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New QTLs involved in the control of stigma position in tomato

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Abstract

Background Tomato mating systems were strongly affected by domestication events. Mutations disrupting self-incompatibility paralleled by changes retracting the stigma position (SP) within the staminal cone conferred strict autogamy and self-fertility to the cultivated forms. Although major genes affecting these changes have been identified, SP control in domesticated forms that retain a constitutive or heat-inducible noninserted SP needs elucidation. To widen the possibility of identifying SP genetic determinants, we analyzed the trait in four populations (two germplasm collections, a multiparental recombinant inbred and a biparental progeny) under different environmental conditions (normal and heat stressed).

Results Overall, 37 markers significantly associated with the trait were identified. Several colocalizations were found, both among regions first reported in this work and among them and previously reported positions. This finding supported the reliability of the analysis. Three such regions, in the long arms of chromosomes 1, 8 and 11, were validated in an independent segregating population, and candidate genes in confidence intervals were identified among transcription factors and hormone-, stress- and cell wall-related genes.

Conclusion Overall, this work supported the hypothesis that the SP phenotype is controlled by different key genes in tomato, paving the way for the identification of novel players and novel mechanisms involved in the regulation of herkogamy.

Keywords Autogamy, GWAS, QTL, Stigma exsertion, Stigma insertion, Tomato

Introduction

To complete their reproductive function, flowers develop specialized structures that allow the fusion of male and female gametes. First, pollination is needed, with the transfer of pollen grains from different plants in outcrossing species (allogamy) or from the same flower or flowers in the same plant in selfing species (autogamy). Modifications of flower morphology during domestication often resulted in changes in mating systems [1].

In tomato (*Solanum lycopersicum* L.), the flower is hermaphrodite, typically with six to eight stamens closed by intermingling lateral hairs, forming a cone around the

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pistil. The pistil is formed by two or more fused carpels and consists of a basal part, the ovary, which contains ovules with sexual germlines, and the style, an elongated structure tipped by the expanded stigma surface. Wild tomato relatives show a stigma protruding from the staminal cone (exserted stigma) and self-incompatibility, two traits that favor allogamy [2, 3]. Domestication events caused the "selfing syndrome", which included the disruption of self-incompatibility and the evolution of homomorphic herkogamy, ultimately transforming the crop into a strict selfer [4–6]. Other floral changes occurred during tomato domestication due to several human-selected mutations affecting ovary morphology, which caused an increase in fruit size and a variation in shape [7].

The "selfing syndrome" affects the relative length of the pistil and stamens, progressively inserting the stigma position (SP) within the antheridial cone [8]. Although SP is a relative concept and is the result of the developmental dynamics of stamens, style and ovary, the correlation between SP and style length is generally greater than that between SP and other reproductive organs [9, 10]. Indeed, quantitative trait loci (QTLs) controlling style length and SP often colocalize [11], indicating that SP is determined mainly by factors affecting style growth.

The "selfing syndrome" is driven by several changes, and polygenic control of SP has been reported [10, 12]. A major QTL was identified on the long arm of chromosome 2 and referred to as *stigma exsertion 2.1* (*se2.1*; [13]). The gene underlying *se2.1* was later cloned and named *Style2.1* (*Solyc02g087860*); it encodes a polypeptide bearing a helix-loop-helix (HLH) motif involved in the control of cell expansion in the style [6]. Recently, two additional genes were involved in SP control in tomato, *Se3.1* (*Solyc03g098070*), which encodes a C2H2 zinc finger transcription factor, emerged from the screening of a population including wild and cultivated germplasm [14], and *SlLst* (*Solyc12g027610*), encoding an ethylene receptor protein identified after functional characterization of a temperature-sensitive mutant [15].

Other SP-associated QTLs have been reported studying interspecific segregating populations. Three loci were found on chromosomes 4, 8 and 9 after a cross within *S. pimpinellifolium* (*sty4.1*, *sty8.1* and *sty9.1*; [5]), and one was found on the long arm of chromosome 5 after a cross with *S. habrochaites* (*se5.1*; [16]). More recently, three positions controlling SP and style length, which are located on chromosomes 1 (*qSP1*/*qSL1*), 2 (*qSL2*) and 3 (*qSP3*/*qSL3*; [10]), were detected after plants were exposed to high temperatures. Notably, *qSL2* mapped to the same region as *Se2.1.* The *Se2.1* locus was also reported in the screening of recombinant inbred and introgression lines derived from a cross between *S. lycopersicum* and *S. pimpinellifolium* [17]. Although an inserted SP is a standard trait of modern tomatoes, traditional cultivars and landraces often retain an exerted or flush stigma [18, 19]. Studies with interspecific crosses have not yet revealed this type of SP trait variation.

