCQLWSP-VPELEIK 223
BTARPC2  QCEGCKRAVINRYRNDETLYVEAKPDRVTVFESTIFRDEDDVILGKVFMOELREGR---RASHTAPQVLESHREPELEIK 210
CEARPC2  EKEGCKRAVINRYRNDETLYVEAKPDRVTVFESTIFRDEDDVILGKVFMOELREGR---RASHTAPQVLESHREPELEIK 210
SCARPC2  AGCEGCKRAVINRYRNDETLYVEAKPDRVTVFESTIFRDEDDVILGKVFMOELREGR---KASCTAPAVLSLGEPELEIK 211

SLARPC2A  EAFT--EALSANAGFVSFVIESRHVE-GRKLDRTVMNLSTEHAYVNYHVKCEGEGMHTRMRRCQVESLIQALDRAKEDPEK 300
STARPC2A  EAFT--EALSANAGFVSFVIESRHVE-GRKLDRTVMNLSTEHAYVNYHVKCEGEGMHTRMRRCQVESLIQALDRAKEDPEK 300
NTARPC2A  EAFT--EALSANAGFVSFVIESRHVE-GRKLDRTVMNLSTEHAYVNYHVKCEGEGMHTRMRRCQVESLIQALDRAKEDSEK 300
ATARPC2A  GAFK--ETLSANAGFVTFFVIFPRHVE-GRKLDRTVMNLSTEHAYVSYHVKCEGEGMHTRMRRCQVESLIQALDRAKEDSEK 296
OSARPC2A  GVPS--DALNANAGFVTFFVIFPRHVE-GRKLDRTVMNLSTEHAYVSYHVKCEGEGMHTRMRRCQVESLIQALDRAKEDSEK 300
DMARPC2  NT---DARVGNIGYITFVLFPFRHTN-KETRDNTINLHMFEDYLYHYHIKCSKAYIHTMRMRKTSDDFKVLNRARE---- 282
BTARPC2  DT---DARVGNIGYITFVLFPFRHTN-KASARDNTINLHMFEDYLYHYHIKCSKAYIHTMRMRKTSDDFKVLNRARE---- 282
CEARPC2  DTP--EARVGNIGYITFVLFPFRHTN-KKTKDNTIDLHMFEDYLYHYHIKCSKVYLHTRMRMRKTSDDFKVLNRARE---- 284
SCARPC2  RLYQPPKVAEQSRRFITFVLFPFRHFTKELQHSICQTLTFNMFHYHIKCSKAYMHSRMRRCQVESLIQALDRAKEDDED 318

SLARPC2A  AKKNSPNRSFKRMSLKDGNNSLGSRSWK 328
STARPC2A  AKKNSPNRSFKRMSLKDGNNSLGSRSWK 328
NTARPC2A  AKRSSPSRSFKRMSLKDGNNSLGSRSWK 328
ATARPC2A  RSMN--NKSFKRLGLENVNHNNSK---- 318
OSARPC2A  LKKLVNGSGPKRLSLNNDGN----- 320
DMARPC2  -----EPKNTEKKIITGRIFKRID-- 301
BTARPC2  -----DAPKEMKTIITGRIFSSR-- 300
CEARPC2  -----EVKG-EKKTIFHGRIFQTQ-- 301
SCARPC2  ENDELSAEGRQQARRTFTGRKIVY---- 342

```

B

Fig. 6 PCR amplification of partial length *ARPC2A* genomic DNA from WT and *ini* plants

(A) Schematic diagram showing PCR-amplified genomic DNA region (1 ~ 9) of *ARPC2A* gene.

(B) Agarose gel showing the PCR-amplified products indicated by (A).

Fig. 7 Flanking PCR to analyze mutated sequence within *ARPC2A* gene of *ini* mutant

(A) Diagram depicting flanking regions adjacent to the left or right region of *ARPC2A* DNA in *ini* mutant. A detailed flanking PCR procedure is described in the flanking PCR section of Materials and Methods. Briefly, *Bfa*I- or *Hpy*CH4IV-digested DNA was ligated with *Bfa*I or *Hpy*CH4IV adapter. The first PCR was carried out with AP1 primer and one of F1, R1, F3, and R3 primers to amplify PCR1 product. Using PCR1 product as a template, the second PCR was carried out with AP2 primer and one of F2, R2, F4, and R4 primers to generate PCR2 product. PCR2 DNA fragment was sequenced to identify mutated sequence within *ARPC2A* gene of *ini* mutant.

(B) Diagram depicting the nature of the DNA fragment insertion in *ARPC2A* of *ini* mutant, as compared to *ARPC2A* of WT. The *ini* mutation corresponds to a ≥ 940 -bp insertion (Solyc11g045230: zinc finger protein-related gene) in the intron 5 region of the gene. One-way arrows depict the directionality of zinc finger protein-related gene DNA segments that are inserted in *ARPC2A* of the mutant. A two-way arrow indicates the length of insertion that are not characterized. E5: Exon 5, E6: Exon 6.

