Theor Appl Genet (2011) 123:625–633
DOI 10.1007/s00122-011-1612-x

ORIGINAL PAPER

Fine-mapping of the *woolly* gene controlling multicellular trichome formation and embryonic development in tomato

Changxian Yang · Hanxia Li · Junhong Zhang · Taotao Wang · Zhibiao Ye

Received: 24 October 2010 / Accepted: 30 April 2011 / Published online: 3 June 2011

Abstract Trichomes are small hairs that originate from the epidermal cells of nearly all land plants, and they exist in unicellular and multicellular forms. The regulatory pathway of unicellular trichomes in *Arabidopsis* is well characterized. However, little is known about the multicellular trichome formation in tomato (*Solanum lycopersicum*). The *woolly* (*Wo*) gene controls multicellular trichome initiation and leads to embryonic lethality when homozygous in tomato. To clone and characterize *Wo*, the gene was fine-mapped to a DNA fragment of ~200 kb using the map-based cloning strategy. A series of sequence-based molecular markers, including simple sequence repeat, sequence characterized amplified region, and cleaved amplified polymorphic sequence were utilized in this study. Analysis of the sequence indicated that this region carries 19 putative open reading frames. These results will provide not only the important information for the isolation and characterization of *Wo* but also the starting point for studying the regulatory pathway responsible for trichome formation and embryonic lethality in tomato.

Communicated by M. Havey.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1612-x) contains supplementary material, which is available to authorized users.

C. Yang · J. Zhang · Z. Ye (✉)
National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
e-mail: zbye@mail.hzau.edu.cn

H. Li · T. Wang · Z. Ye
Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University, Wuhan 430070, China

Introduction

Plant trichomes, which are found in nearly all terrestrial plants, originate from epidermal cells. Trichomes can be divided into two types, unicellular and multicellular (Werker 2000). *Arabidopsis* and cotton (*Gossypium arboretum*) are characterized by their unicellular trichomes, whereas tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana tabacum*) are characterized by multicellular trichomes. Due to the simple cell structure of trichomes, they provide excellent models to study the pattern formation in plants (Schiefelbein 2003). Many important genes involved in the initiation of unicellular trichomes on the epidermis of *Arabidopsis* have been cloned and characterized. The *GLABROUS1* (*GL1*) gene promotes trichome formation, a loss-of-function mutation of which results in a complete absence of trichomes (Marks and Feldmann 1989). This gene encodes a protein ascribed to the R2R3 MYB family featuring two MYB repeats (Oppenheimer et al. 1991). *TRANSPARENT TESTA GLABRA1* (*TTG1*) gene is another key regulator controlling trichome formation in *Arabidopsis* (Koornneef 1981), which encodes a protein containing four conserved WD repeats (Walker et al. 1999). Genetic studies indicate that *GL1* and *TTG1* regulate the same process in trichome initiation (Larkin et al. 1999). Overexpression of *GL3* results in the formation of additional leaf trichomes. *GL3* encodes a transcription factor of the bHLH family (Payne et al. 2000). In addition, a bHLH-like protein encoded by the *R* regulator of maize can also activate leaf trichome formation when overexpressed (Lloyd et al. 1992). Another bHLH gene, *ENHANCER OF GLABRA 3* (*EGL3*) functions in a redundant manner with *GL3* to specify trichome cell fate in *Arabidopsis* (Bernhardt et al. 2003). A WD-bHLH-MYB complex consisting of these four genes triggers trichome formation in *Arabidopsis* by

enhancing the expression of two downstream genes, *GL2* and *EGL2* (Zhao et al. 2008). Cotton fibres, resembling *Arabidopsis* trichomes, are controlled by *GaMYB2*, which shows high sequence homology with *GL1* and can restore trichome formation in a *gl1* mutant (Wang et al. 2004). A common complex possibly regulates trichome formation in *Arabidopsis* and cotton, both of which are Rosids (Serna and Martin 2006).

Whether there is a complex similar to WD-bHLH-MYB controlling trichome formation in tomato, snapdragon (*Antirrhinum majus*), and tobacco (*N. tabacum*) is still unknown, three of which are Asterids (Serna and Martin 2006). Overexpression of *GL1* in tobacco does not affect trichome formation (Payne et al. 1999). *MIXTA*, a MYB-related regulator controls conical cell formation in snapdragon, overexpression of which can trigger the trichome formation (Glover et al. 1998). However, the ectopic expression of *MIXTA* in *Arabidopsis gl1-1* mutants failed to induce trichome initiation (Payne et al. 1999). Hence, the trichomes in snapdragon, tobacco, and tomato may develop through a transcriptional network different from that of *Arabidopsis* and cotton. The *woolly* gene (*Wo*), a spontaneous mutation in tomato, is responsible for trichome formation (Shilling 1959) and embryo lethality of tomato (Huang and Paddock 1962). Plenty of trichomes on the epidermal parts enable them to be easily distinguished from non-woolly plants (Rick and Butler 1956; Shilling 1959; Huang and Paddock 1962). The self-pollinated progenies of woolly plants always exhibit two phenotypes, the woolly and the non-woolly. However, the offspring of non-woolly plants do not segregate. The *Wo* mutation is inferred to be lethal for the embryo when homozygous (Shilling 1959); the lethal action occurs prior to the torpedo stage and no cambial differentiation is found at this period through cytological observation (Huang and Paddock 1962).