An exserted SP is not only a constitutive feature of some tomato types but also a heat stress-related phenotype. Several studies have indicated that style elongation is a reaction to high temperatures, resulting in poor fertilization and low fruit set [9, 15, 20]. Because environmental conditions are important for reproductive success in tomato and other crop species [21], dissecting the genetic basis of stress-related SP sensitivity represents a key point for protecting crop yield in the context of increasing episodes of weather extremes.

Conversely, an exserted SP has been regarded as a positive trait to facilitate hybrid seed production in autogamous cereals [22, 23] and legumes [24]. This possibility was also considered in tomato; harnessing an exerted SP combined with male sterility was proposed to ease the production of hybrid seeds [15, 25]. However, the dominance of exserted over inserted phenotypes and the incomplete penetrance of the exserted SP trait limit the use of herkogamy for producing reliable tomato hybrids [12, 25].

Therefore, there is considerable interest in a deeper dissection of the genetic control of SP in tomato. Whereas QTL analysis was initially carried out using interspecific biparental populations, the development of high-throughput genotyping platforms and the availability of variation collections has opened the perspective for association mapping, which addresses the natural genome-wide distribution of markers and alleles underlying phenotypic traits [5, 26]. Genome-wide association studies (GWASs) have therefore become a powerful tool for studying quantitative traits [27]. GWAS relies on linkage disequilibrium (LD), the nonrandom co-occurrence of two or more alleles between proximal loci eventually broken down by recombination. In cultivated tomato, the extent of LD is relatively high, making it possible to perform GWASs using fewer markers than with species having lower LD [28–30]. As a drawback, high LD allows a lower resolution, and germplasm collections are often strongly structured and present minor frequency alleles.

Multiparent populations, which require crosses between more than two parental lines to generate recombinant inbred progeny, have been developed to increase the rate of LD decay. Among them, multiparent advanced generation intercrossing (MAGIC) has the advantages of the absence of structure and balanced allelic frequencies [31]. Compared with biparental progenies, such populations can therefore increase the length of genetic maps and reduce confidence intervals [32, 33].

To increase the possibility of identifying SP-associated markers, we investigated SP variation in four tomato

populations featuring different genetic backgrounds, encompassing the genetic diversity of wild, semiwild and cultivated tomatoes. SP phenotypic values were collected under different growth conditions and used to detect QTLs via association mapping approaches.

Materials and methods

Plant material

Four different tomato populations were used in the study. The Traditom Core Collection (TRA) was established in the frame of the H2020 European project "Traditional tomato varieties and cultural practices" and included 217 accessions selected to represent most of the phenotypic and genotypic variability of a larger collection [34]. TRA is composed mainly of *S. lycopersicum* landraces from, France, Greece, Italy, and Spain (Additional file 1: Table S1; [35]). The *S. lycopersicum* var. *cerasiforme* population (CER) was previously composed and characterized [36]. In this study, 132 CER accessions, including 12 *S. pimpinellifolium*, 101 *S. lycopersicum* var. *cerasiforme* and 19 admixed accessions, were used to encompass the genetic diversity of the small-fruited tomato (Additional file 1: Table S1). The MAGIC population (MAG) was developed and genotyped at INRAE after intercrossing eight founders, four representing the small-fruited group *S. lycopersicum.* var. *cerasiforme* and four the large-fruited germplasm of cultivated tomato [33]. Of 397 MAGIC lines developed by [33], 255 could be screened in the present study. Finally, the segregant interspecific population (SIP) was an F_2 progeny ($n = 96$) derived from a cross between *S. pimpinellifolium* (LA1589, with exserted stigma) and *S. lycopersicum* (LA1563, with inserted stigma). Seeds of the parent accessions were obtained from the C.M. Rick TGRC, University of California, Davis, and the SIP population was used to validate the QTLs identified via association analyses.

Plant growth conditions

For the TRA population, four plants per accession were grown in open field in Spain (UPV, 39°28' N 0°22' E, ESP), France (INRAE Avignon, 43.560' N, – 4°51' E, FRA) and Italy (University of Tuscia, Viterbo, 42°26′N, 12°04′E, ITA). CER was grown in France under the same conditions as TRA [37], whereas MAG was grown in France for two years in a greenhouse under control (MAG_N; [33]) and high-temperature conditions induced by two months of delayed sowing (MAG_H; [37]). The SIP population, together with four plants each of the two parents and of the F1 hybrid, was grown as ITA.