A

B

Fig. 8 Genomic sequence of the affected region of *ARPC2A* in WT and *ini* plants

(A) Partial genomic sequence of intron 5 region in *ARPC2A* from WT plant. A triangle indicates the mutated spot in *ARPC2A* from *ini* mutant. The flanking PCR result revealed insertion of part of Soly11g045230 in the left (338 bp) and right (598 bp) junction regions. A question mark indicates a DNA fragment which is not sequenced.

(B) Soly11g045230 sequence inserted in *ARPC2A* of *ini* mutant. DNA fragments in red and blue are inserted in the right and left junction regions, respectively, in *ARPC2A* of *ini* mutant.

B

Solyc11g045230 (zinc finger protein-related)

TAGATTTGCTTCAGAAGAAGAGGTCAGTAACATAAAGTATAAGTATATATTGTTGCTATCAACAGGAGTGTCAATTGTTTATTCGTTT
 ACTAATTTATAAGCTAAATTTTCCAAAGGCGACTGAGTAAACGTTAGGAAATGCTTATTTGCTTTTATACCTTTTTCAGATTTAAGCAG
 TATTGGAAGGAAAGACATGCTTCCGAAAGAGCTGAAATGGTGAAACAATCAAAATCTTCTGGGATAACCAAAACAGATATGCTGTG
 GGTTTTTAGATACTGTCTTGGTGCAACAGCGTGATGGAGCGATCTATTTCTCCGAGCTCCGATTTTTTTCACCAATTTCATCAGCG
 AATATCATCAGCTGGTGTGTTTGTCTAATTTAGACATGTTTCTTGATACTAATAGCTTCTGATTCTTACAGCTATTGGAGTTTGOCA
 AAGTTGCTAAGTCACATATTGTGACTTTGGTTTCTTTCTTGCAAAATTTCTCAAAGACTCATTTATCAGATTTCAAAGTGGAATTTT
 TGGCTTGAATTAAGTATCTTAAATCAGGGGCTCAATAAAATCCATTTTGTGTTTCTTAATCTCTTGTGTGCAATTTCTCTCGTTT
 TTATTTATCTGTGCTACTTACTAAAAGTAGATCTTAATATTTTTCATTTTACAAAATTAATGTATGTGATTGCTCTCTCTGTTCTGAT
 TTATATGTTGTTCTTACTAACTAGATGATATTAATATTTGTCAATTTACAAAATTAATGTATGTATATTACTCAGTCTCATTCATATT
 TATATGTCGTTCTTACTAAAATAGATGATCTTAATATTTGTCAATTTACAAAATCATGTATAAATGACACTATCTTCTATTTTCTCT
 TGATAGTTATTAGCTCGAAAGTATGGAATTTTATTACAG

Fig. 9 Southern blot analysis with *ARPC2A* probes on genomic DNA extracted from WT and *ini* plants

(A) Schematic diagram showing relative positions of exons, probes, and *Hind*III restriction enzyme sites in *ARPC2A*. *ARPC2A* of *ini* mutant has a DNA insertion ($\geq$ 6.4 kb) between probe 1 and probe 2 in the intron 5 region. E5: Exon 5, E6: Exon 6.

(B) Southern blot hybridization of *Hind*III-digested DNA with probe1 and probe2 from WT and *ini* plants. DNA maker (M) sizes (bp) are shown in the left. One-way arrows indicate detected bands. Two-way arrows depict the size difference between bands detected in WT and *ini* plants.

Fig. 10 *ARPC2A* complements the trichome morphology defect of the *ini* mutant

(A) The vector construction of *ARPC2A* overexpression

(B) Light microscopy was used to assess the trichome morphology of WT, *ini*, and complemented transgenic lines (*Ini-A*, *Ini-B*). Photographs show the adaxial leaf surface (first row), stem (second row) of each genotype. The Arrows indicate representative type I trichomes. Scale bars: 2mm

Fig. 11 Light micrographs of trichomes in WT and new distorted mutants

Photographs show the adaxial leaf surface (first row) and stem (second row) of wild-type (WT) and distorted mutants (*dis1*, *dis6*, *dis10*, and *dis13*). The Arrows indicate representative type I trichomes. Scale bars: 2mm

Table. 1 Arabidopsis genes encoding subunits of the ARP2/3 and WAVE complexes and their homologs in tomato

Arabidopsis			Tomato		
Genetic name	Gene name	Locus code	Locus code	E value	identity with Arabidopsis homolog (%)
<i>WURM</i>	<i>ARP2</i>	AT3G27000	Solyc02g094320	0	90
<i>DIS1</i>	<i>ARP3</i>	AT1G13180	Solyc05g013940	0	83
