A Unique Mutation in a MYB Gene Cosegregates with the Nectarine Phenotype in Peach

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Abstract
Nectarines play a key role in peach industry; the fuzzless skin has implications for consumer acceptance. The peach/nectarine (G/g) trait was described as monogenic and previously mapped on chromosome 5. Here, the position of the G locus was delimited within a 1.1 cM interval (635 kb) based on linkage analysis of an F₂ progeny from the cross ‘Contender’ (C, peach) x ‘Ambra’ (A, nectarine). Careful inspection of the genes annotated in the corresponding genomic sequence (Peach v1.0), coupled with variant discovery, led to the identification of MYB gene PpeMYB25 as a candidate for trichome formation on fruit skin. Analysis of genomic re-sequencing data from five peach/nectarine accessions pointed to the insertion of a LTR retroelement in exon 3 of the PpeMYB25 gene as the cause of the recessive glabrous phenotype. A functional marker (indelG) developed on the LTR insertion cosegregated with the trait in the CxA F₂ progeny and was validated on a broad panel of genotypes, including all known putative donors of the nectarine trait. This marker was shown to efficiently discriminate between peach and nectarine plants, indicating that a unique mutational event gave rise to the nectarine trait and providing a useful diagnostic tool for early seedling selection in peach breeding programs.

Introduction
Peach (Prunus persica L., Batsch) is one of the most important fruit crops in temperate regions with about 21 million tons produced worldwide and Italy, with over 1.6 million tons, is the second producer after China (FAOSTAT 2011, http://faostat.fao.org/). The presence or absence of skin pubescence (fuzziness) is one of the commercial characteristics used to classify peach fruits along with flesh features (color, adhesion and texture) and fruit shape. Nectarines, characterized by the absence of fruit trichomes, are widely cultivated and play an important role in world peach production (30% in Italy, http://agri.istat.it/, 2013; 20% in USA, http://www.nass.usda.gov/, 2013) and may be associated with decreased allergenic properties. In P. persica two major allergens, Pru p 1 and Pru p 3, are known as responsible for the oral allergy syndrome (OAS) [1]. Indeed, the Pru p 3 protein was undetectable in the nectarine ‘Rita Star’ suggesting that this may be considered as a hypoallergenic variety [2]. Interestingly, in Humulus lupulus and in Nicotiana tabacum, genes encoding proteins highly similar to Pru p 3 and Pru p 3 are mainly expressed in trichomes [3,4].

The peach/nectarine character is monogenic (G/g) with nectarine recessive to fuzzy fruit [5]. The G locus was mapped in the distal part of linkage group (LG) 5 [6,7] spanning a region from 15,126,681 to 16,315,341 (1.189 Mb) of pseudomolecule 5 of the peach reference genome (Peach v1.0) [8]. Peach originated in North-West China and was domesticated there about 4,000–5,000 years ago [8]. From China it spread westwards reaching Persia following the Silk Road, was introduced to Rome in the first century BC and then disseminated to all the Roman Empire [9]. Nectarines have been known in China for over 2,000 years [10] and have been reported in most of the oases of the Tarim Basin (China) and along the Silk Road trade routes in Central Asia and the Caucasus [9,11]. The means and timing of their introduction in Europe are not clear. Likely, Romans did not know this type of peaches [9], but nectarines have been described by several botanists in Europe since the Renaissance period [9]. Old European nectarine varieties include ‘Lord Napier’, ‘Precoce di Cronos’ and ‘Galoquin’. In Southern Italy, traditional local white nectarines, called ‘Sbergie’ (Sicily) or ‘Merendelle’ (Calabria), have been cultivated since the 16th century [12]. Cluster analysis suggested that these local accessions are distinct from the western nectarine germplasm, pointing to a putative different origin of this group of cultivars [13]. Historically, nectarines have had little impact in China’s peach industry [11], and nowadays there are no reports of traditional nectarine cultivars available in China [14]. The timing of introduction of nectarines to the United States (US)
is controversial: their cultivation is reported in the early 20th century, although a newspaper article (New York Gazette March 28, 1768, p. 3) described nectarines being grown in the US prior to the War of Independence. Modern nectarine breeding started in the US in the middle of the 20th century. In 1942, Anderson introduced the nectarine 'Le Grand' using the accession 'Quetta', discovered near the homonymous city in India (now part of Pakistan) in 1906 [15], as the source of the nectarine trait. Other known sources of the nectarine trait used in modern breeding programs were 'Goldmine' and 'Lippia' discovered in New Zealand in 1900 and 1916, respectively [15]. These latter three genotypes are acknowledged as donors of most of the current nectarine cultivars widespread in US and Europe. Modern Japanese breeding programs have extensively used two old European nectarines, 'Precoce di Croncels' and 'Lord Napier', and modern US cultivars [16]. In the last decades, the trait was introduced to Chinese breeding programs directly from western accessions or indirectly using Japanese material [17].

Trichomes are hair-like appendages that derive from the differentiation of epidermal cells and are classified based on their morphology ( unicellular or multicellular), and secretory abilities (glandular or non-glandular) [18,19]. Trichomes may develop on several plant organs (leaf, fruit, seed, etc.). They play an important role in protecting plants against biotic and abiotic stresses [20–22] and can also hold a direct economic relevance. Aromatic substances are often synthesized by glandular trichomes, for example in aromatic plants, such as peppermint ( _Mentha piperita_ ) [23] and basil ( _Ocimum basilicum_ ) [24]. Cotton ( _Gossypium hirsutum_ ) seed fibers are classified as non-glandular trichomes and represent one of the most highly expanded plant cell types [25]. In peach fruit, trichomes are non-glandular and unicellular and first appear on the ovary as early as four weeks before anthesis as observed in the peach 'Contender' [26]. By the time of physiological ripening most fruit skin trichomes are dead cells [27]. In _Arabidopsis_ a number of genes involved in trichome formation and development have been identified by mutant analyses [28] and transcriptome profiling [29], revealing a complex regulatory network. Several transcription factors interact during trichome initiation and formation: in particular members of the R2R3-MYB class are known to act as positive regulators [30–33], while single-repeat MYB genes function in negative control [34–36]. Mutations in known to act as positive regulators [30–33], while single-repeat MYB genes function in negative control [34–36]. Mutations in single-repeat MYB genes function in negative control [34–36].