Reportedly, *Wo* is located near the midpoint of the long arm of chromosome 2 (Rick and Butler 1956) and a classical map on pachytene also demonstrated that *Wo* is located near the mid-region (Khush and Rick 1968). However, no nucleotide information regarding the *Wo* gene is known. Therefore, fine mapping and molecular cloning of *Wo* would significantly enhance our understanding of the mechanism of multicellular trichome formation and embryo lethality when homozygous at this locus in tomato, as well as the relationship of the regulatory pathway controlling trichome initiation between the tomato and tobacco, snapdragon, *Arabidopsis*, and cotton.

In this study, we developed three types of molecular markers: simple sequence repeat (SSR), sequence characterized amplified region (SCAR), and cleaved amplified polymorphic sequence (CAPS), based on the bacterial artificial chromosome (BAC) and RFLP probe sequences. We constructed a high-resolution molecular map of *Wo* and delimited it in an ~200 kb DNA fragment. Sequence analysis indicated that this region contains 19 putative open-reading frames (ORFs). These results set a good foundation for further isolation and characterization of *Wo*.

Materials and methods

Plant materials, mapping populations and phenotyping trichomes

Two introgression lines (ILs), IL2-3 and IL2-5, which contain the middle fragments of chromosome 2 of *Solanum pennellii* (Syn. *Lycopersicon pennellii*) LA716 genome in *S. lycopersicon* cv. M82 background (Eshed and Zamir 1994), and LA3186, a spontaneous woolly mutant (derived from Ailsa Craig, as the "wild-type" for sequence comparisons) (Fig. 1), were provided by the Tomato Genetics Resource Center. Due to embryo lethality when homozygous at *Wo* locus, the mutation is maintained at the heterozygous state. F_2 populations segregated for the woolly phenotype were obtained by crossing IL2-3 and IL2-5 with LA3186 (removed non-woolly F_1 plants and self-pollinated). A total of 106 F_2 recessive non-woolly individuals from the cross of IL2-5 × LA3186 were used for rough mapping analysis. Then, equal amounts of DNA from 6 F_2 recessive individuals (total = 1,035) from the cross of IL2-3 × LA3186 were pooled to construct non-woolly bulk and analysed with the newly developed markers for fine mapping. Young plants (woolly and nonwoolly) were monitored for the presence of trichomes on the surface of leaf and stem by visual observation.

Isolation of genomic DNA and PCR for marker development

Plant DNA was extracted from 3-week-old seedling leaves for PCR-based genotyping using the method described by Fulton et al. (1995). Three kinds of molecular markers were utilized in the mapping of *Wo*, namely SSR, SCAR, and CAPS. The PCR for the SSR markers were performed in 20 μL containing approximately 50 ng of genomic DNA as template, 20 pm/μL of each primer, 1 U of *Taq* DNA polymerase (Invitrogen, USA), and 2 μL of buffer solution (10×). The reaction program consisted of one cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 40 s, 55°C for 40 s, and 72°C for 30 s. The PCR products were separated on a 6% polyacrylamide gel in 1× TBE buffer. After electrophoresis, the gel was stained with 4 g/L silver nitrate.

The SCAR and CAPS markers from BAC and RFLP probe sequences were designed using Primer 3 (http://frodo.wi.mit.edu/primer3/). The PCR mixture contained 1 μL of DNA, 2 μL of 10× PCR buffer, 0.4 μL of 10 mM