			Solyc04g024530	0	82
			Solyc05g006470	0	76
<i>DIS2</i>	<i>ARPC1A</i>	AT2G30910	Solyc05g006470	0	76
	<i>ARPC1B</i>	AT2G31300	Solyc05g006470	0	76
	<i>ARPC2A</i>	AT1G30825	Solyc11g068610	1.00E-162	69
	<i>ARPC2B</i>	AT2G33385	Solyc09g090550	1.00E-136	52
	<i>ARPC3</i>	AT1G60430	Solyc07g007630	1.00E-111	86
			Solyc02g014540	1.00E-60	73
	<i>ARPC4</i>	AT4G14147	Solyc12g098430	1.00E-113	92
<i>CRK</i>	<i>ARPC5A</i>	AT4G01710	Solyc01g090450	3.00E-82	86
<i>GRL</i>	<i>NAP1</i>	AT2G35110	Solyc02g068720	0	76
<i>PIR/KLK</i>	<i>SRA1</i>	AT5G18410	Solyc11g013280	0	78
			Solyc11g013290	0	90
<i>BRK1</i>	<i>BRICK1</i>	AT2G22640	Solyc03g043720	2.00E-50	90
<i>DIS3</i>	<i>SCAR2</i>	AT2G38440	Solyc09g014980	1.00E-115	49
			Solyc02g076840	5.00E-59	51
	<i>ABI1L1</i>	AT2G46225	Solyc01g095280	1.00E-114	60

*: Tomato homologs of Arabidopsis ARP2/3 and WAVE complex genes were identified by a blast search using Tomato genome chromosomes (build SL2.5) in the Sol Genomics Network (<https://solgenomics.net/>). †: E value and identity were obtained by aligning each homolog in tomato and Arabidopsis using blastp in the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>).

Table. 2 Description of PCR-based mapping markers

marker name	forward primer sequence	reverse primer sequence	PCR size (bp)		mapping enzyme	digest size (bp)	
			<i>S. lycopersicum</i> / <i>S. pennellii</i>			<i>S. lycopersicum</i> / <i>S. pennellii</i>	
cTOE-7-B4	CTGGTCGGCTGGAGAAT	CTGGTACTCTGAGATAG	233/233		<i>kpnI</i>	233/192+41	
Tl460	AGTAAAGGCCATCCACTCC	CAAGCTATGTAATTGACTG	311/311		<i>HinfI</i>	221+91/311	
TG400	TCCAAATCCACCACCTATCC	AGCATTGCTCCCTGCTAAAG	404/404		<i>HinfI</i>	404/300+104	
ARPC2A	GAGGCAAGACGAACTGCTGG	AGACAGTTCTGTCCAGCTTTC	246/248		<i>SpeI</i>	142+104/248	
C2_A15g06000	TTGCTTCAAGGTTGCAGAAATGCG	ACCAGGCAAGTGTGAGCTCTTCTCTC	250/250		<i>RsaI</i>	120+70/250	
TG36	TGTTTAAACTGAAGATGTG	TGCTTTTACCATAATCAATTAC	179/188		<i>DraI</i>	173+6/147+41	

Table. 3 Description of primers for RT-PCR and genomic PCR

Primer Name	Forward primer	Reverse primer	Description
ARPC2A-full	TCGATGATATTACTGCAATCTCCCTCTAG	TTCTACTTCCAACTTCGCGAACC	Amplifies full length ARPC2A cDNA and gDNA
GAPDH	GACAAGGCTGCTGCTCACTT	GCTTGACCTGCTGTCACCAAC	housekeeping gene used as control for RT-PCR
ARPC2A-gDNA1	TCGATGATATTACTGCAATCTCCCTCTAG	AACCCATCTCTTGGAGGATCAAG	Amplifies ARPC2A gDNA from exon 1 to exon3
ARPC2A-gDNA2	TCAATTGCCCATACCACCTCCAG	TCATGTTCATTTGAACCTTAAAGCCTATAGAGG	Amplifies ARPC2A gDNA from exon 3 to intron 4
ARPC2A-gDNA3	ACAACTTGTTTACAAGGACAGTTCTTCC	AGTGAAGCTTTCTATCTTTAGTCAGCAAGG	Amplifies ARPC2A gDNA from exon 4 to intron 7
ARPC2A-gDNA4	AGCTGGACAGAACTGTCTGGAACC	TTCTACTTCCAACTTCGCGAACC	Amplifies ARPC2A gDNA from exon 7 to exon 10
ARPC2A-gDNA5	TCTATAGGCTTAAGTTCAAATGAACATGAATCC	AGAACAGTATCTATGGAATCATTGAAATCTCATAG	Amplifies ARPC2A gDNA from intron 4 to exon 5
ARPC2A-gDNA6	AGATTCAATGATTCATAGATAGTGTCTAGC	AGGGAATTGGTTGTAGTATTCTGATGAGATC	Amplifies ARPC2A gDNA from exon 5 to intron 5
ARPC2A-gDNA7	AAGATCTCATCAGAATACTACAAACCAATTCC	TAGGTTCCAGACAGTTCTGTCCAGC	Amplifies ARPC2A gDNA from intron 5 to exon 7
ARPC2A-gDNA8	AAGATCTCATCAGAATACTACAAACCAATTCC	TGAGCCACCTACTCATTCAGG	Amplifies ARPC2A gDNA from intron 5 to intron 5
ARPC2A-gDNA9	ATGTGCCTGAATGAGTAGGGTGG	TAGGTTCCAGACAGTTCTGTCCAGC	Amplifies ARPC2A gDNA from intron 5 to exon 7

Start and stop codon are in bold.