The aims of the present study were to precisely map the G locus, identify a candidate gene, and develop a reliable marker for the nectarine phenotype (glabrous fruit). To these ends, we used an F2 population from a cross between the peach ‘Contender’ (C) and the nectarine ‘Ambra’ (A), segregating for the peach/nectarine trait, [41,42] (CxA F2), was used to develop a SNP map around the G locus. The trees were located in a farm belonging to the Municipality of Castel San Pietro (Bologna, Emilia Romagna, Italy) leased to ASTRA (latitude: from 44°24’44.10”N to 44°24’30.08”N; longitude: from 11°35’47.21”E to 11°36’2.00”E). No specific permission was required because Daniele Bassi is the curator of the peach germplasm collection grown there and no endangered or protected species were involved.

Trees were planted on their own roots with a spacing of 1 m within and 4 m between rows and trained as slender spindle (one stem with short lateral scaffolds). Pruning was performed yearly and standard cultural practices were applied. Scoring of the peach/nectarine trait was carried out in two seasons to confirm correct scoring of the phenotype.

Ninety-five _P. persica_ genotypes, 46 peaches and 49 nectarines grown at the CRA-FRU experimental farm (Rome, Italy) (except for ‘Galopin’ and ‘Lord Napier’ grown at Ivalsa-CNR, Follonica, Italy), were analyzed to validate the functional marker. For each accession, phenotype, pedigree, geographical origin and putative donor of the nectarine trait are reported in Table 1. DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) as per manufacturer’s protocol and quantified with NanoDrop (Thermo Scientific, Waltham, MA, USA).

### Linkage map

In total 305 individuals from the CxA F2 progeny were analyzed to map the G locus using an upgraded version of the CxA map [41,42] covering LG 5 from base 10,192,138 to base 17,544,073 of the peach genome sequence pseudomolecule 5 and spanning the already known G interval [7]. To refine the position of the G locus, SNPs located in this region were selected from those identified through analysis of re-sequencing data from the CxA F2, individual [42] (biosample SRS335631, run SRR502997). About 300 bp of the SNP-flanking sequence were downloaded from the IGA peach Gbrowse [http://www.appliedgenomics.org/] and the Mass AR-RAY Assay Design 3.1 software was used to design multiplex assays for SNP analysis [43]. SNP genotyping was performed using the iPLEX Gold technology available for Sequenom platforms (Sequenom, Inc., San Diego, CA, USA), SNP markers and the G locus (scored as a dominant phenotypic marker) were mapped using Joinmap 3.0 [44] with a minimum LOD score of 10 for grouping; the Kosambi mapping function [45] was used to convert recombination frequencies into map distances. Based on this map, three new SNP markers (S5_1598499, S5_15865556 and S5_15866258) were developed in the G locus interval (scaffold_5, from 15,853,006 to 16,488,104; see Results and Discussion) and genotyped on eight informative recombinants by sequencing 200 bp encompassing the SNP (primer sequences shown in Table 2). Standard PCRs were performed using GoTaq Green Master Mix (Promega, Madison, WI, USA). Each PCR reaction contained 1 X GoTaq Green Master Mix, 0.4 μM of each primer, 20 ng template DNA and sterile Milli-Q water to a final volume of 25 μl. The PCR protocol consisted in an initial step at 95°C (5 min), followed by 40 cycles at 95°C (30 s), 60°C (30 s) and 72°C (1 min), and a final elongation at 72°C (5 min). PCR products were purified with ExoSapIT (Amersham PharmaciaBiotech, Uppsala, Sweden) and sequenced with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). After ethanol precipitation, sequencing products were mixed with 15 μl of HiDi formamide and subjected to capillary electrophoresis in an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Genotyping was performed by