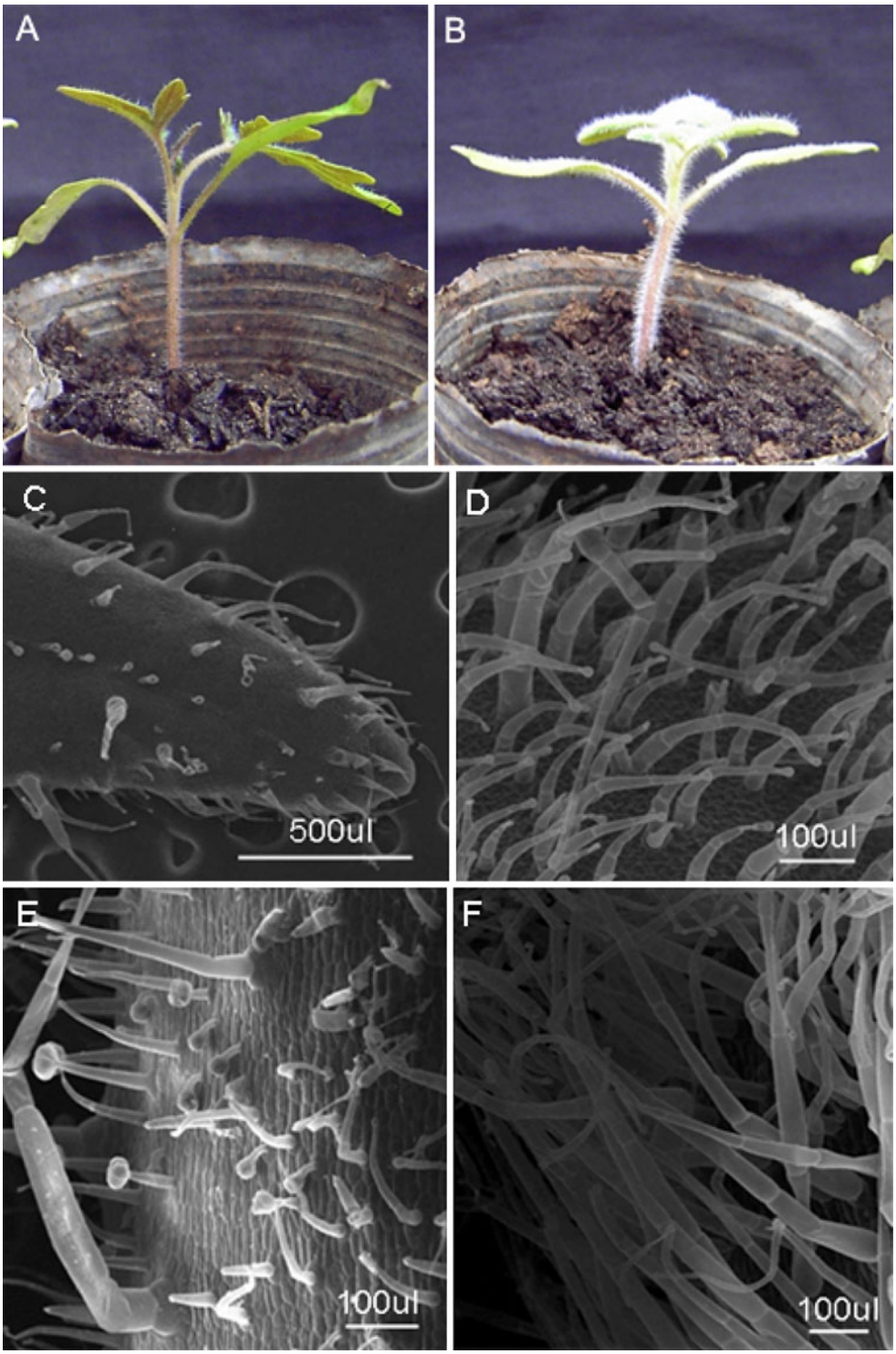

Fig. 1 Trichome phenotypes of non-woolly plant (**a**, **c** and **e**) and woolly mutant LA3186 (**b**, **d** and **f**). Photographs **a** and **b** are magnified images. **c**–**f** Were taken by a scanning electron microscope (**c** and **d**, trichomes on leaves; **e** and **f**, trichomes on stems)

dNTPs, 0.4 μL of 2.5 U/μL *Taq* polymerase (Invitrogen, USA), 0.5 μL of 20 pm/μL of each primer, and 15.7 μL of distilled water. The program used for the PCR machine (Bio-Rad, USA) was as follows: 1 cycle of 4 min at 94°C; 35 cycles of 40 s at 94°C, 40 s at 57°C, 2.5 min at 72°C, and, finally, 10 min at 72°C. The products were analysed with a 1.5% (w/v) agarose gel. The PCR products, which exhibited good polymorphisms between the parents, were

directly used for genetic mapping, and those that showed no polymorphisms were digested with endonucleases with recognition sites in the marker sequences in order to identify the polymorphisms for further mapping.

Genetic mapping

For all the polymorphic markers between IL2-5 and LA3186, an F_2 recessive population of 106 individuals was initially used for mapping *Wo* and a rough map was constructed. Based on this map, new markers were developed according to the BAC and RFLP probe sequences between the two closely linked markers flanking *Wo*. These markers were subsequently analysed for fine mapping in a larger population of 1,035 F_2 recessive non-woolly individuals from the cross IL2-3 × LA3186. Data from the genotype survey of each individual were adopted for linkage analysis by the MAPMAKER/EXP 3.0 program (Lander et al. 1987; Lincoln et al. 1992). The Kosambi function was used to transform the recombinant frequencies into genetic distance (centiMorgan, cM). Map order was estimated based on the maximum likelihood estimates.

Construction of a BAC contig and gene prediction

BAC sequences deposited in the SOL genomics network (SGN), in which the most related markers with *Wo* were located, were aligned with each other using the BLAST software from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The physical map around the *Wo* locus was constructed if completely identical overlapping ends were present between the two BACs. Protein coding genes were predicted from the resultant contig using the FGENESH program (http://linux1.softberry.com/berry.phtml). The predicted proteins were further analysed by GENESCAN (http://genes.mit.edu).

Results

Segregation of the woolly phenotype and trichome phenotype analyses

Progenies of the woolly mutant LA3186 presented two phenotypes (95 woolly and 46 non-woolly plants) at the segregation of a 2:1 Mendelian ratio ($\chi^2 = 0.01$, $P > 0.05$). Nevertheless, the non-woolly plants did not show phenotype segregation. The F_1 plants from the crosses IL2-3/IL2-5 × LA3186 presented two phenotypes. We scored 65 woolly plants versus 61 non-woolly plants from the cross IL2-3 × LA3186, and 53 woolly plants versus 47 non-woolly plants from IL2-5 × LA3186, both of which corresponded at a 1:1 Mendelian ratio ($\chi^2 = 0.07$, $P > 0.05$; $\chi^2 = 0.25$, $P > 0.05$). Previous studies showed that there are five types (I, III, V, VI and VII) of trichomes on the epidermis of cultivated tomato plants (Luckwill 1943). LA3186 was found to have much higher density of type I trichomes on leaves and stems than non-woolly plants (Fig. 1). However, no obvious alteration of other types of trichomes was observed.