Table. 4 Description of flanking PCR primers

Name	Sequence (5'-3')	Description
ADP2	CTAATACGACTCACTATAGGCTCGAGCGGCCGGCAGGT	Used with ADP3 to generate the BfaI adapter or <i>HpyCH4IV</i> adapter
ADP3	TAACTTGCCCA	Used with ADP2 to generate the BfaI adapter
ADP4	CGACCTGCCCA	Used with ADP2 to generate the <i>HpyCH4IV</i> adapter
ini fAP1-F1	GAGGATTGCCCAAGATCTCATC	<i>ini</i> DNA forward primer near WT sequence used with AP1 for PCR1
ini fAP1-R1	CCTCAACAAATTCCTGATGAAATC	<i>ini</i> DNA reverse primer near WT sequence used with AP1 for PCR1
AP1	GGATCCTAATACGACTCACTATAGGGC	Adapter primer for PCR1
ini fAP2-F2	CTCATCAGAACTACTACAACCAATTCC	Nested <i>ini</i> DNA forward primer near WT sequence used with AP2 for PCR2 and used to sequence PCR2 products
ini fAP2-R2	AGCCACCTACTCAATTCAGG	Nested <i>ini</i> DNA reverse primer near WT sequence used with AP2 for PCR2 and used to sequence PCR2 products
AP2	TATAGGGCTCGAGCGGC	Nested adapter primer for PCR2
ini fAP1-F3	TGTCCTTATCTTTTATATCATCCCAATCC	<i>ini</i> DNA forward primer near WT sequence used with AP1 for PCR1
ini fAP1-R3	CAGTCGCCCTTTGGAAAAATTAGC	<i>ini</i> DNA reverse primer near WT sequence used with AP1 for PCR1
ini fAP2-F4	TGAAAAAGGTGTGAGAACTAATAGCTTCC	Nested <i>ini</i> DNA forward primer near WT sequence used with AP2 for PCR2 and used to sequence PCR2 products
ini fAP2-R4	TAGTGACGAATAAACAAAAATGACACTACC	Nested <i>ini</i> DNA reverse primer near WT sequence used with AP2 for PCR2 and used to sequence PCR2 products

Table. 5 Description of PCR primers used for probe synthesis

Name	Sequence (5'-3')	Description
ARPC2A-gDNA6-F	AGATTCAATGATTCCATAGATACTGTTCTAGC	To generate template DNA for probes
ARPC2A-gDNA3-R	AGTGAAAGCTTTCTATCTTTAGTCAGCAAGG	
ini-ProbeT-F1	TTAACTTGGGCCTAATTCAATTCC	To generate probe1
ARPC2ACG-R1	TTCCATCTCTTAACATGGTATCAGAGC	To generate probe2
ini-probeB-F1	TGTGTGGGGCTTAACACATTACAC	
ini-probeB-R1	TGAGCCACCCCTACTCAATTCAGG	

DISCUSSION

The availability of numerous trichome-related mutants of *Arabidopsis* has been instrumental for elucidating the molecular mechanisms underlying the development of simple unicellular trichomes. For example, molecular genetic studies with the ‘distorted group’ (*dis*) of trichome mutants showed that actin filament organization is crucial for cell growth and cell shape in the late stage of trichome development (Basu et al. 2004; Basu et al. 2005; Le et al. 2006; Uhrig et al. 2007). The *Arabidopsis dis* genes encode subunits of two different complexes: the ARP2/3 complex that controls nucleation of actin filaments and the WAVE complex that regulates the activity of ARP2/3. The ARP2/3 complex consists of seven subunits (ARP2, ARP3, ARPC1, ARPC2, ARPC3, ARPC4, and ARPC5), whereas the WAVE complex contains five subunits (NAP1, SRA1, BRICK1, SCAR2, and ABI1L1) (Table 1) (Szymanski 2005). Recently, we reported that the tomato *hl* mutant has abnormally distorted and swollen trichomes very similar to trichomes of *Arabidopsis dis* mutants, and that *Hl* gene encodes the tomato homolog of SRA1, a subunit of WAVE complex (Kang et al. 2016). Here, we provide further genetic evidence that a subunit of ARP2/3 complex is essential for normal trichome development in tomato.