### Materials and Methods

#### Plant materials & DNA extraction

An F2 population of 305 seedlings derived from the cross between the peach ‘Contender’ (C) and the nectarine ‘Ambra’ (A),
| Accession name      | Genotype       | Phenotype   | Pedigree                      | Geographical origin* | Putative donor of nectarine alleleb |
|---------------------|----------------|-------------|-------------------------------|----------------------|-----------------------------------|
| Ambra               | 197-197        | nectarine   | Stark Red Gold x Mayfire      | Italy (B)            | Lippiatt/Goldmine                 |
| Aniversario         | 197-197        | nectarine   | –                             | Argentina (B)        | -/-                               |
| Big Top             | 197-197        | nectarine   | –                             | USA (B)              | -/-                               |
| Branca              | 197-197        | nectarine   | Goldmine x Pala              | Brazil (B)           | Goldmine/Goldmine                 |
| California          | 197-197        | nectarine   | P60-30 x Fantasia            | Italy, 1994 (B)      | Quetta/-                          |
| Centenaria          | 197-197        | nectarine   | Docura 2 op                  | Brazil (B)           | Goldmine/Goldmine                 |
| Chiyodared          | 197-197        | nectarine   | Hirakutsared x Nectarated 5   | Japan (B)            | Lord Napier or Precoce di Croncels/- |
| Crasiommmolo Rosso  | 197-197        | nectarine   | –                             | Italy (L)            | -/-                               |
| Crimson Gold        | 197-197        | nectarine   | Nectarine selection x July Elberta | USA, 1967 (B)  | -/-                               |
| Sabrina             | 197-197        | nectarine   | –                             | Spain (B)            | -/-                               |
| Fairlane            | 197-197        | nectarine   | (Le Grand x Sun Grand) x Fantasia | USA, 1973 (B)  | Lippiatt/Quetta                   |
| Jacquotte           | 197-197        | nectarine   | –                             | France, 1979 (B)     | -/-                               |
| Laura               | 197-197        | nectarine   | –                             | USA, 1995 (B)        | -/-                               |
| JinXia              | 197-197        | nectarine   | (Okubo x Okitsu) x Okitsu F2   | China (B)            | Lord Napier/Precoce di Croncels   |
| Lord Napier         | 197-197        | nectarine   | –                             | Belgium, 1859 (L)    | -/-                               |
| Madonna Di Agosto   | 197-197        | nectarine   | –                             | Italy (L)            | -/-                               |
| Madonna Di Giugno   | 197-197        | nectarine   | –                             | Italy (L)            | -/-                               |
| Magali              | 197-197        | nectarine   | –                             | France, 1988 (B)     | -/-                               |
| Max                 | 197-197        | nectarine   | –                             | Italy, 1995 (B)      | -/-                               |
| Mayfire             | 197-197        | nectarine   | Armking op                   | USA, 1984 (B)        | Goldmine/Goldmine                 |
| Nectarross          | 197-197        | nectarine   | Stark Red Gold x Le Grand    | Italy, 1983 (B)      | Lippiatt/Quetta                   |
| Nettarina Pendula   | 197-197        | nectarine   | –                             | Italy (L)            | -/-                               |
| Nico                | 197-197        | nectarine   | –                             | Italy (B)            | -/-                               |
| Phn 91-12           | 197-197        | nectarine   | –                             | USA (B)              | -/-                               |
| Phn 91-14           | 197-197        | nectarine   | –                             | USA (B)              | -/-                               |
| Phn 91-17           | 197-197        | nectarine   | –                             | USA (B)              | -/-                               |
| Quetta              | 197-197        | nectarine   | –                             | Pakistan, 1906 (L)   | -/-                               |
| Ricci 2             | 197-197        | nectarine   | Stark Red Gold x Tastyfree    | Italy, 1993 (B)      | Lippiatt/Quetta                   |
| Romamer 1           | 197-197        | nectarine   | –                             | Romania, 1983 (B)    | -/-                               |
| Romamer 2           | 197-197        | nectarine   | City 29-245 x RR48-153        | Romania, 1983 (B)    | -/-                               |
| Russian Nectarine 592-B1 | 197-197   | nectarine   | –                             | Ukrainy, 1980 (L)    | -/-                               |
| Russian Nectarine 598-B1 | 197-197   | nectarine   | –                             | Ukrainy, 1980 (L)    | -/-                               |
| Shizukured          | 197-197        | nectarine   | Okitsu x NJN17                | Japan (B)            | Lord Napier or Precoce di Croncels/- |
| Silver Lode         | 197-197        | nectarine   | (Goldmine x Rio Oso Gem) x (Goldmine x July Elberta) | USA, 1951 (B)  | Goldmine/Goldmine                 |
| Sirio               | 197-197        | nectarine   | Flamekist x Fantasia         | Italy, 1987 (B)      | Quetta/Quetta                     |
| Snow Queen          | 197-197        | nectarine   | –                             | USA, 1975            | -/-                               |
| Souvenir Nikitski   | 197-197        | nectarine   | Lola op                      | Ukrainy, 1988 (B)    | -/-                               |
| StarkRedgold        | 197-197        | nectarine   | Sun Grand op                 | USA (B)              | Lippiatt/Lippiatt                 |
| Summer Beauty       | 197-197        | nectarine   | Red Diamond x Sun Grand       | USA, 1979 (B)        | Quetta/Lippiatt                   |
| Vania               | 197-197        | nectarine   | –                             | Italy, 1990 (B)      | -/-                               |
| ZeeGlo              | 197-197        | nectarine   | (Red Grand op) x (Sun Grand x Merrill Gem) | USA, 1988 (B)  | Quetta/Lippiatt                   |
| Zephyr              | 197-197        | nectarine   | –                             | France, 1992 (B)     | -/-                               |
| Accession name   | Genotype | Phenotype | Pedigree                        | Geographical origin* | Putative donor of nectarine allele‡ |
|------------------|----------|-----------|---------------------------------|----------------------|-------------------------------------|
| Zinca 5          | 197-197  | nectarine | –                               | Spain (B)            | -/-                                 |
| Acireale         | 941-941  | peach     | –                               | Italy (L)            |                                     |
| Amber Gold       | 941-941  | peach     | Red Grand x Royal May           | USA, 1973 (B)        |                                     |
| Amsden           | 941-941  | peach     | –                               | USA, 1868 (B)        |                                     |
| Autumniglo       | 941-197  | peach     | (Candoka x Tennessee Natural) x Merrill Fiesta | USA, 1977 (B)        | -                                   |
| Baekmulosaeng    | 941-941  | peach     | Mishima x Nunome Wase           | Republic of Korea, 1983 (B) |                                     |
| Baldagena   | 941-197  | peach     | –                               | France               |                                     |
| Changbang Mutant | 941-941  | peach     | –                               | Republic of Korea, 1986 (B) |                                     |
| Chiyomaru        | 941-941  | peach     | –                               | Japan, 1988 (B)      |                                     |
| Chul Huang Tao   | 941-941  | peach     | –                               | China (B)            |                                     |
| Ciccio Petrino   | 941-941  | peach     | –                               | Italy                |                                     |
| City 32-82       | 941-197  | peach     | –                               | USA (B)              | -                                   |
| Contender        | 941-941  | peach     | Wiblo x [Norman x (Candoka x Summercrest x USA, 1987 (B) Redhaven)] |                                     |                                     |
| Cp 88/2          | 941-941  | peach     | –                               | Mexico (B)           |                                     |
| Early Gold       | 941-941  | peach     | –                               | Japan, 1980 (B)      |                                     |
| Elberta          | 941-941  | peach     | Chinese Cling op (perhaps x Early Crawford) | USA, 1889 (B)        |                                     |
| CxA F1           | 941-197  | peach     | Contender x Ambra               | Italy (B)            | Lipiatt or Goldmine                 |
| Fairtime         | 941-197  | peach     | (Rodeo x Kirkman Gemi op        | USA, 1968 (B)        |                                     |
| Fayette          | 941-941  | peach     | Fay Elberta x (Fireglow x (Fireglow x Hitley)) | USA, 1966 (B)        |                                     |
| FeiChing Bai Li 17 | 941-941 | peach     | –                               | China (B)            |                                     |
| FeiChing Tao     | 941-941  | peach     | –                               | China, 1909 (B)      |                                     |
| Fidella          | 941-197  | peach     | (O'Henry x Giant Babcock) x (May Grand x SamUSA, 1986 (B) Houston) |                                     |                                     |
| Frau Maria Rudolf| 941-941  | peach     | Top Red Delicious op            | Germany (B)          |                                     |
| Grosse Mignonne  | 941-941  | peach     | –                               | France, 1667 (L)     |                                     |
| Higama           | 941-941  | peach     | Japanese seed op                | France, 1970 (B)     |                                     |
| Itwendo 1        | 941-941  | peach     | –                               | Republic of Korea, 1977 (B) |                                     |
| J H Hale         | 941-941  | peach     | putative selfpollination of Elberta | USA (B)              |                                     |
| JingYu           | 941-197  | peach     | Okubo x Okitsu                  | China (B)            | Lord Napier or Precoce di Croncels |
| KurakataWase     | 941-941  | peach     | –                               | Japan (B)            |                                     |
| PLove2- 2N       | 941-941  | peach     | di-haploids of Lovell           | USA, 1882 (B)        |                                     |
| O'Henry          | 941-197  | peach     | Merrill Bonanza op              | USA, 1970 (B)        | -                                   |
| Pantao 20-58     | 941-941  | peach     | –                               | China, 1869 (B)      |                                     |
| Pillar           | 941-941  | peach     | –                               | Japan (B)            |                                     |
| Redhaven         | 941-941  | peach     | Halehaven x Kalhaven            | USA, 1940 (B)        |                                     |
| Reginella II     | 941-941  | peach     | –                               | Italy (B)            |                                     |
| Rou Tao          | 941-941  | peach     | –                               | China (L)            |                                     |
| Russotto         | 941-941  | peach     | –                               | Italy (B)            |                                     |
| Sahua Hong Pantao| 941-941  | peach     | –                               | China (L)            |                                     |
| Sanguinella      | 941-941  | peach     | –                               | Italy (L)            |                                     |
| Shen Zhou Mi Tao | 941-941  | peach     | –                               | China (B)            |                                     |
| Siberian C       | 941-941  | peach     | selection of a China seedling   | Canada, 1967 (B)     |                                     |
| Summer Pearl     | 941-197  | peach     | [(Candoka x Tennessee natural op) x (CandokaUSA, 1979 (B) x Flaming Gold) x NJNS op] x (PI119844 x White Hale) | USA, 1990 (B)        | -                                   |
| TaturaDawn       | 941-941  | peach     | Levis selfpollination          | Australia, 1960 (B)  |                                     |
| Yoshihime        | 941-197  | peach     | (Nakatsu Hakuto x Nunome Wase) x Akatsuki | Japan, 1990 (B)        | -                                   |
| Yu Bai           | 941-941  | peach     | –                               | China (L)            |                                     |
| Yumyeong         | 941-941  | peach     | Yamato-Wase x Nunome Wase       | Republic of Korea, 1977 (B) |                                     |
### Table 1. Cont.

