Fine mapping of a major locus representing the lack of prickles in eggplant revealed the availability of a 0.5-kb insertion/deletion for marker-assisted selection

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As prickles cause labour inefficiency during cultivation and scratches on the skin of fruits during transport, they are considered undesirable traits of eggplant (Solanum melongena L.). Because the molecular basis of prickle emergence has not been entirely revealed in plants, we mapped an eggplant semi-dominant Prickle (Pl) gene locus, which causes the absence of prickles, on chromosome 6 of a linkage map of the F2 population derived from crossing the no-prickly cultivar ‘Togenashi-senryo-nigo’ and the prickly line LS1934. By performing synteny mapping with tomato, the genomic region corresponding to the eggplant Pl locus was identified. Through bacterial artificial chromosome (BAC) screening, positive BAC clones and the contig sequence that harbour the Pl locus in the prickly eggplant genome were revealed. The BAC contig length was 133 kb, and it contained 16 predicted genes. Among them, a characteristic 0.5-kb insertion/deletion was detected. As the 0.5-kb insertion was commonly identified with the prickly phenotype worldwide, a primer pair that amplifies the insertion/deletion could be used for marker-assisted selection of the no-prickly phenotype. Such findings contribute to map-based-cloning of the Pl gene and the understanding of gene function, ultimately providing new insights into the regulatory molecular mechanisms underlying prickle emergence in plants.

Key Words: prickle, eggplant, fine mapping, marker-assisted selection.

Introduction

Eggplant (Solanum melongena L.) belongs to the Solanaceae family and is the third most important food crop in the family, following potato and tomato. Today, the total production of eggplant has reached 52 million tons (FAO 2017; http://faostat.fao.org) and its cultivation is widely performed in Asia, the Middle East, and Mediterranean countries (Daunay 2008). In the present decade, eggplant has grown in importance and its increased rate of production has surpassed that of the top two produced species (FAO 2017; http://faostat.fao.org).

In addition to the increased demand for eggplant as a food source, it has been recognised as a target for molecular genetics research. As a result, an exponential progress in molecular genetics study with eggplant has begun with the development of random amplified polymorphic markers (Nunome et al. 2001). Continuous efforts have also been dedicated to accumulating its molecular genetic information (Barchi et al. 2010, 2011, 2012, Daunay 2008, Fukuoka et al. 2010, 2012, Nunome et al. 2001, 2003a, 2003b, 2009, Stągęł et al. 2008, Wu et al. 2009). Today, whole-genome sequencing and the construction of a draft genome dataset have been achieved (Barchi et al. 2019, Hirakawa et al. 2014). With the accumulation of genetic information, molecular genetic studies that elucidate useful characteristics of eggplant have rapidly increased. These studies focused on disease resistance (Barchi et al. 2018, Lebeau et al. 2012, Miyatake et al. 2015, Mutlu et al. 2008, Salgon et al. 2017), nutritional properties (Gramazio et al. 2014, 2016, Toppino et al. 2016), parthenocarpy (Miyatake
et al. 2015), and morphological traits, such as fruit shape, colour, or emergence of prickles (Barchi et al. 2012, 2019, Cericola et al. 2014, Frary et al. 2014, Portis et al. 2014, 2015). As many of these characteristics are common targets in solanaceous model species, such as tomato, the results of in-depth studies with model species could be used to better understand the genetics. However, one of the morphological traits, “prickly phenotye”, is unique to eggplant and is thus difficult to accurately estimate on a molecular basis. Such characteristic could therefore contribute to eggplant’s utility as a useful model for research on prickle development.

Several mapping reports (Doganlar et al. 2002, Frary et al. 2014, Gramazio et al. 2014, Portis et al. 2015, Yoshida et al. 1999, Zhang et al. 2006) on eggplant, rose, and citrus fruits can directly reveal an aspect of the molecular basis of prickle emergence, however, these maps are insufficient for delineating the responsible genes. A series of studies on a similar protruding organ, trichome, might therefore enable the discovery of the regulatory network of eggplant prickles. In the early era of trichome studies, the unicellular trichome in Arabidopsis was thoroughly investigated. Many studies have revealed that the regulatory pathway in the development of Arabidopsis trichome is comprised of positive and negative transcription factors. The transcriptional activation of GLABRA2 (GL2) (Rerie et al. 1994) by a molecular complex formed from GLABROUS1 (GL1) (Oppenheimer et al. 1991), TRANSPARENT TESTA GLABRA1 (TTG1) (Walker et al. 1999), and GLABRA3/ENHANCER OF GLABRA3 (GL3/EGL3) (Payne et al. 2000, Zhang and Oppenheimer 2004), which encode the homeodomain-leucine zipper (HD-ZIP) proteins, play a key role as positive factors in the initiation of Arabidopsis trichome. Another group of genes, including TRIPTYCHON (TRY), CAPRICE (CPC), ENHANCE OF TRY AND CPC1, 2 and 3 (ETC1, ETC2 and ETC3), CPC-LIKE MYB3 (CPL3), TRICHOMELESS1 (TCL1), and TRICHOMELESS2 (TCL2) (Kirik et al. 2004a, 2004b, Schellmann et al. 2002, Wada et al. 1997), which encodes the R3 MYB factors, acts as negative regulators by competing with GL1 and GL3/EGL3. Based on prior studies on trichome in Arabidopsis, intensive research has recently been conducted in multicellular trichome. In addition, many genetic and molecular biological studies on cucumber prickles have been reported. These studies revealed that Cucumis sativus Glabra 1 (CsGL1) and Micro-trichome (Mict), which encode a class I HD-ZIP protein, control the development of cucumber trichome (Liu et al. 2016, Zhao et al. 2015), and the TRICHOME-LESS/Cucumis sativus Glabra 3 (TRIL/CsGL3) gene, which encodes a class IV HD-ZIP protein, acts as a positive regulator of trichome development (Cui et al. 2016) and is epistatic to CsGL1/Mict (Wang et al. 2015). Another important member of the trichome pathway is Cucumis sativus TRANSPARENT TESTA GLABRA1 (CsTTG1), which directly interacts with Mict and controls the density and initiation of trichome (Chen et al. 2017). Similar to cucumber trichome, which is also found in tomato, trichome is a multicellular structure of epidermal origin. The development of trichome in tomato and cucumber share a similar mechanism (Yang et al. 2011).