In all the experiments, the plants were grown in plots following standard agronomic practices. The temperature data were recorded during plant growth, and the weekly average minimum, mean and maximum values were reported.

Plant phenotyping

In all the experimental fields except MAG_N, the SP was recorded on a scoring scale as follows: 1, stigma inserted; 2, stigma at the level of the anther cone (flush stigma); 3, stigma slightly exserted (≤ 2 mm); and 4, stigma highly exserted (> 2 mm; [38]). SP scoring in MAG_N was carried out in a previous trial, where a 1 to 3 scoring scale was adopted (1, stigma inserted; 2, flush stigma; 3, stigma exserted). In TRA, CER and SIP, SP scoring was carried out two times during cultivation: at the beginning of flowering (from the 1st to 3rd truss) and during the late season (from the 6th to 9th truss). As temperatures increased during the growing season (Additional file 2: Fig. S1), the two SP measurements corresponded to increased heat stress experienced by the plants and were thus referred to as normal (N) and heat stress (H) conditions. The scoring was thus coded with the name of the trial followed by the growth condition abbreviation (i.e., in the TRA population, ESP_N describes the SP recorded in the ESP under N conditions). MAG was grown in two different years, which represented the N and H conditions [33, 37].

To study the correlation of SP with other fertility-related traits, the number of commercial fruits collected from the 1st to 4th truss (FN) and the mean commercial fruit weight (FW) were retrieved from the Traditom project dataset [35] for 186 accessions phenotyped in Italy after taxa with missing data were removed. In the same dataset, fruit fasciation (FASC) was scored as follows: 1, absent; 3, scarce, less than 5% of fruits affected; 5, intermediate, between 5% and 20%; and 7, abundant, more than 20%. Finally, the fruit shape index (SI, ratio between the polar and equatorial diameters measured on eight representative fruits) and the number of seeds per fruit (SxF, estimated after all seeds extracted from a sample of 5 to 15 fruits were weighed) were calculated. All the data were taken on an accession basis.

The SIP population was phenotyped for SP on a single-plant basis with the 1–4 scoring scale. As in the other trails, in the SIP field temperatures increased with season (Additional file 2: Fig. 1f), and we could refer to SP scoring as normal (SIP_N) and heat stress (SIP_H) conditions.

Statistical analysis of phenotypic data

SP phenotypic plasticity was calculated for each accession/line as the difference between the SP value at H and N temperatures, to obtain the ΔSP variable. For MAG, the ΔSP value was calculated after converting the MAG_H data into a 1–3 scale. Moreover, for the TRA and CER, the SP means were calculated by averaging the SPs under N and H conditions (ESP, ITA, FRA and CER). For SIP, SP values were mediated for each genotype under the two evaluation conditions (SIP_N and SIP_H).

The reproductive and fertility-related traits retrieved from the ITA trial were analyzed for pairwise Spearman correlations using the PROC CORR procedure of the SAS software package [39], and correlation coefficients were reported via Heatmapper [40].

The TRA accessions were grouped into 12 typologies according to their fruit shape, as described in [35], with slight modifications. Typologies included genotypes with flat (big or medium according to fruit size), rectangular, ellipsoid, obovoid, round (big, medium and small), oxheart, long (San Marzano and horn) and bell pepper fruits. Since the SP data did not meet the assumption of normality of residuals and no data transformation could correct this departure, a nonparametric Kruskal–Wallis's test was performed using the PROC NPAR1WAY procedure with the DSCF option to compute Dwass, Steel, Critchlow–Fligner multiple comparison analysis [39] to evaluate the differences in SP within each field.

Genotypic data

The TRA was genotyped by genotyping-by-sequencing analysis as previously reported [41]. After filtering for a minor allele frequency threshold of 5%, a maximum missing value per site and per accession of 30% and 25%, respectively, and a maximum heterozygosity per site of 50%, the final dataset included 2,708 single-nucleotide polymorphism (SNP) markers and 195 accessions. In addition, only biallelic loci were retained, and sites with fewer than 0.05% heterozygotes were set to missing.

For the CER, SolCAP genotypic data were retrieved from [36]. The maximum rate of missing data was fixed at 10% and 25% per site and per accession, respectively, and a minor allele frequency threshold of 4% was applied to discard markers with rare alleles. Sites with more than 50% and less than 0.05% heterozygotes were also discarded, giving a final dataset of 5,875 SNPs.

The MAG genotypic data was also retrieved from a previous publication [33]. Briefly, 1,536 SNPs were selected from more than four million markers detected by resequencing the genomes of the eight founder lines [42] to construct a genetic map. SNP genotyping was performed via KASPar and Fluidigm technology as described previously [33].