| Accession name | Genotype | Phenotype | Pedigree | Geographical origin* | Putative donor of nectarine allele* |
|----------------|----------|-----------|----------|----------------------|-------------------------------------|
| ZanetsuShidare | 941-941  | peach     | –        | Japan (B)            | b- = unknown donor of the nectarine allele. |

*TB = breeding materials (L) = landrace. 

<sup>n</sup> = unknown donor of the nectarine allele. 

For each accession the following information is reported: name, genotype at the indelG marker (941 bp reference peach allele, 197 bp nectarine allele carrying the retrotransposon insertion), phenotype, pedigree, country of origin and year of release/discovery when known, putative donor of the nectarine allele in *P. persica* if pedigree information was available.

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visual inspection of the resulting electropherograms using 4PEAKS freeware (Nucleobytes Inc.).

### Variant discovery from NGS data

In order to identify genetic variants putatively involved in the control of the nectarine trait publicly available paired-end (PE) whole-genome re-sequencing data of *P. persica* accessions from study SRP013437 [8] were downloaded from the NCBI Sequence Read Archive (SRA) [46]. Five accessions were considered for this study: ‘Bolero’ (biosample SRS335629, run SRR501836), ‘OroA’ (biosample SRS335633, run SRR502998), ‘Lovell’ Clone PLov2-2N (biosample SRS335634, run SRR502985), ‘Quetta’ (biosample SRS335636, runs SRR502989 and SRR502987) and F1 ‘Contender’ × ‘Ambra’ (biosample SRS335631, run SRR502997). ‘Quetta’ was included as the reference nectarine accession. The CxA F1 individual originated the CxA F2 population used to map the G locus. The peach ‘Lovell’ Clone PLov2-2N is the doubled haploid used to generate the reference peach genome sequence (Peach v1.0) [8], providing an internal control for false variant calling. Finally, the peaches ‘Bolero’ and ‘OroA’ were chosen as controls for nectarine segregation, as it has been demonstrated that the nectarine trait does not segregate in the ‘Bolero’ × ‘OroA’ F1 population [42].

SRA data of each run were dumped in fastq format using the fastq-dump tool of NCBI sratoolkit v2.1.16 software (http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view = software), splitting forward and reverse paired reads for each sample into two separate files. Reads were quality filtered on a single sample basis using Trimomatic v0.22 [47], first trimming leading and trailing bases below a quality threshold of 20, and then removing trimmed reads shorter than 24 bp or having an average quality below 20 (calculated on 8 bp long sliding windows). For each sample, only reads passing the quality filtering as matching pairs were retained and aligned to the whole *P. persica* reference genome Peach v1.0 using the Burrows-Wheeler Alignment Tool (BWA v0.6.2) [48]. The abu (IS linear-time algorithm) and sampe (all default options except -n 25 –N 23) commands were applied, respectively, for finding suffix array (SA) coordinates of each individual read and to convert them to chromosomal coordinates and to pair the reads. The resulting alignment SAM files were converted by Picard Tools version 1.77 (http://picard.sourceforge.net/) to sorted BAM files compliant to the Genome Analysis Toolkit (GATK) format, using the tools CleanSam, SamFormatConverter and AddOrReplaceReadGroups. GATK-compliant BAM files were submitted to GATK version 2.5-3 [49] for pre-processing procedures, i.e. indel realignment, duplicate removal and base quality score recalibration (BQSR). The data table needed for the recalibration step in BQSR was manually generated upon validated SNP data from the Peach 9K chip array [50]. Variant discovery procedures were then applied using whole-genome recalibrated alignments of all five samples simultaneously. Genotypes for SNP and small INDEL variants were called through the GATK HaplotypeCaller tool applying hard filtering parameters [51]. Structural variants were also independently called on the same recalibrated alignment data by Pindel software v0.2.4t [52] following standard procedures.

Reads from the resequencing of ‘Quetta’ (biosample SRS335636, runs SRR502989 and SRR502987) were also analyzed using the CLC genomic workbench (6.0.1). The 75 bp pair-end fragments were trimmed for quality retaining only nucleotides with Phred values higher than 30, and trimmed reads were aligned against the *P. persica* reference genome (Peach v1.0) [8] using the read mapping tool. Only reads with over 90% identity over at least 92% of their length were mapped on the reference. All variant discovery searches were limited to the locus G mapping interval defined by informative recombinants in the CxA F2 mapping population (scaffold_5, from 15,853,006 to 16,480,104, see Results and Discussion).