Based on DNA marker resources, eggplant BAC libraries, and a microsynteny with the tomato genome sequence, we sought to report the fine mapping of a Prickle (Pl) gene locus of the Japanese commercial eggplant cultivar, ‘Togenashi-senryo-nigo’ (Takii & Co., Ltd., Kyoto, Japan). Thereafter, we used the obtained genomic sequence, including the Pl gene, for candidate gene prediction and to derive the putative molecular mechanism of prickle development in eggplant. Based on breeding, a unique polymorphism located in the Pl locus, which could be easily detected by electrophoresis on an agarose gel, was also presented.

Materials and Methods

Plant materials for genetic mapping

The F2 mapping population, T2LF2, was employed for primary mapping. The T2LF2 population was derived via a cross between an F1 commercial eggplant cultivar, ‘Togenashi-senryo-nigo’ (P1), developed in a private company (Takii & Co., Ltd.), and the line, LS1934 (P2), collected in Malaysia (Sakata et al. 1996). The maternal parent, ‘Togenashi-senryo-nigo’, does not have prickles on organs such as the calyxes, leaves, and stems. Conversely, the paternal parent, LS1934, has hard prickles while the F1 hybrid (‘Togenashi-senryo-nigo’ × LS1934) has weak prickles on the above organs (Fig. 1). The F1 hybrid was crossed with both parents to develop the backcross populations, BC1P1 and BC1P2. Moreover, for further detailed mapping, the selected F2 and F3 progenies that displayed genetic recombination near the responsible locus were self-crossed to develop the F3 (T2LF3) and F4 (T2LF4) populations.

Plant growth conditions and prickle strength measurement

The eggplant materials, ‘Togenashi-senryo-nigo’ (P1), LS1934 (P2), F1 (P1 × P2), T2LF2 (n = 93), BC1P1 (n = 35), and BC1P2 (n = 35) were tested in a genetic analysis for the emergence of prickles. Seeds were sown in October of 2008. The tests were conducted in a warm greenhouse at a minimum air temperature of 15°C. Approximately one month after sowing, seedlings were transplanted into 12-cm-diameter plastic pots. After two months, the presence of prickles was visually confirmed on calyxes and leaves. The prickle strength of each plant was scored on a scale of 0 to 2: 0 = no prickles; 1 = weak prickles (half size of hard prickles scored as “2”); and 2 = hard prickles (Fig. 1). The evaluation tests were performed three times. Each F2 progeny was replicated by grafting with the rootstock cultivar, ‘Daitaro’ (Monma et al. 1997). The advanced mapping
The prickly phenotype on leaf veins (upper) and calyces (lower) of LS1934 (a1, a2), F1 plant (b1, b2) and ‘Togenashi-senryo-nigo’ (c1, c2), respectively. White bars mean scales of 10 mm.

**Fig. 1.** The prickly phenotype on leaf veins (upper) and calyces (lower) of LS1934 (a1, a2), F1 plant (b1, b2) and ‘Togenashi-senryo-nigo’ (c1, c2), respectively. White bars mean scales of 10 mm.

populations, T2LF3 and T2LF4, were tested on April 2012 and December 2012, respectively.

**Primary mapping of the Pl locus**

For the first step of genetic analysis, primary mapping was performed with already published genomic simple sequence repeat (SSR) and expressed sequence tag-single nucleotide polymorphism (EST-SNP) markers (Fukuoka et al. 2012, Hirakawa et al. 2014, Nunome et al. 2009). The polymorphic markers were applied to 93 F2 progenies of the T2LF2 population. Methods for applying the DNA markers and map construction were previously described (Miyatake et al. 2012, 2015).

**Discovery of microsynteny with tomato genome for secondary mapping of the Pl locus**

Based on the synteny between eggplant and tomato genome, we developed novel markers flanking to the Pl locus, mainly using the reported method (Fukuoka et al. 2012). First, the EST-SNP markers close to the Pl locus on the eggplant LWAE2012 map (Hirakawa et al. 2014) were mapped according to the reciprocal-best-hit homology search (Fukuoka et al. 2012) on tomato genome (SL3.0) using the BLASTN program. The corresponding tomato sequence was then extracted. Thereafter, a BLASTN search was performed using the eggplant 16k unigene set (Fukuoka et al. 2012) as a query against the extracted tomato sequence. The eggplant query sequences displaying a homology with the extracted tomato sequence were subjected to alignment using the T-COFFEE program (Notredame et al. 2000) to predict intron positions. The polymerase chain reaction (PCR) primers listed in Supplemental Table 1 were designed to amplify intron-containing genomic sequences using the Primer3 software (Rozen and Skaletsky 2000), which was denoted as the SmTgn1-series. SNPs and Indels were screened via direct sequencing of the amplified genomic DNA fragments using a BigDye v3 sequencing premix and a 3730xl DNA sequencer (Life Technologies Corporation, Carlsbad, CA, USA). Sequence data were processed using the phred/phrap/cross_match package (Ewing et al. 1998). The obtained SNP markers were tested to derive the T2LF2 population using either the modified Tm-shift PCR method (Fukuoka et al. 2008) or direct sequencing with the same primer sets used for SNP discovery. Secondary mapping of the Pl locus was conducted with the method used for primary mapping.