Generation of PCR-based markers

Although *Wo* is located near the mid-region of the long arm of chromosome 2 (Rick and Butler 1956), we do not know the interval in which *Wo* is delimited. Twelve markers, evenly distributed across the mid-region of the long arm of chromosome 2, were applied to screen polymorphism between the two parents IL2-5 and LA3186, and eight of them showed stable polymorphism. The primer sequences of U237440, C2_At5g64670, HBa44O16SP6, and SSR287 were downloaded from the SGN (http://www.sgn.cornell.edu). STS45 was designed according to the published sequence of C02HBa0008G02. W124 and SSRD69 were kindly provided by Dr. Wang. For fine-mapping, we developed several new markers based on the published BAC and RFLP probe sequences. Five markers exhibited stable polymorphism between IL2-3 and LA3186. WY42, STS64, STS64, and STS62 were designed based on the sequences of C02HBa0323A14, C02HBa0006L05, C02HBa0204D01, and C02HBa0175O20, respectively. STS4 was converted from an RFLP marker-TG494 probe sequence. Detailed information regarding these markers is listed in Table 1.

Rough mapping of Wo

To determine the genetic interval of *Wo*, 8 markers were used to screen 106 F_2 non-woolly individuals from the cross IL2-5 × LA3186. Recombination events between these markers and *Wo* were statistically analysed. The linkage analysis indicated that all these markers span a genetic region of 12.1 cM (Fig. 2). Among these flanking markers of *Wo*, W124 and C2_At5g64670 were the most closely linked. The SSR markers SSRD69 and W124 were on one side, approximately 5.3 and 3.9 cM from the *Wo* gene, respectively (Fig. 2). On the other side, the SSR marker SSR287, the SCAR marker C2_At5g64670, and the CAPS markers U237440, HBa44O16SP6, and STS45 were at a distance of 5.8, 1.9, 2.9, 3.9, and 4.4 cM from the *Wo* gene, respectively (Fig. 2). Simultaneously, we observed that the relative position between U237440, HBa44O16SP6, and C2_At5g64670 had been inverted by comparing the local map of *Wo* with the published EXPEN 2000 map (http://www.sgn.cornell.edu/) (Fig. 2). The genetic distance between SSR287 and C2_At5g64670 in the local map (4.9 cM) was much shorter than in the published EXPEN 2000 map (41.0 cM) (Fig. 2).

Table 1 Primer sequences and PCR parameters for SSR, SCAR, and CAPS markers

Marker type	Marker name	Primer sequence (5′–3′)	PCR product length (bp)	Annealing temp. (°C)	No. of cycles
SSR	SSRD69	F ATAGTTTATCTGCAATGTATTTAGTTC			
		R TGGAAATTTCACGGACTGGT	204	55	33
SSR	W124	F ATGTATTGTGAAGAAGAGCAGTTTG			
		R GATAACTGCGATTCAATAGGTGG	194	55	33
SSR	SSR287	F GCATCCCAAACAATCCAATC			
		R TCCACTTTCAAGATCAGAGCAA	168	55	33
SSR	WY42	F GGTTTCGCCAGCATAAAATG			
		R CAACAAGAGTCCCAAGCAAA	234	55	33
SCAR	C2_At5g64670	F TGATAAATGCTGGGAAGATTGACTC			
		R ATCAACCTGGCTCCATCTTCTATTTG	200,220[a]	57	35
SCAR	STS33	F GCATCGGAGCTTGCTAAAAG			
		R ACTTTGGTGGAGGCAAAATG	~1800–2450[a]	58	35
SCAR	STS64	F TTACGGGTGTAATCGCACAA			
		R AGGGAGCAGCATGGTTAAAA	~2,500	58	35
CAPS	U237440[b]	F TCCACACCTCCACCAATTTT			
		R AACCAAGTTGGACGCACTTC	218 + 209 + 106,614[a]	57	35
CAPS	HBa44O16SP6[c]	F CTTGTTGGCAATGCAAGAGA			
		R AAGGCCGTGAATCATTGAAC	265 + 204,469[a]	57	35
CAPS	STS45[d]	F GCAGGGTGAAAAATCTGGAA			
		R AAATCAACGCTTTGCTGCTT	~650–450[a]	58	35
CAPS	STS4[e]	F ATTTGAGGCCGGTTTAGCTT			
		R TGCCTGCAGTTCCCTTTCTA	~1,200–1,500[a]	58	35
CAPS	STS62[f]	F TTTGACTGGGCAAGAACCTT			
		R TTGGGACTTTCCAGTTGAGG	~2,400–1,000 + 1400[a]		

[a] Product length on IL2-3 or IL2-5 and LA3186, respectively

[b] Polymorphic after digestion with *Rsa*I

[c] Polymorphic after digestion with *Map*I

[d] Polymorphic after digestion with *Dra*I

[e] Polymorphic after digestion with *Dra*I

[f] Polymorphic after digestion with *Ava*II

According to the bin-mapping of these markers in IL2-3 and IL2-5 (Fig. 2), SSRD69 and W124 were located in the introgression region of IL2-3, and U237440, HBa44O16SP6, SSR287, and STS45 were located in the introgression region of IL2-5 (Fig. 2). C2_At5g64670 was located in the overlapping region of these two lines (Fig. 2).