Our map-based cloning study positioned *Ini* in a region spanning markers T1460 and C2_At5g60660 on chromosome 11. Based on the similar trichome phenotype of the *hl* mutant and the *dis* mutants of *Arabidopsis*, we hypothesized that *Ini* may encode a component of the ARP2/3 or WAVE complex. This approach

identified the tomato ortholog of *ARPC2A* as a strong candidate gene for *Ini*. Our results demonstrate that the *ini* mutant harbors a major polymorphism in *ARPC2A* and that this indel is associated with lack of detectable expression of normal *ARPC2A* transcripts in the mutant. The crystal structure of bovine ARP2/3 complex revealed that the C-terminal domain of ARPC2 interacts with ARPC4 (Robinson et al. 2001). El-Assal et al. demonstrated that ARPC2 physically interacts with ARPC4 in *Arabidopsis* (2004). We speculate that disrupted interaction between *ARPC2A* and ARPC4 in the *ini* mutant may impair actin filament nucleation to give rise the aberrant trichome morphology. To validate this hypothesis, We performed functional complementation of the *ini* mutant with the normal *ARPC2A* gene. These indicate that the defects in trichome morphology in the *ini* mutant result from a defective *ARPC2A* gene.

In addition to tomato and *Arabidopsis*, several studies have demonstrated that genes encoding subunits of the ARP2/3 and WAVE complexes in other plants are also important for epidermal cell morphogenesis. For example, the rice mutants *less pronounced lobe epidermal cell (lpl)2* and *lpl3* that encode SRA1 and NAP1, respectively, show an epidermal cell defect (Zhou et al. 2016). The *arpc1* mutant in *Lotus japonicas* and the *required for infection thread (rit)* mutant encoding NAP1 in *Medicago truncatula* also show a trichome distortion phenotype (Hossain et al. 2012; Miyahara et al. 2010). A soybean *gnarled* trichome mutant is impaired in *NAP1* gene (Campbell et al. 2016). These collective observations imply that the function of ARP2/3-WAVE complex in epidermal cell development is evolutionarily conserved in higher plant species. In contrast, trichome initiation in *Arabidopsis* and tomato may

be regulated by distinct transcriptional networks (Serna and Martin 2006). For example, overexpression of Arabidopsis *GL1*-encoding MYB protein in tobacco does not induce trichome formation (Payne et al. 1999). Likewise, overexpression in Arabidopsis of the tomato *Woolly* gene, which encodes a HD-ZIP protein, also does not alter trichome initiation (Yang et al. 2011). Identification of additional tomato mutants that are defective in trichome initiation promises to provide a better understanding of genetic regulatory networks underlying multicellular trichome development.

Several previous studies indicate that the function of ARP2/3 and WAVE complexes is not limited to trichome development. For example, Arabidopsis ARP2, ARP3, and ARPC2 play an important role in stomatal movement by reorganizing actin filaments and vacuoles in guard cells (Jiang et al. 2012; Li et al. 2013). Arabidopsis ARP2 regulates mitochondrial-dependent calcium signaling in response to salt stress (Zhao et al. 2013). Arabidopsis NAP1 acts as a regulator of autophagy in response to nitrogen starvation and salt stress (Wang et al. 2016). The Arabidopsis ARP2/3 and WAVE complexes also modulate light-induced root elongation by controlling the expression of longitudinal F-actin through photoreceptors and the 26S proteasome (Dyachok et al. 2011). In rice, the *early senescence1* mutant encoding SCAR-LIKE PROTEIN2 exhibits higher stomatal density, resulting in excessive water loss (Rao et al. 2015). The tomato *Hl* gene encoding SRA1 is involved in the production of terpenoids and flavonoids (Kang et al. 2016). In addition, ARP2/3-WAVE complex is also required for rhizobial infection of root hair in leguminous plants such as *Lotus japonicas* and *Medicago truncatula* (Hossain et al. 2012; Gavrin et al. 2015; Miyahara

et al. 2010; Qiu et al. 2015).

To identify more genes related the trichome structure in tomatoes, we selected four new trichome mutant lines (*dis1*, *dis6*, *dis10*, *dis13*) with highly distorted and bent trichomes compared to wild-type plants. The trichome phenotype of these four mutants is very similar to that of the *ini* and *hl* mutants. Previously, it was reported that *Hl* gene encodes a subunit (SRA1) of the highly conserved WAVE regulatory complex that controls nucleation of actin filaments (Kang et al. 2010a). Here, we demonstrated that *Ini* is *ARPC2A* gene encoding a protein involved in nucleating the polymerization of actin filaments. In addition, Arabidopsis distorted mutants were also defective in genes involved in the polymerization of actin filaments (Basu et al. 2004; Basu et al. 2005). Therefore, we predict that the new distorted mutants in tomato may encode a component of the ARP2/3 or WAVE complex. To prove this hypothesis, a map-based cloning and candidate gene approaches should be employed to identify genes causing the distorted trichome phenotype in the mutants. Finally, these new distorted mutants will help to define the role of WAVE-ARP2/3 complex and provide better understanding of genetic regulatory networks in development of trichome structure in tomato.