**BAC library construction, PCR screening, and BAC end sequencing**

Two BAC libraries were developed in this study. The prickly eggplant line, AE-P03 (Miyatake et al. 2012), and the no-prickly eggplant cultivar, ‘Togenashi-senryo-nigo’, were used. Partial genomic DNA was cloned into a pCC1BAC vector at a BamHI site in AE-P03 and pIndigo BAC5 vector at a HindIII site in ‘Togennashi-senryo-nigo’. The BAC library consists of 40,320 (AE-P03) and 57,943 (‘Togennashi-senryo-nigo’) clones, with an average insert size of 115 kb (AE-P03) and 140 kb (‘Togennashi-senryo-nigo’), thereby representing approximately four (AE-P03) and eight (‘Togennashi-senryo-nigo’) genomic equivalents of the S. melongena genome estimated to be 1,127 Mb in size (Barchi et al. 2011, Hirakawa et al. 2014). For PCR screening of the AE-P03 BAC library, three dimensional pools (plate, row, column) were developed GenoTechs (Tsukuba, Ibaraki, Japan), however, only a plate pool was developed for the ‘Togennashi-senryo-nigo’ BAC library. PCR screening was carried out using Blend Taq plus polymerase (TOYOBO Co., Ltd., Osaka, Japan). Plasmid isolation was carried out using a QIAGEN Large Construct kit (QIAGEN, Valencia, California, USA). BAC end sequencing was conducted with the primers, pIndigo BAC5 Forward Sequencing Primer (5'-GGAGTGTCATGGCAGAG) and pIndigo BAC5 Reverse Sequencing Primer (5'-CTCGCTAGATGTGGAAATGGTGGTGACG-3') (Epicentre, Madison, WI, USA), using a 3730xl DNA sequencer (Life Technologies Corporation).

**Size selection of BAC DNA and shot-gun sequencing**

BAC clones derived from the AE-P03 genome were sequenced by shot-gun sequencing. The BAC DNA was sonicated using a Bioruptor XL water bath sonicator (Diagenode). The size-selected DNA (2 kb-4 kb) was gel-purified using a QIAquick Gel Extraction Kit (QIAGEN). Thereafter, the products were cloned into the pUC118 vector. The ligated DNA was electropropored into the Escherichia coli strain, DH10B, and the plasmid DNA was extracted by the alkaline lysis method (Birnboim and Doly 1979). Escherichia coli was inoculated in 100 μl of LB containing 50 μg/ml ampicillin. Escherichia coli cells were incubated for 18 h with continuous shaking at 37°C before harvesting. Cell pellets were resuspended in 30 μl of solution I (50 mM Tris-HCl, 10 mM EDTA, pH 8.0, 50 mg/l
RNase A) and the subsequent lysis and neutralisation were achieved by respectively adding 30 μl of solution II (0.2 M NaOH, 1% SDS) and 30 μl of solution III (0.627 M potassium acetate, pH 4.6 and 4 M Guanidinium chloride, pH 5.5). After centrifugation for 15 minutes at 4,800 rpm, the supernatant was transferred to the clearing filter, UNIFILTER (Whatman, Brentford, UK), stacked on the DNA BINDING UNIFILTER (Whatman) and the receiver, UNIPLATE (Whatman). Thereafter, the DNA BINDING UNIFILTER was washed with solution III and the plates were centrifuged for 15 minutes at 4,800 rpm. The same washing procedure was repeated twice with 80 μl of wash solution (50 mM Tris-HCl, pH 8.0, 0.2 M NaCl, 60% EtOH) and 80 μl of 80% EtOH. After washing, the DNA absorbed on the DNA BINDING UNIFILTER was eluted in 15 μl of 1 mM Tris-HCl (pH 8.0). Plasmid DNA sequencing was then conducted with the primers, fw-dtSeq (5’-GTA AAA CGA CGG CCA GTG CC-3’) and rv-dtSeq (5’-GCG GAT AAC AAT TTC ACA CAG G-3’), using a 3730xl DNA sequencer (Life Technologies Corporation). The accumulated sequences were assembled using phred/phrap/cross_match package (Ewing et al. 1998).

**Results**

**Absence of prickles is controlled by a single semi-dominant gene locus**

Hard prickles on the calyxes, leaves, and stems were clearly detected in the paternal parent of T2LF2, LS1934 (P2) (scored as “2”). Conversely, no prickles were found in the maternal parent, ‘Togenashi-senryo-nigo’ (P1) (scored as “0”). F1 plants had weak prickles, which could be detected via prudent inspection (Fig. 1) (scored as “1”). The segregation ratio in the F2 (n = 93) and BC1/P1/P2 (n = 35 each) populations displayed a good fit at ratios of 1:2:1 and 1:1 (Table 1), thereby confirming that the no-prickly phenotype is controlled by a single semi-dominant gene locus, denoted as Pl. The biological replicated experiments and the repeated experiments for a three-season period could reveal the same prickle scores in the control plants (both parents and F1 plants). Overall, prickles were most clearly detected on calyxes (Fig. 1).

**Primary mapping of the Pl locus**

Among the 1,745 DNA markers reported by Hirakawa et al. (2014), a total of 116 polymorphic markers between ‘Togenashi-senryo-nigo’ and LS1934 were screened to construct a linkage map of T2LF2. By using the linkage map, the Pl locus was mapped on the edge of chromosome 6 and was flanked by markers, SOL6046 and emd16C09, at a genetic distance of 7.3 cM and 1.1 cM, respectively (Fig. 2a). The eggplant LWAE2012 map (Hirakawa et al. 2014) revealed that SOL6046 and emd16C09 were mapped on the genetic positions of 99.8 cM and 102.9 cM, respectively.

### Table 1. Genetic analysis for prickle emergence

| Populations          | Number of individuals for each prickle strength | Expected ratio | χ² |
|----------------------|-----------------------------------------------|----------------|----|
|                      | no prickles | weak prickles | hard prickles | 1:0:0 | 0:1:0 | 1:2:1 | 1:1:0 | 0:1:1 | 0:2:1 | 0:3:1 |
| P1 (“Togenashi senryo nigo”) | 15 | 0 | 0 | 1:0:0 | – | – | – | – | – |
| P2 (LS1934)          | 0 | 0 | 15 | 0:0:1 | – | – | – | – | – |
| F1 (P1 x P2)         | 0 | 15 | 0 | 0:1:0 | – | – | – | – | – |
| F2 (P1 x P2)         | 26 | 48 | 19 | 1:2:1 | 1.15* | – | – | – | – |
| BC1P1                | 16 | 19 | 0 | 1:1:0 | 0.26* | – | – | – | – |
| BC1P2                | 0 | 17 | 18 | 0:1:1 | 0.03* | – | – | – | – |