Fine mapping of Wo

According to the result of rough mapping, *Wo* has been delimited to an interval of 5.8 cM flanked by W124 and C2_At5g64670. This region was located in the introgression segment of IL2-3. In order to narrow the interval spanning the target locus, we carried out fine mapping based on 1,035 F_2 progenies derived from the cross IL2-3 × LA3186 using the newly exploited markers between W124 and C2_At5g64670 according to the published BAC sequences and RFLP probe sequences. Fifteen individuals displayed recombination between *Wo* and WY42, and 12 individuals between *Wo* and STS33 (Fig. 3). STS64, STS4, and STS62 were further analysed with these recombinants. We delimited *Wo* in the region between markers STS64 and STS62, which were both closely linked to *Wo* with just one recombination event identified (Fig. 3). One marker STS4 showed cosegregated with *Wo* (Fig. 3).

BAC contig construction spanning the Wo region

As the cosegregating marker STS4 was located in the interval between STS64 and STS62, we did BAC-blast search in SGN using the probe sequence of TG494, which has been successfully converted into the CAPS marker STS4. Fortunately, a BAC clone (C02HBa0204D01) displaying 100% identity with this probe sequence was found. Therefore, we

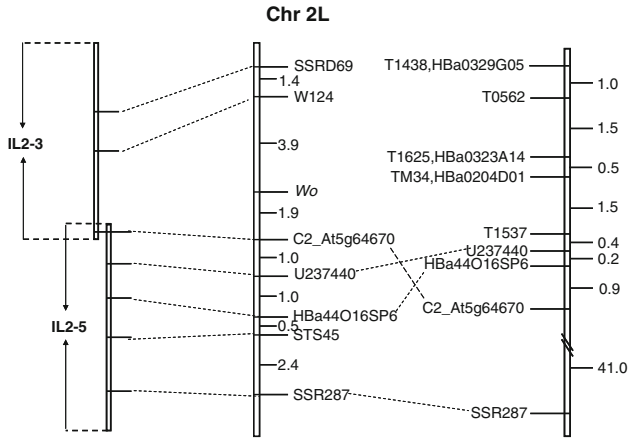

Fig. 2 Bin-mapping of molecular markers in the introgression fragments of IL2-3 and IL2-5 (*left*); genetic linkage map spanning the *Wo* locus derived from the cross IL2-5 × LA3186 (*mid*); the tomato EXPEN 2000 molecular linkage map of the long arm of chromosome 2 (*right*) published in the Solanaceae Genomics Network (http://www.sgn.cornell.edu/). *Dotted lines* indicate the common markers during these three maps. Genetic distances are shown in centimorgans (cM)

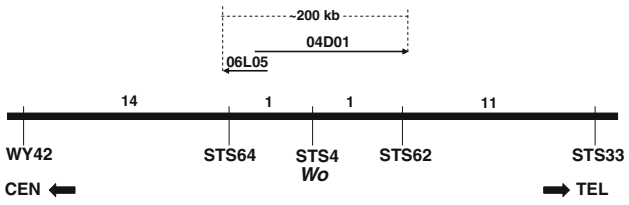

Fig. 3 Tomato BAC contig encompassing the *Wo* gene. *S. lycopersicum* BAC contig encompassing the *Wo* region. The *thick horizontal line* represents the region of chromosome 2 encompassing the *Wo* gene in *S. lycopersicum*. The centromere is to the *left* and the telomer to the *right*, as indicated by the *arrows*. *Markers* below the *thick line* are developed from BAC sequences (except for STS4, which is from probe sequence of RFLP marker TG494). The *numbers* above the line represent the recombinants from the F2 population (derived from the cross IL2-3 × LA3186) in each interval. The *thin arrow lines* represent the contig spanning the *Wo* gene with the direction indicated by *arrow lines*, which consists of C02HBa0006L05 (06L05, 35,580 bp) and C02HBa0204D01 (04D01, 179,860 bp) (with a 15,328 bp identical overlapping end)

concluded that STS4 is located in C02HBa0204D01. Simultaneously, as the flanking markers STS64 and STS62 were designed based on the sequences of C02HBa0006L05 and C02HBa0204D01 separately, we tried to align these two BAC sequences. These two BAC clones formed a ~200 kb contig with ends overlapping by 15,328 bp (Fig. 3). We confirmed the location of *Wo* in this approximately 200 kb contig (Fig. 3).

Candidate genes of Wo

The BAC clones used for contig construction were not from Ailsa Craig (cultivar, background material of the woolly mutant LA3186). However, all the markers that were designed according to the BAC sequences derived from the cultivar Heinz 1706 were used in the physical map, indicating that the sequence content does not differ greatly in these two genetic backgrounds. Therefore, *Wo* (recessive allele) must be found in this region. Analysis of the ~200 kb sequence revealed that this fragment contains 19 ORFs as automatically predicted by FGENESH (http://softberry.com) and GENESCAN (http://genes.mit.edu) (Table 2). The best hits of these ORFs include copia LTR rider (*Solanum lycopersicum*), COBRA-LIKE PROTEIN 10 PRECURSOR (COBL10) (*Arabidopsis thaliana*), PROTODERMAL FACTOR 2 (PDF2) (*A. thaliana*), VPS28-2 (*A. thaliana*), beta-glucosidase (*Oryza sativa*), Cytochrome P450 (*Populus trichocarpa*), tobamovirus multiplication 1 homolog 3 (*S. lycopersicum*), abnormal spindle-like protein (*O. sativa*), RNA Binding Protein 45 (*N. plumbaginifolia*), MEDIATOR 21 (MED21) (*A. thaliana*), ATP BINDING CASSETTE PROTEIN 1 (ATABC1) (*A. thaliana*), putative heat shock protein (*A. thaliana*), multi-antimicrobial extrusion family protein (*N. tabacum*), MATE efflux family protein (*O. sativa*), hypothetical proteins. We designed 19 primer pairs for full length amplification of these ORFs to test for polymorphism between the woolly mutant LA3186 and its segregated non-woolly plants (Supplementary Table 1). No amplification polymorphisms were found in these PCR products generated by the 19 primer pairs. This result demonstrated that the woolly phenotype may be conferred by a single nucleotide polymorphism or small fragment mutation. Therefore, all PCR products from the woolly and non-woolly plants were sequenced and compared. Sequence alignment showed that one putative homeodomain gene (JF518780) has 41% amino acid sequence identity to GL2, a trichome related gene regulated by the WD-bHLH-MYB complex (Zhao et al. 2008), and 73% to PDF2, a shoot epidermal cell differentiation related gene in *Arabidopsis* (Abe et al. 2003). Both of these genes contain a homeobox and a bZIP motif. And the putative homeodomain gene of these 19 ORFs may be a good candidate for *Wo*. We expect to find the gene from the woolly mutant that has two allelic sequences. Subsequent studies, including sequence analysis and functional verification, are under way.