REFERENCES

- Basu D, El-Assal SED, Le J, Mallery EL, Szymanski DB** (2004) Interchangeable functions of *Arabidopsis* PIROGI and the human WAVE complex subunit SRA1 during leaf epidermal development. *Development* **131**:4345-4355
- Basu D, Le J, El-Essal SED, Huang S, Zhang CH, Mallery EL, Koliantz G, Staiger CJ, Szymanski DB** (2005) DISTORTED3/SCAR2 is a putative Arabidopsis WAVE complex subunit that activates the Arp2/3 complex and is required for epidermal morphogenesis. *Plant Cell* **17**:502-524
- Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A, Kant MR, Prins M, de Vos M, Haring MA, Schuurink RC** (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. *P Natl Acad Sci* **109**:20124-20129
- Campbell BW, Hofstad AN, Sreekanta S, Fu F, Kono TJY, O'Rourke JA, Vance CP, Muehlbauer GJ, Stupar RM** (2016) Fast neutron-induced structural rearrangements at a soybean *NAP1* locus result in *gnarled* trichomes. *Theor Appl Genet* **129**:1725-1738
- Chang J, Yu T, Gao SH, Xiong C, Xie QM, Li HX, Ye ZB, Yang CX** (2016) Fine mapping of the *dialytic* gene that controls multicellular trichome formation and stamen development in tomato. *Theor Appl Genet* **129**:1531-1539
- Deeks MJ, Kaloriti D, Davies B, Malho R, Hussey PJ** (2004) *Arabidopsis* NAP1

is essential for Arp2/3-dependent trichome morphogenesis. *Curr Biol* **14**:1410-1414

Dempsey WH, Sherif THI (1987) Genetic and morphological comparisons of 7 "hairless" mutants in the tomato. *Rep Tomato Genet Coop* **37**:43-44

Dyachok J, Zhu L, Liao FQ, He J, Huq E, Blancaflor EB (2011) SCAR mediates light-induced root elongation in *Arabidopsis* through photoreceptors and proteasomes. *Plant Cell* **23**:3610-3626

Ehleringer JR, Mooney HA (1978) Leaf hairs: Effects on physiological activity and adaptive value to a desert shrub. *Oecologia* **37**:183-200

El-Assal SE, Le J, Basu D, Mallery EL, Szymanski DB (2004) DISTORTED2 encodes an ARPC2 subunit of the putative *Arabidopsis* ARP2/3 complex. *Plant J* **38**:526-538

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* **141**:1147-1162

Gan YB, Kumimoto R, Liu C, Ratcliffe O, Yu H, Broun P (2006) GLABROUS INFLORESCENCE STEMS modulates the regulation by gibberellins of epidermal differentiation and shoot maturation in *Arabidopsis*. *Plant Cell* **18**:1383-1395

Gavrin A, Jansen V, Ivanov S, Bisseling T, Fedorova E (2015) ARP2/3-mediated actin nucleation associated with symbiosome membrane is essential for the development of symbiosomes in infected cells of *Medicago truncatula* root nodules. *Mol Plant Microbe In* **28**:605-614

- Goffreda JC, Steffens JC, Mutschler MA** (1990) Association of epicuticular sugars with aphid resistance in hybrids with wild Tomato. *J Am Soc Hortic Sci* **115**:161-165
- Grebe M** (2012) The patterning of epidermal hairs in *Arabidopsis* - updated. *Curr Opin Plant Biol* **15**:31-37
- Hossain MS, Liao JQ, James EK, Sato S, Tabata S, Jurkiewicz A, Madsen LH, Stougaard J, Ross L, Szczylowski K** (2012) *Lotus japonicus* *ARPC1* is required for rhizobial infection. *Plant Physiol* **160**:917-928
- Jiang K, Sorefan K, Deeks MJ, Bevan M, Hussey PJ, Hetherington AM** (2012) The ARP2/3 Complex mediates guard cell actin reorganization and stomatal movement in *Arabidopsis*. *Plant Cell* **24**:2031-2040
- Juvik JA, Shapiro JA, Young TE, Mutschler MA** (1994) Acylglucoses from wild tomatoes alter behavior and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera, Noctuidae). *J Econ Entomol* **87**:482-492
- Kang JH, Shi F, Jones AD, Marks MD, Howe GA** (2010a) Distortion of trichome morphology by the *hairless* mutation of tomato affects leaf surface chemistry. *J Exp Bot* **61**:1053-1064
- Kang JH, Campos ML, Zemelis-Durfee S, Al-Haddad JM, Jones AD, Telewski FW, Brandizzi F, Howe GA** (2016) Molecular cloning of the tomato *Hairless* gene implicates actin dynamics in trichome-mediated defense and mechanical properties of stem tissue. *J Exp Bot* **67**:5313-5324