* Significant at 5% probability.
Microsynteny with tomato genome and secondary mapping of the Pl locus

Sixteen EST-SNP markers (est_rbw02m04, SOL6100, SOL6046, SOL8209, gg5559_714, gg1893_307, gg8669_1065, gg8669_1237, gg5785_166, gg17144_396, gg13092_328, gg12487_1848, SOL8437, gg9544_252, SOL1019, SOL5205) were identified on the eggplant LWAE2012 map (Hirakawa et al. 2014) around the Pl locus. Excluding 2 markers (SOL8209 and SOL8437), all source EST sequences for the 14 markers displayed a reciprocal-best-hit homology to the SL3.0 tomato genome ranging from 45,754,569 bp to 48,252,211 bp on chromosome 6 (Supplemental Table 2). The 14 eggplant markers on the LWAE2012 map were essentially arranged in the same order as the tomato genome sequences, thus, the microsynteny could be detected between eggplant and tomato (Supplemental Table 2). A BLASTN search was performed with 16 k eggplant unigenes as a query against the extracted tomato genome sequence. As a result, 486 unigenes were screened (data not shown). Based on the reported method (Fukuoka et al. 2012), intron positions on the extracted eggplant unigenes were predicted and PCR primers were designed to amplify the intron-containing genomic sequences (SmTgn1_series; Supplemental Table 1). By comparing the intron-containing sequences of LS1934 and ‘Togenashi-senryo-nigo’, 6 novel SNP markers were developed (SmTgn1_03, SmTgn1_14, SmTgn1_19, SmTgn1_22, SmTgn1_29, SmTgn1_33) and used for secondary mapping with the T2LF2 population (n = 93). As a result, the Pl locus was mapped on the site flanked by the markers, SmTgn1_19/SmTgn1_22 and SmTgn1_33/SmTgn1_29, at a genetic distance of 2.2 cM and 1.1 cM, respectively. The marker, SmTgn1_29, was demonstrated to be co-segregated with the prickly phenotype (Fig. 2b).
To obtain the eggplant genomic sequence of the Pl locus, BAC clone screening with the AE-P03 library was performed with 5 SmTgn1_series primer pairs (SmTgn1_25, SmTgn1_26, SmTgn1_28, SmTgn1_29, SmTgn1_30), including SmTgn1_29 which was co-segregated with the prickly phenotype (Supplemental Table 1). As a result, 3 BAC clones (35L02, 13E22, 50N20) were screened and sequenced by shot-gun sequencing (Fig. 2c). Thereafter, the accumulated sequences were assembled into a single contig, including the above 5 BAC screening primer sites (Fig. 2c). The contig sequence length was 264,330 bp (hereinafter referred to as “pl_contig_264330”; Fig. 2c). For fine mapping of the Pl locus, novel markers and mapping population were prepared. First, PCR primer pairs for direct sequencing were designed at an appropriate interval on the “pl_contig_264330” sequence and eight novel SNP markers (SmTgn2_31k, SmTgn2_113k, SmTgn2_133k, SmTgn2_155k, SmTgn2_163k, SmTgn2_190k, SmTgn2_253kA, SmTgn2_253kB) were developed (SmTgn2_series) (Fig. 2c, Supplemental Tables 1, 2). Secondly, among the T2LF2 population (n = 93), 7 individuals displaying a heterozygous genotype at both sides of the Pl locus (SmTgn1_22 and emd16C09) were selected, and their self-pollinated F3 progenies (T2LF3; n = 1918) were genotyped by both neighbouring markers. Thereafter, 11 T2LF3 individuals displayed recombination between the two markers. These individuals were genotyped with SmTgn1_29 and the above eight SmTgn2_series markers. Their prickly phenotype was determined by the self-pollinated F4 progeny test (T2LF4) using the method described above (Fig. 2c). According to the genotype and phenotype of the 11 individuals, the Pl locus was reduced to the interval between the markers, SmTgn2_31k and SmTgn2_163k (hereinafter called “pl_contig_133313”), with 133,313 bp as the interval distance (Fig. 2c).

Gene prediction within the Pl locus sequence
Gene prediction using the “pl_contig_133313” sequence was performed with the Genscan software (http://genes.mit.edu/GENSCAN.html). As a result, 16 genes, namely GS01-GS16, were predicted (Fig. 3, Supplemental Table 3). A homologous gene search was then performed using the BLASTP program and the 16 predicted protein sequences. These genes encoded carbonic anhydrase, nudix family hydrolase, GATA transcription factor, Auxin Response Factor 10B, etc. (Supplemental Table 3). The homologous genes of the tomato ITAG SL3.2 genes and the corresponding eggplant genes reported by Barchi et al. (2019) are listed in Supplemental Table 3.

Finding sequence variants between prickly and no-prickly accessions on the Pl locus
To obtain the ‘Togenashi-senryo-nigo’ genome sequence of the Pl locus, PCR BAC screening was performed with the ‘Togenashi-senryo-nigo’ library using two primer pairs (SmTgn1_31k and SmTgn1_163k) located on both sides of the Pl locus (Fig. 2c). As a result, one BAC clone was screened and sequenced on Illumina MiSeq. Thereafter, the accumulated sequences were mapped to the “pl_contig_133313” sequence of AE-P03 and the sequence variants were listed. A total of 335 SNPs/64 short indels (<50 bp) were detected (data not shown). Among them, 2 SNPs were positioned on the promoters of GS06 and GS16, and 28 SNPs were positioned on exons of GS06, GS07, GS09, GS10, GS11, GS12, GS15, and GS16, respectively. Although no stop codon changes could be identified, the above SNPs could induce amino acid changes (Supplemental Table 3). An insertion (0.5-kb) was also identified on the prickly eggplant genome (Figs. 2c, 3, Supplemental Table 3). The inserted site, which was 1.2-kb upstream of the GS08, was homologous to the tomato Solyc06g075140.3 gene and encoded a GATA transcription factor (Fig. 3, Supplemental Table 3).
A PCR primer pair, denoted as pl_indel_0.5k, was developed to amplify the above 0.5-kb insertion (Supplemental Table 1). By using the primer pair, the amplified fragments could be clearly detected by electrophoresis (Fig. 4). In fact, ‘Togenashi-senryo-nigo’ and LS1934 had fragment sizes of 229 bp and 768 bp, respectively. Supplemental Fig. 1 shows the amplicon sequence obtained with the pl_indel_0.5k primer pair and the 539-bp insertion site in LS1934 compared to ‘Togenashi-senryo-nigo’. The inserted sequence in LS1934 was conserved in the Solanaceae family and there were no features, such as the evidence of genome duplication or transposon transition, which are worthy of mention (data not shown). As both fragments were detected in the F1 plant (Fig. 4), this primer pair could be used as a selective marker that co-segregates with the no-prickly phenotype. Therefore, pl_indel_0.5k was tested with other combinations of the F2 progenies derived from a cross between the no-prickly line, ES384 (reported by Sakata et al. (2008)) and the prickly accession, WEC066 (reported as S10 LS2436 by Saito et al. (2010)). As a result, we successfully confirmed the availability of pl_indel_0.5k as a selective marker at least with one more additional combination (data not shown).