### Validation of candidate variant

To validate the putative variant individuated among the resequenced genotypes long-range PCRs were performed on five nectarines genotypes (‘Quetta’, ‘Goldmine’, ‘Madonna di Agosto’, ‘Stark Red Gold’ and ‘Ambra’) and on the peach ‘Contender’, with primers Seq16F and Seq4R (Table 3) designed flanking the putative insertion, using Hercule DNA Polymerase (Agilent Technologies, Santa Clara, CA, USA). Each reaction contained 1× Hercule reaction buffer, 0.3 mM dNTP mix, 0.5 μM each Seq16F and Seq4R primer, 3% DMSO, 2.5 U Herculase polymerase, 300 ng template DNA, and sterile Milli-Q water to a final volume of 50 μl. The following PCR protocol was performed on an Esco Swift Maxi thermocycler (Esco GB Ltd, Downton, UK) or an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA); 95°C for 5 min; 28 cycles of 95°C (1 min), 59°C (1 min), 72°C (12 min) followed by a step at 72°C for additional 12 min. All PCR amplicons were checked on 1% agarose gel in an overnight run in SB buffer. A standard ethidium bromide staining was used for band visualization.

For restriction analysis, 5 μl of long-range PCR products from ‘Quetta’, ‘Goldmine’, ‘Madonna di Agosto’, ‘Stark Red Gold’ and ‘Ambra’ were digested with EcoRI and HindIII (Fermentas, Vilnius, Lithuania) in a single overnight reaction at 37°C. Master mix was calculated by double digest tool (http://www.thermoscientificbio.com/webtools/doubledigest/) with 0.5 U/sample of each restriction enzyme and buffer R. Restriction products were separated on 1% agarose gels and stained with ethidium bromide for band visualization.

The ‘Quetta’ long-range PCR product was first purified with the Macherey-Nagel PCR clean-up kit (Carlo Erba reagents, Italy) and quantified by Picogreen (Quant-IT PicoGreen dsDNA kit,
Life Technology, US) in preparation for sequencing using the Illumina MiSeq platform with a 150 bp paired end sequencing strategy. Preparation of Nextera XT library was performed with 1 ng of genomic DNA according to the Nextera XT protocol (Ver. Oct 2012, rev C). Briefly, the DNA was fragmented in 5 nl of Amplicon Tagment Mix and 10 nl of Tagment DNA buffer (Illumina, San Diego, CA, USA). Tagmentation reactions were performed by incubation at 55°C for 5 min followed by neutralization with 5 nl of Neutralize Tagment Buffer for 5 min. Tagmented DNA (25 nl) was used as the template in a 50 nl limited-cycle PCR (12 cycles) and processed as outlined in the Nextera XT protocol. Amplified DNA was purified using 90 nl of AMPure XP beads then normalized with 45 nl of combined Library Normalization beads/additives. In preparation for cluster generation and sequencing, the normalized library was diluted in hybridization buffer and heat denatured. Due to the low diversity of the library a phiX spike-in (30%) was added to the final denatured 10 pM library. The sample was sequenced using the MiSeq Personal Sequencer (Illumina Inc., San Diego, CA, USA) running MiSeq Control Software Version 2.0. A total of 20 M reads were obtained and analyzed using the CLC genomic workbench (6.0.1). Trimming and De Novo assembly tools were used with default parameters. The assembly obtained was filtered retaining only contigs with a length greater

| Markers* | cm³ | Peach v1.0 position (nt) | SNP allele | Forward primer | Reverse primer | SNP Extension primer |
|----------|-----|--------------------------|------------|---------------|-----------------|---------------------|
| S5_10192138 | 0 | 10,192,138 | G/A | GATGAAATGTTAGGTAAGTGTGAA | TTTGCGAATAAAAAAACATATC | ttgAgGCAATCGTGAAGTTCATGTTTACCA |
| S5_11640083 | 4.2 | 11,640,083 | T/G | CCTACTAACAATTTGCTTATA | GTCGGTGCACTCTTCTTATG | gcACCTTGAATCTCTTTAACCAAGTA |
| S5_12847567 | 11.4 | 12,847,567 | T/A | TCGGATTGTCTTTCTGACTAC | CCITCTCCCATTCTCACTG | ATTAGGATGTATGATTAT-TATAT |
| S5_13449464 | 14.3 | 13,449,464 | G/C | CGGTGATGATGATGATGATGATG | CACTCAAAATGCTCTTCCC | GTATTGATGATGATGATTATA-TATAT |
| S5_13852617 | 16.7 | 13,852,617 | A/C | ATACCTTGTTACACTCCCCG | TTGCTGTAGTAAAGTGGTGGG | TGTACTGATGTTGAATCTAATG |
| S5_14584095 | 24.3 | 14,584,095 | G/C | AAGTTGATGAGGGTGCACACA | TATAGGTTGCACTCTTCTCTCATCAG | GGGCTGGAATCTCTTCTCTCAGA |
| S5_14984563 | 25.5 | 14,984,563 | C/A | GCAGGAGAAATTTTCCCTAC | TTAGGAGGAGGACTATG | ATGTTGAGATAATTATGTTG |
| S5_14994332 | 26.0 | 14,994,332 | T/C | AGAAGTTGATGAGGGTTAGAG | TTTCCGATCCGAAAGGAT | TTTCCGATCCGAAAGGAAT |
| S5_15731107 | 28.4 | 15,731,107 | T/G | CTGTTGTTAAGACAGTTTGGG | AACATGTTGCTGCAGCTCCTC | GGGCTGGAATCTCTTCTCTCAGA |
| S5_15853006 | 28.8 | 15,853,006 | T/A | TAGTTGCTTGCTAACAAGCCG | CCCAAGAAGTGAAGTATG | GCTTTAAGAACATATAGG |
| S5_15865556 | 29.1 | 15,865,556 | A/T | GGTGCGGGCTCTGTATTC | TAAAGGCAACACATTGCA | TAAAGGCAACACATTGCA |
| S5_15866285 | 29.1 | 15,866,285 | A/G | TCAGGCTTGTTACAGGATTT | GGGCAGGAGGCTCTTTCTC | ATAGTTGAGATAATTATGTTG |
| S5_15984942 | 29.4 | 15,982,499 | C/T | GCAGGAGAAATTTTCCCTAC | TTAGGAGGAGGACTATG | ATGTTGAGATAATTATGTTG |
| S5_16488104 | 29.9 | 16,488,104 | A/G | TTGCGGTTGATGAGGGTTAGAG | TTTCCGATCCGAAAGGAT | TTTCCGATCCGAAAGGAAT |
| S5_17544073 | 36.2 | 17,544,073 | G/A | GCCATCTCTGTGCTTCTCT | GTAGATCAGAGCGAGCTAG | cTTAACATACATGACATACATACA- |

*SNP detection was performed by Sequenom MassArray technology, except for the SNPs in italics that were genotyped by Sanger sequence.