Discussion

Transcriptional regulatory network controlling the initiation of unicellular trichome in *Arabidopsis* and unicellular fibre in cotton has been well characterized. However, the mechanism of multicellular trichome formation in the Solanaceous family, which is different from that in *Arabidopsis* and cotton, remains to be elucidated (Serna and Martin

Table 2 Summary of gene prediction for the contig C02HBa0006L05 and C02HBa0204D01

ORF No.	Accesion No.	BAC gene ID	Start	End	Best hits in GenBank (accession/species)	*E* value
ORF1	JF518777	06L05.1	5990	11206	Copia LTR rider (ABO36622/*Solanum lycopersicum*)	0.00E+00
ORF2	JF518778	06L05.2	11648	12884	COBL10 (NP_188694 *Arabidopsis thaliana*)	3.00E−113
ORF3	JF518779	06L05.3	13836	17332	Hypothetical protein (XP_002305604/*Populus trichocarpa*)	2E−19
ORF4	JF518780	0204D01.4	4357	7758	PROTODERMAL FACTOR 2 (NP_567274/*Arabidopsis thaliana*)	0.00E+00
ORF5	JF518781	0204D01.5	15839	18406	VPS28-2; transporter (NP_567281/*Arabidopsis thaliana*)	9E−106
ORF6	JF518782	0204D01.6	25721	39520	Beta-glucosidase (NP_001053302/*Oryza sativa*)	4.00E−160
ORF7	JF518783	0204D01.7	40410	43654	Hypothetical protein (NP_001060531/*Oryza sativa*)	0.00E+00
ORF8	JF518784	0204D01.8	49605	53967	Cytochrome P450 (XP_002305592/*Populus trichocarpa*)	0.00E+00
ORF9	JF518785	0204D01.9	63089	74650	Hypothetical protein (CAN68639/*Vitis vinifera*)	0.00E+00
ORF10	JF518786	0204D01.10	79122	85647	Tobamovirus multiplication 1 homolog 3 (BAE43840/*Solanum lycopersicum*)	6.00E−144
ORF11	JF518787	0204D01.11	99544	110543	Abnormal spindle-like protein (BAC56022/*Oryza sativa*)	3.00E−133
ORF12	JF518788	0204D01.12	111506	112033	Hypothetical protein (XP_002271195/*Vitis vinifera*)	1.00E−50
ORF13	JF518789	0204D01.13	115806	121310	RNA Binding protein 45 (CAC01237/*Nicotiana plumbaginifolia*)	3.00E−126
ORF14	JF518790	0204D01.14	122072	125002	MED21 (NP_192387/*Arabidopsis thaliana*)	3.00E−38
ORF15	JF518791	0204D01.15	125548	129990	ATABC1 (NP_192386/*Arabidopsis thaliana*)	0.00E+00
ORF16	JF518792	0204D01.16	145409	152364	Putative heat shock protein (NP_172631/*Arabidopsis thaliana*)	0.00E+00
ORF17	JF518793	0204D01.17	154058	156512	Hypothetical protein (XP_002317001/*Populus trichocarpa*)	4.00E−76
ORF18	JF518794	0204D01.18	157542	160222	Multi antimicrobial extrusion family protein (BAF47752/*Nicotiana tabacum*)	0.00E+00
ORF19	JF518795	0204D01.19	165679	169171	MATE efflux family protein (ABF97209/*Oryza sativa*)	0.00E+00