**Development of a selective marker and marker validation with other F2 progenies**

By confirming the versatility of the pl_indel_0.5k primer pair, the World Eggplant Core (WEC) collection (Miyatake et al. 2019) was used for amplification. The collection was constructed with 100 eggplant accessions consisting of 12 no-prickly and 88 prickly accessions. Based on PCR amplification, the 12 no-prickly accessions displayed a band size similar to ‘Togenashi-senryo-nigo’ via electrophoresis (i.e., 0.2-kb genotype). Although there were 79 accessions, most of the above 88 prickly accessions (89.8%) had a band size similar to LS1934 (i.e., 0.8-kb genotype). The remaining 9 accessions were however exceptions and they had a 0.2-kb genotype similar to ‘Togenashi-senryo-nigo’. In conclusion, most of the prickly eggplant accessions in the WEC collection, excluding 9 accessions, could be used as breeding materials in marker assisted selection (MAS) with a set of ‘Togenashi-senryo-nigo’ and the pl_indel_0.5k. The number of each phenotype (no-prickly/prickly) and genotype (0.2-kb/0.8-kb) in the worldwide eggplant collection is shown in Fig. 5 while the tested eggplant accessions, their selective marker genotype with pl_indel_0.5k, and the prickly phenotype are listed in Supplemental Table 4.

**Discussion**

In the present study, the prickly phenotype could be easily determined, and the responsible gene locus for the absence of prickles, Pl, was evidently mapped relative to that presented in previous studies (Doganlar et al. 2002, Frary et al. 2014, Gramazio et al. 2014, Portis et al. 2014, 2015). The major reason for the success of fine mapping was not only due to appropriate cross combination, but also better phenotyping conditions. The prickly phenotype of the eggplant was unstable in the summer, but stable from autumn to spring. Indeed, most of the phenotyping data, which represent 64% of the collection tested worldwide in the winter, differed from that in the summer (data not shown). Every phenotyping test presented herein was performed from autumn to spring.
Among previous mapping studies on the prickly phenotype, Doganlar et al. (2002), Frary et al. (2014), and Gramazio et al. (2014) used interspecific mapping populations for quantitative trait locus (QTL) analyses via crossing between Solanum melongena and Solanum lycopersicum or Solanum incanum. Portis et al. (2015) used 191 Solanum melongena accessions for genome-wide association analysis. In each study, a major gene or QTL was mapped on the same position as the Pl locus on chromosome 6. The findings revealed that the Pl locus plays an important role in many eggplant accessions and its relative species. One of the mapping studies reported by Portis et al. (2014) was however an exception, as the mapping positions were located on chromosomes 7 and 8. This might be due to both parents displaying a certain degree of prickly phenotype. Therefore, the detected QTLs on chromosomes 7 and 8 displayed the genetic locus that controls prickle strength. To derive some supporting data, the same QTLs were mapped in the subsequent report (Portis et al. 2015) by using the genome-wide association mapping strategy with prickle strength phenotyping data.

Our success in fine mapping of the Pl locus could be a result of our ability to find and substantially utilize microsynteny with the tomato genome. Despite published data on the eggplant draft genome by Hirakawa et al. (2014) and although many past studies (Doganlar et al. 2002, Frary et al. 2014, Rinaldi et al. 2016, Wu et al. 2009) have revealed the synteny between the genome of eggplant and tomato, such data are insufficient for obtaining objective eggplant sequences without any data analysis. This is because the complete pseudomolecule has not been fully revealed. The success of the microsynteny mapping strategy used in this study could however indicate the potential for fine mapping other important traits in eggplant. Most recently, Barchi et al. (2019) reported an updated genome sequence of eggplant, which depicts the anchored sequence to each chromosome. This data therefore serve as a fundamental solution for developing novel markers in the detailed mapping of objective traits.

The main purpose of the present study was to detect the characteristic sequences available for MAS of the non-prickly phenotype. As a result, we identified the 0.5-kb deletion within the Pl locus of the ‘Togenashi-senryo-nigo’ genome and its amplification with the primer pair, pl_indel_0.5k (Fig. 3, Supplemental Table 1). Interestingly, in the WEC collection, all 79 worldwide accessions with a 0.5-kb insertion in the same locus displayed the prickly phenotype, conversely, the 12 no-prickly accessions had no insertion, similar to ‘Togenashi-senryo-nigo’ (Supplemental Table 4). Based on this result, a set of ‘Togenashi-senryo-nigo’ with the pl_indel_0.5k could be widely used for marker-assisted selection. For further speculation, the above 91 eggplant accessions (79 prickly and 12 no-prickly accessions) were found to be controlled by the same locus as the Pl in the absence/presence of prickles. An additional linkage analysis with the F2 population, crossing two eggplant lines, ES384 and WEC066, could reveal the contribution of the Pl locus to the presence/absence of prickles, thereby supporting the above speculation. In these days, surveys of eggplant genetic resources were performed in Lao People’s Democratic Republic (Lao PDR), and identified many prickleless germplasms unlike in Japan (Hamato et al. 2018, Saito et al. 2015, 2016, 2017). If the speculation for the commitment of the Pl locus was correct, many of the prickleless germplasms collected in Lao PDR were available with the selective marker, pl_indel_0.5k. In contrast, the prickly phenotype of the 9 accessions shown in Supplemental Fig. 2 was inconsistent with the selective marker genotype. This may indicate that their prickly phenotype might be derived from another origin.