A MYB Gene for the Nectarine Trait

Table 2. SNP markers.

Table 3. List of Primers.
than 200 bp and formed by more than 500 reads. The filtered contigs were mapped using BWA v0.7.5a [53] with the MEM algorithm against the Peach v1.0 genome. Identification of conserved domains on contigs was performed using HHMER v3.1 [54] against the PFAM [55] database v27.0. Blast search of contigs was done using NCBI-BLAST+ v2.2.27 [56] against the NT database downloaded from NCBI FTP site on Oct 23 2013.

Expression analysis
Total RNA was extracted from floral buds collected from ‘Contender’ and ‘Ambra’ at different developmental stages (seven, five, four and one week before anthesis) using the RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) and treated with DNase I (Sigma-Aldrich, St. Louis, MI, USA) following manufacturers’ instructions; 1 μg of RNA was reverse-transcribed using the GoScript Reverse Transcription System (Promega, Fitchburg, WI, USA) with oligo (dT)15 according to the manufacturer’s protocol. For reverse transcription analysis, primers were designed on exon 2 (Seq15F) and exon 3 (Seq15R) of the PpeMYB25 gene and the RNA polymerase II sequence was used as reference gene [57] (Table 3). For RT-PCR, 1 μL of cDNA was used with 1x GoTaq Green Master Mix, 0.4 μM of each primer and sterile Milli-Q water to a final volume of 10 μL. The PCR protocol consisted of an initial denaturation at 95°C for 2 min, followed by 35 cycles at 95°C (20 s), 62°C (20 s) and 72°C (30 s), followed by a final elongation at 72°C (5 min). PCR products were checked on 1% agarose gel at 5 V/cm in TBE buffer. A standard ethidium bromide staining was used for band visualization.

Functional marker design and genotyping
To perform association studies for the nectarine trait and provide a tool for marker assisted breeding (MAB) a codominant marker (indelG), consisting of a three-primer PCR assay (primers indelG-F, indelG-1R and indelG-2R; Table 3), was developed for genotyping of the candidate insertion: two outer primers (one forward and one reverse), designed on opposite sides of the insertion were combined in a single reaction with an inner reverse primer (designed on the reconstructed left sequence of the insertion).

PCRs were carried out in 10 μL containing 10 ng of template DNA, 1x PCR buffer, 1.5 mm MgCl2, 200 μM each dNTP, 0.2 μM each primers and 0.5 U of Platinum Taq DNA polymerase (Life Technologies, Carlsbad, CA, USA). Amplifications were performed on a Veriti thermal cycle (Life Technologies, Carlsbad, CA, USA) with the following temperature profile: 95°C (5 min) followed by 35 cycles at 94°C (30 s), 61°C (30 s), 72°C (30 s) and a final extension at 72°C (10 min). PCR products were separated on an ethidium bromide stained 1% agarose gel. The 305 seedlings of the CxA F2 population, the parents and the hybrid CxA F1, as well as 46 peach and 49 nectarine accessions, were analyzed (Table 1).

Results and Discussion
Mapping of the G locus
Scoring of the CxA F2 progeny revealed the presence of 246 peach and 59 nectarine plants, indicating a slight distortion from the expected 3:1 segregation (χ² = 5.41, p<0.05 with 1 d.f.). In agreement with a previous report [7], the peach/nectarine phenotype was regarded as a dominant trait (G locus). A total of 12 SNPs, covering about 7 Mb of LG 5 around the G locus, were mapped on the whole progeny (Figure 1). Consistent with the observed distortion of the phenotypic trait, a skewed segregation was also found for all the markers around the G locus from

![Figure 1. LG 5 CxA map around the G locus.](image)

**Variant identification in the locus G genomic region**
In order to identify variants putatively underlying the nectarine phenotype, genome-wide recalibrated alignment data from five P. persica accessions were examined in detail around the G locus (scaffold_5, from 15,853,006 to 16,488,104). In this region, GATK HaplotypeCaller detected 291 SNP and indel variants above the chosen minimum phred-scaled score quality threshold of 200, out of which 67 mapped within predicted genes in the peach genome (Peach v1.0) [8]. Of the latter, 20 variants, distributed in seven genes, were heterozygous in CxA F1 with non-segregating joint
genotype combinations in ‘Bolero’ and ‘OroA’. In the nectarine ‘Quetta’ the same analysis identified two variants, both indels, homozygous for the non-reference allele and located in two distinct genes (ppa023682m and ppa023143m). Both of them were heterozygous in CxA F1, with no other variants found were located in these two genes, and were thus considered as putative candidates for the G locus. The first candidate variant is a deletion of 3 motives in a known (AAC)₃ microsatellite at position 16,463,040 which maps within the only intron of the predicted gene ppa023143m (best Arabidopsis thaliana blast match AT5G15310.1, ABI five binding protein AFB9, e-value 5×10⁻¹⁸). ABI Five Binding Proteins (AFPs) are members of a small plant-specific protein family, characterized by three conserved domains of unknown function. AFPs act as negative regulators ofABA signaling [58] and have no known involvement in trichome formation. Next we focused on the second variant, reported by 

**Figure 2. Alignment of Quetta reads against a 635 kb interval of Peach v1.0 pseudomolecule** 5. Alignment results of reads, obtained by 

the resequencing of ‘Quetta’, against the peach genome region identified by the mapping interval in LG5 (from 15,853,006 bp to 16,488,104 bp). Top panel: intron-exon structure of ppa023143m. Central panel: plot of ‘Quetta’ paired-end distance (blue) and frequencies of single reads (yellow) at the ppa023143m locus. Bottom panel: blue lines are paired reads, green and red lines correspond to single reads with missing mate on the right and left side, respectively. The orange arrow points to the putative insertion inside exon 3 of ppa023143m.