2006). *Wo* triggers multicellular trichome formation in woolly mutant, which is an excellent basis for studying the molecular events underlying multicellular trichome formation and further defining the relationship between *Wo* and homozygous embryo lethality. *Wo* was reportedly located near the mid-region of chromosome 2 (Khush and Rick 1968). By comparing the classic map (Khush and Rick 1968) with the published Tomato/*pannellii* IL map, we inferred that *Wo* is in the introgression fragments of IL2-3 and IL2-5. Thus, our mapping populations were constructed by crossing these two ILs with woolly mutant LA3186. Abundant genome data can be downloaded from the SGN FTP server (ftp://ftp.sgn.cornell.edu) as the International Tomato Genome Sequencing Project is ongoing. The data contain BAC end sequences and full BAC sequences, which facilitate molecular marker development, mapping, isolation, and characterization of new genes. Several important genes in tomato had been isolated by map based cloning (Chen et al. 2007; Cong et al. 2008; Xiao et al. 2008). Using a normal mapping strategy, *Wo* was finally delimited in the interval between STS64 and STS62, and cosegregating with STS4. Sequence analysis showed that this interval includes 19 putative ORFs. None of these proteins belong to the well-known gene families related to trichome formation, such as MYB, bHLH, and WD. One putative homeodomain gene of these 19 ORFs showed much higher sequence similarity to PDF2 than GL2, the former of which participates in regulating epidermal cell differentiation, but not in trichome formation (Abe et al. 2003). Therefore, we speculated that trichome formation in tomato may be regulated by a novel pathway distinct from that of *Arabidopsis* and cotton, even snapdragon and tobacco. Future functional studies of the predicted genes will provide us direct evidence on the role of *Wo* in tomato trichome formation, and the relationship with the key genes in *Arabidopsis*, cotton, snapdragon, and tobacco. In addition, regarding its lethal action on the *WoWo* embryos, prospective studies will uncover many new genes participating in embryonic development.

Plant trichomes play an important role in protecting plants from damage caused by herbivores and pathogens (Ashraf et al. 1999; Kang et al. 2010). Many researchers have demonstrated a close relationship between trichome and resistance against whiteflies, aphids, and viruses in many species, such as potato (Gregory et al. 1986), poplar (Philippe and Bohlmann 2007), and tomato (Dimock and

Kennedy 1983; Channarayappa et al. 1992). Several genes controlling trichome initiation in some of these plants have been mapped and cloned, such as *PtaMYB186* in poplar (Plett et al. 2010) and trichome locus 1 (*Ptl1*) (Kim et al. 2010) in pepper. Trichome functions as the first line of defence by two mechanisms, through spatial hindrance and secreting toxins (Duffey 1986; Wagner 1991). The mechanism of non-glandular trichome defence against insect attack belongs to the former and glandular to the latter. Previous studies showed that these two trichome types are distributed on the epidermis of tomato plants (Luckwill 1943). This character proves that tomato can protect itself by both spatial hindrance and secreting toxins. Moreover, the ability of woolly resistance also remains unexplained. Our study will provide an efficient approach for resistance breeding using the woolly phenotype conferred by *Wo*.

A comparison between the rough map of *Wo* with the corresponding region of the tomato EXPEN 2000 molecular linkage map reveals considerable disparity. The tomato EXPEN 2000 molecular linkage map places U237440 and HBa44O16SP6 up from C2_At5g64670. However, the relative position in the rough map of *Wo* had been inverted. Paracentric inversion between tomato and potato has been reported by Tanksley et al. (1992). Giovannoni also observed this phenomenon in the same introgression fragment (private communication). We inferred that this position inversion may occur during the process of introgression. Comparing with the published EXPEN 2000 map, we observed that the recombination frequency between SSR287 and C2_At5g64670 is much lower in the local map. Canady et al. (2006) found that recombination rate positively correlates with the length of *S. lycopersicoides* introgression fragment in *Solanum. esculentum*. Suppression of recombination is also observed in the *Solanum. pannellii* introgression segment of chromosome 7 in *Solanum. lycopersicum* with respect to the whole chromosome (Lim et al. 2008). Therefore, the lower recombination frequency in the local map of *Wo* was obtained using the F_2 population from the cross of IL2-5 × LA3186 than the published EXPEN 2000 map based on the F_2 individuals from the cross *S. lycopersicum* × *S. pennellii*. It suggests that construction of mapping population for map-based cloning using the ILs containing large introgression segments is more efficient than those containing short introgression segments.

Acknowledgments The authors are grateful to Dr. G. P. Wang from the South China Agricultural University and Dr. H. H. Kuang from our College for the helpful advice. The authors acknowledge the Tomato Genetics Resource Center, University of California, USA, for providing the seed stocks of the introgression lines and the woolly mutant LA3186. This project was supported by grants from the 973 project (No. 2011CB100600), the National Natural Science Foundation of China (Nos. 30971997 and 30921002) and the Research Fund for the Doctoral Program of Higher Education of China (4010-081023).

References

Abe M, Katsumata H, Komeda Y, Takahashi T (2003) Regulation of shoot epidermal cell differentiation by a pair of homeodomain proteins in *Arabidopsis*. Development 130:635–643

Ashraf M, Zafar ZU, McNeilly T, Veltkamp CJ (1999) Some morpho-anatomical characteristics of cotton (*Gossypium hirsutum* L.) in relation to resistance to cotton leaf curl virus (CLCuV). Angew Bot 73:76–82

Bernhardt C, Lee MM, Gonzalez A, Zhang F, Lloyd A, Schiefelbein J (2003) The bHLH genes *GLABRA3* (*GL3*) and *ENHANCER OF GLABRA3* (*EGL3*) specify epidermal cell fate in the *Arabidopsis* root. Development 130:6431–6439

Canady MA, Ji YF, Chetelat RT (2006) Homeologous recombination in *Solanum lycopersicoides* introgression lines of cultivated tomato. Genetics 174:1775–1788

Channarayappa SG, Muniyappa V, Frist RH (1992) Resistance of *Lycopersicon* species to *Bemisia tabaci*, a tomato leaf curl virus vector. Can J Bot 70:2184–2192