Through further studies, 16 genes were predicted within the Pl locus interval (133 kb). Among them, the candidate genes, GS08 and GS09, were identified (Supplemental Table 3). The former candidate, GS08, is homologous to Solyc06g075140.3 and encodes the GATA transcription factor (Supplemental Table 3) located downstream of the 0.5-kb insertion/deletion. Previously, many studies (Chen et al. 2017, Cui et al. 2016, Kirik et al. 2004a, 2004b, Liu et al. 2016, Oppenheimer et al. 1991, Payne et al. 2000, Rerie et al. 1994, Schellmann et al. 2002, Wada et al. 1997, Walker et al. 1999, Wang et al. 2015 Yang et al. 2011, Zhang and Oppenheimer 2004, Zhao et al. 2015) reported the important functions of transcription factors in trichome formation. Furthermore, the GATA transcription factors were found to affect phytohormone signalling, which might be related to plant cell formation, including that of trichome (Barchi et al. 2010, Naito et al. 2007, Richter et al. 2010). Based on the stereo microscope images, trichome formation occurred separately from that of prickles, ultimately revealing that the prickle in the eggplant would not be the same organ as trichome (Supplemental Figs. 3, 4). Nonetheless, trichome is indisputably an excellent model for understanding the protruding epidermal organ (Hulskamp 2004), therefore, the evidence of trichome formation is not only highly suggestive, but could also be used to yield helpful information. The latter candidate gene, GS09, which is homologous to Solyc06g075150.3, encoded an Auxin Response Factor 10B. Trichome formation is known to be regulated by the ARF family of genes in Arabidopsis and tomato, AUXIN RESPONSE FACTOR 3 (ARF3) (Fahlberg et al. 2006) and Solanum lycopersicum AUXIN RESPONSE FACTOR 3 (SIARF3) (Zhang et al. 2015), respectively. Based on this information, it is conceivable that the auxin-related gene plays an important role in prickle formation. Through additional analysis of the sequence comparison between prickly accessions and a no-prickly cultivar, many SNPs and Indels were found around the predicted genes. The previously ascribed 0.5-kb deletion in the ‘Togenashi-senryo-nigo’ genome might affect the gene expression level of neighbouring genes, particularly the immediate downstream positioning gene, GS08. With the exception of the
To completely isolate the PI gene, further studies, including gene expression analysis and transgenic plant tests, should be performed. Through this isolation, the molecular basis for the absence of prickles in eggplant could be demonstrated. Based on the insights that could be derived from future studies, research on prickles may not only be promoted in eggplant germplasms, but also in other prickly important plants that remain poorly-understood, such as rose or citrus fruits.

Author Contribution Statement

KM conducted genetic experiments, analysed data, and drafted the manuscript. TS, TN, SN and EY were involved in the development of the mapping populations. HY, AO, EY, JW, YK and HF contributed to the creation of genetic linkage maps, construction of BAC libraries and conducting fine mapping. HY and HF supervised the trait evaluation of the presence of prickles. All the members helped discuss and guide through all steps of the experiments. TN and HF contributed to the preparation of the final version of the manuscript.

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Literature Cited

Barchi, L., S. Lanteri, E. Portis, A. Stägel, G. Valè, L. Toppino and G.L. Rotino (2010) Segregation distortion and linkage analysis in eggplant (Solanum melongena L.). Genome 53: 805–815.

Barchi, L., S. Lanteri, E. Portis, A. Acquaro, G. Valè, L. Toppino and G.L. Rotino (2011) Identification of SNP and SSR markers in eggplant using RAD tag sequencing. BMC Genomics 12: 304.

Barchi, L., S. Lanteri, E. Portis, G. Valè, A. Volante, L. Pulcini, T. Ciriaci, N. Acciarri, V. Barbierato, L. Toppino et al. (2012) A RAD tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. PLoS ONE 7: e43740.

Barchi, L., L. Toppino, D. Valentino, L. Bassolino, E. Portis, S. Lanteri and G.L. Rotino (2018) QTL analysis reveals new eggplant loci involved in resistance to fungal wilts. Euphytica 214: 20.

Barchi, L., M. Pietrella, L. Venturini, A. Minio, L. Toppino, A. Acquaro, G. Andolfo, G. Aprea, C. Avanzato, L. Bassolino et al. (2019) A chromosome-anchored eggplant genome sequence reveals key events in Solanaceae evolution. Sci. Rep. 9: 11769.

Birnboim, H.C. and J. Doly (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7: 1513–1523.

Cericola, F., E. Portis, S. Lanteri, L. Toppino, L. Barchi, N. Acciarri, L. Pulcini, T. Sala and G.L. Rotino (2014) Linkage disequilibrium and genome-wide association analysis for anthocyanin pigmentation and fruit color in eggplant. BMC Genomics 15: 896.

Chen, M., T. Yan, Q. Shen, X. Lu, Q. Pan, Y. Huang, Y. Tang, X. Fu, M. Liu, W. Jiang et al. (2017) GLANDULAR TRICHOME-SPECIFIC WRKY 1 promotes artemisinin biosynthesis in Artemisia annua. New Phyol. 214: 304–316.

Cui, J.Y., H. Miao, L.H. Ding, T.C. Wehner, P.N. Liu, Y. Wang, S.P. Zhang and X.F. Gu (2016) A new Glabrous gene (cgsl13) identified in trichome development in cucumber (Cucumis sativus L.). PLoS ONE 11: e0148422.

Daunay, M.C. (2008) Handbook of plant breeding. Vegetables. Doganlar, S., A. Frary, M.C. Daunay, R.N. Lester and S.D. Tanksley (2002) A comparative genetic linkage map of eggplant (Solanum melongena) and its implications for genome evolution in the solanaceae. Genetics 161: 1697–1711.