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Validation and reconstruction of a long insertion in exon 3 of gene ppa023143m

The physical presence of the long insertion within exon 3 of gene ppa023143m was confirmed by long-range PCR using a primer pair designed on intron 2 (Seq16F) and exon 3 (SeqHR) flanking the insertion site. An amplification product of about 7 kb was obtained in five nectarines, ‘Madonna di Agosto’, ‘Quetta’, ‘Stark Red Gold’, ‘Goldmine’ and ‘Ambra’ (Figure 3). In contrast, in a peach genotype (‘Contender’) the same primer pair gave an amplification product of 960 bp (data not shown). ‘Quetta’ and ‘Goldmine’ are two donors of the trait in modern breeding and ‘Stark Red Gold’ is known to carry the nectarine allele of ‘Lippiat’, the third donor of the trait. ‘Madonna di Agosto’ belongs to a group of landraces not directly related to modern breeding germplasm [12,13]. The double digestion of the five amplicons, with EcoR/HindIII shows the same restriction pattern for all the accesses [Figure 3].

The amplicon obtained in ‘Quetta’ was sequenced by Next Generation Sequencing (NGS). Following filtering and assembly 90% of the reads were collected in three major contigs. Contig1 (GenBank accession number KJ150676), formed by a consensus sequence of 5,836 bp, was mapped on scaffold 3 of peach genome v1.0 and showed a perfect match for 5,713 bp (scaffold_3:13,409,926,13,415,638) corresponding to the predicted LTR_1684 region (http://services.appliedgenomics.org/gbrowse/prunus_public/). The missing 123 bp that do not align on scaffold 3 showed a perfect match on scaffold 5 (scaffold_5:15,898,458,15,898,581), confirming that this contig represents an insertion in the third exon of predicted gene ppa023143m. When the LTR_1684 region, found in correspondence of Contig1 alignment on scaffold 3, was submitted to CENSOR [60] (Release
(18.01, 16 Jan 2013) high similarity was found to a 6,033 bp annotated Ty1-copia retrotransposon (Copia-24_FV-I) from strawbery (Fragaria vesca). A conserved protein domain analysis on the complete sequence of Contig_1 also revealed the presence of different domains: UBN2 (gag-polypeptide of LTR copia-type) from position 1,354 to 1,689 of Contig_1, GAG-pre-integrase domain from position 2,329 to 2,511, RVE (Integrase core domain) from position 2,551 to 2,928 and RVT2 (Reverse transcriptase domain) from position 3,694 to 4,431. The four domains identified showed a high prediction confidence, with E-values of 3.8e-16, 4.3e-13, 6.8e-28 and 1.6e-98 respectively. All these predicted domains are typical of retrotransposable elements.

The remaining two contigs, Contig_2 and Contig_3, 300 and 876 bp respectively, were also mapped on the peach genome. Both were split on scaffold 5 (scaffold_5:15,898,340.15,898,361; scaffold_5:15,898,458.15,899,262) in exon 3 of predicted gene ppa023143m and on scaffold 3 in the same LTR_1684 region of Contig_1. Thus, these contigs span the point of insertion of the Ty-copia retroelement within exon 3 of the gene. Flanking this insertion point, we found a characteristic Target Site Duplication (TSD) (AC)3 [61] produced by the retroelement upon insertion into a new site. Together these results confirm the insertion of a Ty1-copia retrotransposon in the ‘Quetta’ allele of gene ppa023143m.

A BLASTN search of the retrotransposon sequence of Contig_1 against the peach reference genome returned 5 highly similar hits (from 87.1% to 100% sequence identity), two on chromosome 3, and one each on chromosomes 4, 7 and 8. All five hits were about 6 kb long and were precisely delimited by highly similar (from 97.7% to 100.0%) LTR sequences, each flanked by characteristic Target Site Duplications, thus confirming the existence of other copies of this LTR-retroelement in the reference peach genome. In particular LTRs found in the ‘Quetta’ allele of ppa023143m are identical to those present in scaffold 3 (scaffold_3:14,488,093.14,488,522) and scaffold 4 (scaffold_4:27,503,652.27,504,081). These data are consistent with previous analyses showing that 12.6% of LTR-retrotransposons have identical LTRs, indicating recent and ongoing retrotransposition activity in the peach genome [8].

Transposable elements (TEs) are known to cause many kinds of genetic variations in plants and played an important role in plant evolution and domestication [62]. In a survey of allelic variants at 60 genes involved in crop domestication and diversification, 15% were caused by TE insertions [63]. For example, an LTR insertion in a regulatory region of the teosinte branched1 (tb1) gene resulted in overexpression of the gene, causing the conversion from highly branched wild teosinte to the single culm architecture of domesticated maize [64,65]. However, the most common effect of TE insertion is the loss of gene function [57] and recessive TE-
induced mutations have played an important role in plant domestication [62]; examples include "sticky" foxtail millet [66], Mendel's wrinkled peas [67], seedless apple [68], fruit color in grape [69–71] and peach flesh [72,73]. In some cases, multiple independent mutational events were selected as demonstrated by insertion of TEs in the \textit{waxy} locus resulting in the "sticky" phenotype in foxtail millet [66]. Another example is the yellow peach phenotype, which is associated with three different mutational events (an LTR insertion, a SNP and a frameshift mutation at a microsatellite locus) that occurred independently in a carotenoid cleavage dioxygenase gene directly involved in pigment degradation [72,73]. In contrast with the situation for "sticky" foxtail millet [66] and peach color [72,73] our results suggest that a unique mutational event has originated the nectarine phenotype, i.e. the loss of trichomes in peach fruit. The occurrence of unique mutations affecting single genes selected by humans during domestication and diversification of crop species is not rare. In a recent review of 60 such genes, 26 display a unique mutational event selected and spread by humans [63].