Kang JH, Liu GH, Shi F, Jones AD, Beaudry RM, Howe GA (2010b) The tomato *odorless-2* mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. *Plant Physiol* **154**:262-272

Kang JH, McRoberts J, Shi F, Moreno JE, Jones AD, Howe GA (2014) The flavonoid biosynthetic enzyme chalcone isomerase modulates terpenoid production in glandular trichomes of tomato. *Plant Physiol* **164**:1161-1174

Karabourniotis G, Papadopoulos K, Papamarkou M, Manetas Y (1992) Ultraviolet-B radiation absorbing capacity of leaf hairs. *Physiologia Plantarum* **86**:414-418

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. *Annu Rev Entomol* **48**:51-72

Le J, Mallery EL, Zhang CH, Brankle S, Szymanski DB (2006) *Arabidopsis* BRICK1/HSPC300 is an essential WAVE-complex subunit that selectively stabilizes the Arp2/3 activator SCAR2. *Curr Biol* **16**:895-901

Lee DK, Park SH, Seong SY, Kim YS, Jung H, Do Choi Y, Kim JK (2016) Production of insect-resistant transgenic rice plants for use in practical agriculture. *Plant Biotechnol Rep* **10**:391-401

Li LJ, Ren F, Gao XQ, Wei PC, Wang XC (2013) The reorganization of actin filaments is required for vacuolar fusion of guard cells during stomatal opening in *Arabidopsis*. *Plant Cell Environ* **36**:484-497

Luckwill LC (1943) *The genus Lycopersicon: a historical, biological and taxonomic survey of the wild and cultivated tomatoes*. UK: University of Aberdeen

Miyahara A, Richens J, Starker C, Morieri G, Smith L, Long S, Downie JA, Oldroyd GED (2010) Conservation in function of a SCAR/WAVE component during infection thread and root hair growth in *Medicago truncatula*. *Mol Plant Microbe In* **23**:1553-1562

Oppenheimer DG, Pollock MA, Vacik J, Szymanski DB, Ericson B, Feldmann K, Marks MD (1997) Essential role of a kinesin-like protein in *Arabidopsis* trichome morphogenesis. *P Natl Acad Sci* **94**:6261-6266

Payne T, Clement J, Arnold D, Lloyd A (1999) Heterologous myb genes distinct from *GL1* enhance trichome production when overexpressed in *Nicotiana tabacum*. *Development* **126**:671-682

Qian P, Hou S, Guo G (2009) Molecular mechanisms controlling pavement cell shape in *Arabidopsis* leaves. *Plant Cell Rep* **28**:1147-1157

Qiu LP, Lin JS, Xu J, Sato S, Parniske M, Wang TL, Downie JA, Xie F (2015) SCARN a novel class of SCAR protein that is required for root-hair infection during legume nodulation. *PLoS Genet* **11**:

Ramsay NA, Glover BJ (2005) MYB-bHLH-WD40 protein complex and the evolution of cellular diversity. *Trends Plant Sci* **10**:63-70

Reeves AF (1977) Tomato trichomes and mutations affecting their development. *Am J Bot* **64**:186-189

Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S, Pollard TD (2001) Crystal structure of Arp2/3 complex. *Science* **294**:1679-1684

Rodriguez AE, Tingey WM, Mutschler MA (1993) Acylsugars of *Lycopersicon pennellii* deter settling and feeding of the green peach Aphid (Homoptera, Aphididae). *J Econ Entomol* **86**:34-39

Sallaud C, Rontein D, Onillon S, Jabes F, Duffe P, Giacalone C, Thoraval S, Escoffier C, Herbette G, Leonhardt N, Causse M, Tissier A (2009) A novel pathway for sesquiterpene biosynthesis from Z,Z-Farnesyl Pyrophosphate in the wild tomato *Solanum habrochaites*. *Plant Cell* **21**:301-317

Schilmiller AL, Charbonneau AL, Last RL (2012) Identification of a BAHD acetyltransferase that produces protective acyl sugars in tomato trichomes. *P Natl Acad Sci* **109**:16377-16382

Schilmiller AL, Schauvinhold I, Larson M, Xu R, Charbonneau AL, Schmidt A, Wilkerson C, Last RL, Pichersky E (2009) Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. *P Natl Acad Sci* **106**:10865-10870

Serna L, Martin C (2006) Trichomes: different regulatory networks lead to convergent structures. *Trends Plant Sci* **11**:274-280