Ewing, B., L. Hillier, M.C. Wendl and P. Green (1998) Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res. 8: 175–185.

Fahlgren, N., T.A. Montgomery, M.D. Howell, E. Allen, S.K. Dvorak, A.L. Alexander and J.C. Carrington (2006) Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. Curr. Biol. 16: 939–944.

FAO (2017) “FAOSTAT” [online database]; http://faostat.fao.org

Frary, A., A. Frary, M.-C. Daunay, R.N. Lester and S.D. Tanksley (2014) QTL hotspots in eggplant (Solanum melongena) detected with a high resolution map and CIM analysis. Euphytica 197: 211–228.

Fukuoka, H., K. Miyatake, S. Negro, T. Nunome, A. Ohyama and H. Yamaguchi (2008) Development of a routine procedure for single nucleotide polymorphism marker design based on the Tm-shift genotyping method. Breed. Sci. 58: 461–464.

Fukuoka, H., H. Yamaguchi, T. Nunome, S. Negro, K. Miyatake and A. Ohyama (2010) Accumulation, functional annotation, and comparative analysis of expressed sequence tags in eggplant (Solanum melongena L.), the third pole of the genus Solanum species after tomato and potato. Gene 450: 76–84.

Fukuoka, H., K. Miyatake, T. Nunome, S. Negro, K. Shirasawa, S. Isobe, E. Asamizu, H. Yamaguchi and A. Ohyama (2012) Development of gene-based markers and construction of an integrated linkage map in eggplant by using Solanum orthologous (SOL) gene sets. Theor. Appl. Genet. 125: 47–56.

Gramazio, P., J. Prohens, M. Plazas, I. Andújar, F.J. Herranz, E. Castillo, S. Knapp, R.S. Meyer and S. Vilanova (2014) Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant.
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BMC Plant Biol. 14: 350.

Gramazio, P., J. Blanca, P. Ziarosolo, F.J. Herraz, M. Plazas, J. Prohens and S. Vilanova (2016) Transcriptome analysis and molecular marker discovery in Solanum incanum and S. aethiopicum, two close relatives of the common eggplant (Solanum melongena) with interest for breeding. BMC Genomics 17: 300.

Hamato, N., K. Miyatake, T. Vilayphone, M. Simeaungkhoun, T. Sisaphaithong and T. Saito (2018) Collaborative survey of eggplant genetic resources in Lao PDR. 2017. AREIPGR 34: 81–101.

Hirakawa, H., K. Shirasawa, K. Miyatake, T. Nunome, S. Negoro, A. Ohyama, H. Yamaguchi, S. Sato, S. Isobe, S. Tabata et al. (2014) Draft genome sequence of eggplant (Solanum melongena L.): the representative solanum species indigenous to the old world. DNA Res. 21: 649–660.

Hulskamp, M. (2004) Plant trichomes: a model for cell differentiation. Nat. Rev. Mol. Cell Biol. 5: 471–480.

Kirik, V., M. Simon, M. Huelskamp and J. Schiefelbein (2004a) The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis. Dev. Biol. 268: 506–513.

Kirik, V., M. Simon, K. Wester, J. Schiefelbein and M. Hulskamp (2004b) ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis. Plant Mol. Biol. 55: 389–398.

Lebeau, A., M. Gouy, M.C. Daunay, E. Wicker, F. Chirelou, P. Prior, A. Frary and J. Dintinger (2012) Genetic mapping of a major dominant gene for resistance to Ralstonia solanacearum in eggplant. Theor. Appl. Genet. 126: 143–158.

Liu, X., E. Bartholomew, Y. Cai and H. Ren (2016) Trichome-related mutants provide a new perspective on multicellular trichome initiation and development in cucumber (Cucumis sativus L.). Front. Plant Sci. 7: 1187.

Miyatake, K., T. Saito, S. Negoro, H. Yamaguchi, T. Nunome, A. Ohyama and H. Fukuoka (2012) Development of selective markers linked to a major QTL for parthenocarpic in eggplant (Solanum melongena L.). Theor. Appl. Genet. 124: 1403–1413.

Miyatake, K., T. Saito, S. Negoro, H. Yamaguchi, T. Nunome, A. Ohyama and H. Fukuoka (2015) Detailed mapping of a resistance locus against Fusarium wilt in cultivated eggplant (Solanum melongena). Theor. Appl. Genet. 129: 357–367.

Miyatake, K., Y. Shimamura, H. Matsunaga, H. Fukuoka and T. Saito (2019) Construction of a core collection of eggplant (Solanum melongena L.) based on genome-wide SNP and SSR genotypes. Breed. Sci. 69: 498–502.

Momma, S., S. Akazawa and K. Simsoka (1997) “Daitaro”, a bacterial wilt and Fusarium wilt resistant hybrid eggplant for rootstock. Bull. Natl. Inst. Veg. & Tea Sci. 12: 73–83.

Mutlu, N., F.H. Boyaci, M. Gocmen and K. Abak (2008) Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. Theor. Appl. Genet. 117: 1303–1312.

Naito, T., T. Kiba, N. Koizumi, T. Yamashino and T. Mizuno (2007) Characterization of a unique GATA family gene that responds to both light and cytokinin in Arabidopsis thaliana. Biosci. Biotechnol. Biochem. 71: 1557–1560.

Notredame, C., D.G. Higgins and J. Heringa (2000) T-coffee: a novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 302: 205–217.

Nunome, T., K. Ishiguro, T. Yoshida and M. Hirai (2001) Mapping of fruit shape and color development traits in eggplant (Solanum melongena L.) based on RAPD and AFLP Markers. Breed. Sci. 51: 19–26.

Nunome, T., K. Suwabe, H. Iketani and M. Hirai (2003a) Identification and characterization of microsatellites in eggplant. Plant Breed. 122: 256–262.

Nunome, T., K. Suwabe, A. Ohyama and H. Fukuoka (2003b) Characterization of trinucleotide microsatellites in eggplant. Breed. Sci. 53: 77–83.

Nunome, T., S. Negoro, I. Kono, H. Kanamori, K. Miyatake, H. Yamaguchi, A. Ohyama and H. Fukuoka (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (Solanum melongena L.). Theor. Appl. Genet. 119: 1143–1153.