Chen KY, Cong B, Wing R, Vrebalow J, Tanksley SD (2007) Changes in regulation of a transcription factor lead to autogamy in cultivated tomatoes. Science 318:643–645

Cong B, Barrero LS, Tanksley SD (2008) Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. Nat Genet 40:800–804

Dimock MB, Kennedy GG (1983) The role of glandular trichomes in the resistance of *Lycopersicon hirsutum* f. *glabratum* to *Heliothis zea*. Entomol Exp Appl 33:263–268

Duffey SS (1986) Plant glandular trichomes: their partial role in defence against insects. In: Juniper BE, Southwood TRE (eds) Insects and the plant surface. Edward Arnold, London, pp 151–172

Eshed Y, Zamir D (1994) A genomic library of *Lycopersicon pennellii* in *L. esculentum*: a tool for fine mapping of genes. Euphytica 79:175–179

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13:207–209

Glover BJ, Perez-Rodriguez M, Martin C (1998) Development of several epidermal cell types can be specified by the same MYB-related plant transcriptional factor. Development 125:3497–3508

Gregory P, Tingey WM, Ave DA, Bouthyette PY (1986) Potato glandular trichomes: a physicochemical defense mechanism against insects. Nat Resist Plants Pests 13:160–167

Huang PC, Paddock EF (1962) The time and site of the semidominant lethal action of 'Wo' in *Lycopersicon esculentum*. Amer J Bot 49:388–393

Kang JH, Shi F, Jones AD, Marks MD, Howe GA (2010) Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. J Exp Bot 61:1053–1064

Khush GS, Rick CM (1968) Cytogenetic analysis of the tomato genome by means of induced deficiencies. Chromosoma 23:452–484

Kim HJ, Han JH, Kwon JK, Park M, Kim BD, Choi D (2010) Fine mapping of pepper trichome locus 1 controlling trichome formation in *Capsicum annuum* L. CM334. Theor Appl Genet 120: 1099–1106

Koornneef M (1981) The complex syndrome of *ttg* mutants. Arabidopsis Inf Serv 18:45–51

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Larkin JC, Walker JD, Bolognesi-Winfield AC, Gray JC, Walker AR (1999) Allele-specific interactions between *ttg* and *gl1* during trichome development in *Arabidopsis thaliana*. Genetics 151:1591–1604

Lim GTT, Wang GP, Hemming MN, McGrath DJ, Jones DA (2008) High resolution genetic and physical mapping of the I-3 region of tomato chromosome 7 reveals almost continuous microsynteny with grape chromosome 12 but interspersed microsynteny with duplications on *Arabidopsis* chromosomes 1, 2 and 3. Theor Appl Genet 118:57–75

Lincoln S, Daly M, Lander E (1992) Constructing genetic linkage maps with Mapmaker/exp 3.0: a tutorial and reference manual, 3rd edn. Whitehead Institute Technical Report, Cambridge

Lloyd AM, Walbot V, Davis RW (1992) *Arabidopsis* and *Nicotiana* anthocyanin production activated by maize regulators *R* and *C1*. Science 258:1773–1775

Luckwill LC (1943) The genus *Lycopersicon*: a historical, biological and taxonomic survey of the wild and cultivated tomatoes. Aberd Univ Stud 120:44

Marks MD, Feldmann KA (1989) Trichome development in *Arabidopsis thaliana* I T-DNA tagging of the *GLABROUS1* gene. Plant Cell 1:1043–1050

Oppenheimer DG, Herman PL, Sivakumaran S, Esch J, Marks MD (1991) A myb gene required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. Cell 67:483–493

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

Payne CT, Zhang F, Lloyd AM (2000) *GL3* Encodes a bHLH protein that regulates trichome development in *Arabidopsis* through interaction with GL1 and TTG1. Genetics 156:1349–1362

Philippe RN, Bohlmann J (2007) Poplar defense against insect herbivores. Can J Bot 85:1111–1126

Plett JM, Wilkins O, Campbell MM, Ralph SG, Regan S (2010) Endogenous over-expression of *Populus MYB186* increases trichome density, improves insect pest resistance, and impacts plant growth. Plant J 64(3):419–432

Rick CM, Butler L (1956) Cytogenetics of the tomato. Adv Genet 8:267–382

Schiefelbein J (2003) Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot. Curr Opin Plant Biol 6:74–78

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

Shilling PR (1959) An investigation of the hereditary character, woolly in the tomato. Ohio J Sci 59:289–302

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovanonni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder M, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Wagner GJ (1991) Secreting glandular trichomes: more than just hairs. Plant Physiol 96:675–679

Walker AR, Davisona PA, Bolognesi-Winfielda AC, Jamesa CM, Srinivasanb N, Blundellb TL, Eschc JJ, Marksc MD, Graya JC (1999) The *TRANSPARENT TESTA GLABRA1* locus, which regulates trichome differentiation and anthocyanin biosynthesis in *Arabidopsis*, encodes a WD40 repeat protein. Plant Cell 11:1337–1349

Wang S, Wang JW, Yu N, Li CH, Luo B, Gou JY, Wang LJ, Chen XY (2004) Control of plant trichome development by a cotton fiber MYB gene. Plant Cell 16:2323–2334

Werker E (2000) Trichome diversity and development. Adv Bot Res 31:1–35

Xiao H, Jiang N, Schaffner E, Stockinger EJ, Knaap E (2008) A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Science 319:1527–1530

Zhao MZ, 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