Shepherd RW, Bass WT, Houtz RL, Wagner GJ (2005) Phylloplanins of tobacco are defensive proteins deployed on aerial surfaces by short glandular trichomes. *Plant Cell* **17**:1851-1861

- Stubbe H** (1964) Mutanten der kulturtomate *Lycopersicon esculentum* Miller VI. *Kulturpflanze* **12**:121-152
- Szymanski DB** (2005) Breaking the WAVE complex: the point of *Arabidopsis* trichomes. *Curr Opin Plant Biol* **8**:103-112
- Szymanski DB, Jilk RA, Pollock SM, Marks MD** (1998) Control of *GL2* expression in *Arabidopsis* leaves and trichomes. *Development* **125**:1161-1171
- Tanksley SD, Ganai MW, Prince JP, Devicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND** (1992) High density molecular linkage maps of the tomato and potato genomes. *Genetics* **132**:1141-1160
- Thole V, Alves SC, Worland B, Bevan MW, Vain P** (2009) A protocol for efficiently retrieving and characterizing flanking sequence tags (FSTs) in *Brachypodium distachyon* T-DNA insertional mutants. *Nat Protoc* **4**:650-661
- TomatoGenomeConsortium** (2012) The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **485**:635-641
- Uhrig JF, Mutondo M, Zimmermann I, Deeks MJ, Machesky LM, Thomas P, Uhrig S, Rambke C, Hussey PJ, Huelskamp M** (2007) The role of *Arabidopsis* *SCAR* genes in ARP2-ARP3-dependent cell morphogenesis. *Development* **134**:967-977
- Walker JD, Oppenheimer DG, Concienne J, Larkin JC** (2000) *SIAMESE*, a gene

controlling the endoreduplication cell cycle in *Arabidopsis thaliana* trichomes. *Development* **127**:3931-3940

Wang PW, Richardson C, Hawes C, Hussey PJ (2016) *Arabidopsis* NAP1 regulates the formation of autophagosomes. *Curr Biol* **26**:2060-2069

Williams WG, Kennedy GG, Yamamoto RT, Thacker JD, Bordner J (1980) 2-Tridecanone: A naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum f. Glabratum*. *Science* **207**:888-889

Yan A, Wu MJ, Zhao YQ, Zhang AD, Liu BH, Schiefelbein J, Gan YB (2014) Involvement of C2H2 zinc finger proteins in the regulation of epidermal cell fate determination in *Arabidopsis*. *J Integr Plant Biol* **56**:1112-1117

Yang C, Li H, Zhang J, Luo Z, Gong P, Zhang C, Li J, Wang T, Zhang Y, Lu Ye, Ye Z (2011) A regulatory gene induces trichome formation and embryo lethality in tomato. *P Natl Acad Sci* **108**:11836-11841

Yang C, Ye Z (2013) Trichomes as models for studying plant cell differentiation. *Cell Mol Life Sci* **70**:1937-1948

Zhao M, Morohashi K, Hatlestad G, Grotewold E, Lloyd A (2008) The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. *Development* **135**:1991-1999

Zhao Y, Pan Z, Zhang Y, Qu XL, Zhang YG, Yang YQ, Jiang XN, Huang SJ, Yuan M, Schumaker KS, Guo Y (2013) The Actin-related protein2/3 Complex regulates mitochondrial-associated calcium signaling during salt stress in

Arabidopsis. Plant Cell **25**:4544-4559

Zhou WQ, Wang YC, Wu ZL, Luo L, Liu P, Yan LF, Hou SW (2016) Homologs of SCAR/WAVE complex components are required for epidermal cell morphogenesis in rice. *J Exp Bot* **67**:4311-4323

Zobel RW, Opena RT, Rick CM (1970) Additional linkages of the stubbe series. *Rep Tomato Gen Coop* **20**:69-71

ABSTRACT IN KOREAN

토마토 *inquieta* 돌연변이에서 모상체 발달과 관련된 ARP2/3 복합체의 유전적 연구

정나래

서울대학교 국제농업기술대학원 국제농업기술학과

지도교수 강진호

모상체는 식물체의 표면에 발달된 털 구조이며, 초식 곤충에 대해 물리적, 화학적 방어 역할을 수행한다. 여기에서, 본 연구진은 *inquieta (ini)* 라는 토마토(*Solanum lycopersicum*) 단일 열성 돌연변이의 특징에 대해 설명을 하고자 한다. *ini* 식물의 모든 모상체는 형태학적으로 뚜렷한 결점이 보이며 이는 액틴 세포골격 형성과 관련이 있다. 유전자 지도 분석으로 *Ini* 유전자가 11번 염색체내 1.5cM 영역에 위치하는 것을 발견하였으며, 이 위치에는 액틴 섬유의 핵형성 및 중합에 관여하는 애기장대의 *ARPC2A* 유전자와 상동인 토마토 유전자가 있음을 확인하였다. 135개의 F2 세대를 대상으로 *ARPC2A* 유전자를 표지로 사용하였을 때, 목표 유전자와 *ARPC2A* 표지가 같이 분리되는 것을 확인 하였다. *ARPC2A* 유전자 전체를 야생종 토마토 및 *ini* 돌연변이 토마토로 RT-PCR과 gDNA PCR을 수행하였을 때, *ini* 돌연변이는 증폭이 되지 않았다. Flanking PCR과 Southern blot

분석은 *ini* 돌연변이에 약 6Kb 이상의 염기가 ARPC2A 5번 intron에 삽입된 것을 확인하였다. *ini* 돌연변이에 야생종 *ARPC2A* 유전자를 발현시킨 결과, 정상적인 모상체가 발달되었다. 이러한 결과는 *ini* 돌연변이가 액틴 세포골격 형성에 문제가 있었다는 것을 증명한다.

키워드 : ARP2/3 복합체, *ARPC2A*, *Inquita*, 지도 기반 클로닝, 토마토, 모상체