Oppenheimer, D.G., P.L. Herman, S. Sivakumaran, J. Esch and M.D. Marks (1991) A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. Cell 67: 483–493.

Payne, C.T., F. Zhang and A.M. Lloyd (2000) GL3 encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. Genetics 156: 1349–1362.

Portis, E., L. Barchi, L. Toppino, S. Lanteri, N. Acciarri, N. Felicioni, F. Fusari, V. Barbierato, F. Cericola, G. Valè et al. (2014) QTL mapping in eggplant reveals clusters of yield-related loci and orthology with the tomato genome. PLoS ONE 9: e89499.

Portis, E., F. Cericola, L. Barchi, L. Toppino, N. Acciarri, L. Pulcini, T. Sala, S. Lanteri and G.L. Rotino (2015) Association mapping for fruit, plant and leaf morphology traits in eggplant. PLoS ONE 10: e0135200.

Rerie, W.G., K.A. Feldmann and M.D. Marks (1994) The GLABRA2 gene encodes a homeo domain protein required for normal trichome development in Arabidopsis. Genes Dev. 8: 1388–1399.

Richter, R., C. Behringer, I.K. Muller and C. Schwechheimer (2010) The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTCHROME-INTERACTING FACTORS. Genes Dev. 24: 2093–2104.

Rinaldi, R., A. Van Deynze, E. Portis, G.L. Rotino, L. Toppino, T. Hill, H. Ashrafi, L. Barchi and S. Lanteri (2016) New insights on eggplant/tomato/pepper synteny and identification of eggplant and pepper orthologous QTL. Front. Plant Sci. 7: 1031.

Rozen, S. and H. Skalesky (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132: 365–386.

Saito, T., H. Matsunaga and A. Saito (2010) Development of Solanum melongena breeding lines as resistant rootstocks to Verticillium, bacterial, and Fusarium wilts. XIVth EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant, 30 August–1 September 2010, Valencia Spain. In: Advances in Genetics and Breeding of Capsicum & Eggplant: 513–520.

Saito, T., T. Iwahori, P. Sengounkeo, T. Vilayphone, T. Sisaphaithong and H. Okuizumi (2015) Collaborative exploration of vegetable genetic resources in Laos, 2014. AREIPGR 31: 203–223.

Saito, T., T. Sisaphaithong, N. Hamato, K. Ogasawara and T. Vilayphone (2016) Collaborative survey of eggplant genetic resources in Laos, 2015. AREIPGR 32: 183–213.

Saito, T., S. Noda, N. Kishimoto, T. Vilayphone, S. Mounnalath and T. Sisaphaithong (2017) Collaborative survey of eggplant genetic resources in Lao PDR, 2016. AREIPGR 33: 115–141.

Sakata, Y., S. Momma, T. Narikawa and S. Komochi (1996) Elevation of resistance to bacterial wilt and Verticillium wilt in eggplants (Solanum melongena L.) collected in Malaysia. J. Japan. Soc. Hort. Sci. 65: 81–88.
Sakata, Y., K. Kato, T. Saito, K. Tanaka and C. Deuanhaksa (2008) Collaborative exploration of vegetables genetic resources in Laos, 2007. Annual Report on Exploration and Introduction of Plant Genetic Resources 24: 161–183.

Salgon, S., C. Jourda, C. Sauvage, M.-C. Daunay, B. Reynaud, E. Wicker and J. Dintinger (2017) Eggplant resistance to the Ralstonia solanacearum species complex involves both broad-spectrum and strain-specific quantitative trait loci. Front. Plant Sci. 8: 828.

Schellmann, S., A. Schnittger, V. Kirik, T. Wada, K. Okada, A. Beermann, J. Thumfahrt, G. Jurgens and M. Hulskamp (2002) TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. EMBO J. 21: 5036–5046.

Stàgel, A., E. Portis, L. Toppino, G.L. Rotino and S. Lanteri (2008) Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9: 357.

Toppino, L., L. Barchi, R. Lo Scalzo, E. Palazzolo, G. Francese, M. Fibiani, A. D’Alessandro, V. Papa, V.A. Laudicina, L. Sabatino et al. (2016) Mapping quantitative trait loci affecting biochemical and morphological fruit properties in eggplant (Solanum melongena L.). Front. Plant Sci. 7: 256.

Wada, T., T. Tachibana, Y. Shimura and K. Okada (1997) Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC. Science 277: 1113–1116.

Walker, A.R., P.A. Davison, A.C. Bolognesi-Winfield, C.M. James, N. Srinivasan, T.L. Blundell, J.J. Esch, M.D. Marks and J.C. Gray (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, Y.-L., J.-T. Nie, H.-M. Chen, C.-L. Guo, J. Pan, H.-L. He, J.-S. Pan and R. Cai (2015) Identification and mapping of Tril, a homeodomain-leucine zipper gene involved in multicellular trichome initiation in Cucumis sativus. Theor. Appl. Genet. 129: 305–316.

Wu, F., N.T. Eannetta, Y. Xu and S.D. Tanksley (2009) A detailed syntenic map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theor. Appl. Genet. 118: 927–935.

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

Yoshida, T., K. Kawase and H. Nesumi (1999) Inheritance of thornlessness in trifoliate orange [Poncirus trifoliata (L.) Raf.]. J. Japan. Soc. Hort. Sci. 68: 1104–1110.

Zhang, L.H., D.H. Byrne, R.E. Ballard and S. Rajapakse (2006) Microsatellite marker development in rose and its application in tetraploid mapping. J. Am. Soc. Hortic. Sci. 131: 380–387.

Zhang, X. and D.G. Oppenheimer (2004) A simple and efficient method for isolating trichomes for downstream analyses. Plant Cell Physiol. 45: 221–224.

Zhang, X., F. Yan, Y. Tang, Y. Yuan, W. Deng and Z. Li (2015) Auxin response gene SlARF3 plays multiple roles in tomato development and is involved in the formation of epidermal cells and trichomes. Plant Cell Physiol. 56: 2110–2124.