Gene ppa023143m encodes an R2R3-MYB transcription factor putatively involved in trichome formation

Careful inspection of predicted gene ppa023143m led us to reannotate the coding sequence (CDS) extending exon 3 compared to the Peach v1.0 annotation [8]. The reannotated CDS is predicted to encode a peptide of 330 aminoacids showing similarity to the R2R3-MYB transcription factors GhMYB25 from allotetraploid cotton \textit{Gossypium hirsutum} (58.4% similarity) [40] and MIXTA-like1 from \textit{Antirrhinum} (AmMYBML1, 55.3% similarity) [59] (Figure 4). In eukaryotes, MYB factors represent one of the largest and most functionally diverse gene families, which dramatically expanded in plants [74–77] playing a central role in a variety of processes from plant development to responses to biotic and abiotic stresses [74,78,79]. MYB family members share a highly conserved DNA binding domain (the MYB domain) usually composed of up to three amino acid repeats (R1, R2, R3) [80]. \textit{GhMYB25} and \textit{AmMYBML1} belong to R2R3-MYB sub-group 9 [40,77] along with \textit{PpeMYB25} and other genes involved in regulating epidermal cell shape [81]. Analysis of \textit{Antirrhinum} R2R3-MYB genes from subgroup 9 indicated that the C-terminal domain folds as an amphipathic \(	ext{a} \)-helix with putative transactivation ability [84]. An insertional mutant in the MIXTA gene, resulting in loss of this C-
terminal region has been shown to cause recessive phenotypic alteration in epidermal cell differentiation [85]. The recessiveness of the nectarine trait indicates that it corresponds to a loss-of-function mutation. According to this reasoning and by analogy with observations in cotton, we propose that the observed insertion in the PpeMYB25 gene results in a non-functional form of the MYB transcription factor that normally promotes trichome formation in fuzzy peaches. If this were correct, all nectarines should be non-functional homozygous mutants at this gene.

In order to evaluate the timing of PpeMYB25 transcript expression with respect to trichome development, RT-PCR analyses were performed on ‘Contender’ and ‘Ambra’ floral buds sampled at seven, five, four and one week before anthesis. A forward primer designed on exon 2 was used in combination with a reverse primer on exon 3 (downstream of the LTR insertion) in order to evaluate the expression profiles of the gene. Expression was evident in ‘Contender’ from five weeks before anthesis, just before trichomes begin to appear [26] and continued through to one week before anthesis accompanying trichome differentiation (Figure 5). In contrast, the expression of PpeMYB25 was never visible in ‘Ambra’ floral buds, consistent with the presence and the position of the large insertion (Figure 5). Together sequence and expression analyses support the proposed involvement of PpeMYB25 gene in promoting trichome differentiation. Mutational events in regulatory genes have played a major role during crop domestication and breeding; in a recent overview of domestication and diversification genes, 37 out of 60 (~62%) encoded transcription factors [63].

Association study and origin of the nectarine trait in peach germplasm

The genotype at the LTR retrotransposon insertion in gene PpeMYB25 was assessed in the CxA F2 population as well as in a panel of 95 peach and nectarine accessions by means of a functional marker based on a three primers PCR assay (indelG) (Figure 6). As expected, the marker co-segregated with the G locus in the CxA F2 progeny (Figure 1), with all the nectarines displaying a unique fragment of 197 bp. Similarly, all nectarines in the germplasm panel were characterized by a fragment of the same length (Figure 6). Peach accessions fell into two categories: those homozygous for the reference allele (941 bp) and those heterozygous (197 bp, 941 bp). Taking into account pedigree information [15,86], we confirmed all the known heterozygotes (‘Autumnglo’, ‘Fairtime’, ‘Fidelia’, ‘Jing Yu’, ‘O’Henry’, ‘Summer Pearl’ and CxA F1, Table 1) and we also detected three previously unknown heterozygous genotypes (‘Baldagenais’, ‘City 32–82’ and ‘Yoshihime’) carrying the nectarine allele originated from the LTR insertion. The complete association between the indelG marker and the trait confirms the presence of the Ty1-copia retrotransposon within exon 3 of the PpeMYB25 gene in all the nectarines analyzed.

Figure 6. Functional Marker indelG. A marker assay was developed based on sequence information on the PpeMYB25 gene and the Ty1-copia insertion. Three primers were designed to discriminate peach and nectarine genotypes (A, B). A panel of nectarines including the putative donors of the trait, show a unique fragment of about 200 bp (C). A set of peaches, of diverse pedigree and origins (Table 1) (D), shows homozygous or heterozygous patterns.

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Modern western nectarines trace back to three founders discovered at the beginning of the last century (‘Quetta’ in Pakistan, and ‘Lippiatti’ and ‘Goldmine’ in New Zealand). The founders and their modern descendants show the presence of the retrotransposon insertion. The insertion is also present in Southern Italian landraces (‘Madonna di Giuomo’ and ‘Madonna di Agosto’) cultivated since the 16th century [12]. In addition to these genotypes, we confirmed the presence of the retrotransposon in several non-related accessions including old European landraces (‘Lord Napier’ and ‘Galopin’) [15], modern Asian cultivars (Chinese and Japanese) and different nectarine cultivars of unknown pedigree (from Italy, East Europe, USA, Mexico and South America). The modern Asian nectarines analyzed in this study (‘Chiyodare’, ‘Shizukured’ and ‘Jin Xia’) have two old European landraces, ‘Lord Napier’ and ‘Precoce di Cronclees’, as donors of the nectarine trait. No traditional nectarine landraces have been reported in China [14] and all modern Chinese nectarines inherited the trait from western germplasm [17].

Together these results indicate that all known nectarine germplasm derives from a unique mutational event in the draft genome of peach (Prunus persica (L.) Batsch] and characterization of morphological traits affecting flower and fruit. Tree Genet Genomes 3: 1–13.

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Conclusions

Nectarines play an important role in the peach industry. In the present study, using a candidate gene approach coupled with fine mapping and NGS-based variant discovery, we provide strong evidence that the transcription factor gene PpMYB25 acts as a positive regulator of trichome formation in peach fruit. The insertion of a Tyl-copia retrotransposon within the third exon of PpMYB25 was identified as the putative cause of a loss-of-function mutation underlying the nectarine phenotype, further supporting the importance of transposition in plant genome evolution and phenotypic variability in domesticated crops. Finally, the development of a functional marker, indelG, provides an efficient diagnostic tool for the early selection of the peach/nectarine trait in marker assisted breeding (MAB).

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Author Contributions

Conceived and designed the experiments: GP ST IV LR. Performed the experiments: EV GP ST IV LR. Contributed reagents/materials/analysis tools: DB ST IV LR. Wrote the paper: EV GP ST IV LR. Critically revised the manuscript: LD IP MTD SS DB LG.
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