SIP genotyping was carried out by single-marker analysis with PCR and restriction analysis as detailed in the "QTL validation" section below.

Association mapping and linkage disequilibrium

For TRA, the analysis was carried out using 195 accessions and 12 variables, corresponding to the SP mean value, the values under N and H conditions and the ΔSP values of each field. GWAS was performed via a General Linear Model (GLM) considering the structure (Q) obtained by Principal Component Analysis (PCA, 5 components) and via a Mixed Linear Model (MLM), considering Q and the kinship matrix (K) as described previously [43]. The calculation of Q, K and subsequent GWAS analysis were performed using TASSEL v. 5.2.52 [44]. Comparison of QQ plots generated by the two analyses was used to select the model minimizing inflation. Finally, a Benjamini and Hochberg [45] procedure was adopted to control for a false discovery rate of 0.05. Significant SNP markers were pruned when R^2 was above 0.50, retaining only the one with highest determination coefficient. The GWAS for CER was performed as in TRA, using four variables (CER_N, CER_H, CER and CER_ΔSP).

The 255 MAG lines were analyzed using the two variables MAG_N and MAG_H. QTL mapping was carried out by the interval mapping procedure with the R package mpMap as reported by [46]. The mpIM command was used to perform simple interval mapping based on the regression of phenotype on the parental probabilities and estimated allelic effect for each parent.

All significant markers were converted and reported according to their position in the SL4.0 version of the genome. Each marker was named with the number of chromosome and its position (i.e., SL4.0ch01:02349292 for a SNP at base pair 2,349,292 on SL4.0 chromosome 1). The original marker name/positions, that referred to the tomato genome version SL2.50 for TRA and CER and to SL2.40 for MAG were maintained in supplementary material for proper reference.

QTL validation

To validate the selected QTLs, primer pairs targeting five chromosomal regions falling in QTL positions or in their proximity were designed for PCR testing of the SIP population (Additional file 1: Table S2). DNA was extracted from the two parents, the F_1 hybrid and 96 F_2 progeny plants, according to [47]. PCR was carried out in a volume of 10 µL, containing 5 µL of GoTaq® Green Master Mix (Promega, Madison, WI, USA), 1 µL of each primer (10 µM) and 1 µL of template DNA, and a MyCycler Thermal Cycler (BioRAD, Hercules, CA, USA) with the following program: 95 °C for 2 min; 95 °C for 30 s; 55–58 °C depending on the primer pair for 30 s; and 72 °C for 20–60 s depending on the primer pair, looping 30–40 times (Additional file 1: Table S2). For markers developed as cleaved amplified polymorphic sequences (CAPSs), a final volume of 20 µL, containing 0.5 µL of the appropriate enzyme, 2 µL of buffer and 5 µL of PCR product, was digested and analyzed via agarose gel electrophoresis. All the markers were checked for Mendelian segregation, and Spearman's correlation was performed to estimate the significance of the validation.

To estimate confidence intervals (CIs) in TRA and CER, the LD threshold baseline for each population was

calculated among all unlinked loci across different chromosomes using Plink 1.09 [48]. The 95th percentile of the unlinked r^2 distribution was designated as the LD threshold baseline for subsequent analyses. The r^2 values were plotted against the base pair genetic distance, and a second-degree locally weighted scatterplot smoothing (LOESS) analysis was conducted to fit curves of LD decay. The extent of LD across each chromosome was estimated by intersecting the LD curve with the calculated LD threshold baseline to derive the observed CIs. In MAG, the CIs were calculated as previously reported [46].

CI related to validated markers were examined in more detail. All the genes included in each CI, identified from the tomato genome annotation (ITAG4.0), were screened for style specificity according to expression data reported in CoNekT [49] or for flower specificity according to TomExpress [50]. Genes reported to be differentially expressed in previous analyses involving tomato pistils have also been identified [20, 38, 51]. For all genes in CI intervals, the number of variants retrieved in the tomato Varitome collection [8] and those polymorphic between *S. pimpinellifolium* LA1589 and the reference genome were reported. Raw reads alignment to the tomato reference genome (SL4.0, available at http://www.solgenomics.net) was performed using Burrow-Wheeler Alignment - Maximal Exact Matches with default parameters selecting uniquely aligned reads. At the end of this process, the VCF (Variant Call Format) files were obtained using VcfTools 0.1.13 [52]. Then, gene annotation and SNP effect were predicted using SNPeff v. 4.3T [53].

In addition, for the regions *se1.1* and *sty8.1*, which target MAG-derived markers, the number of candidate genes was narrowed down by contrasting the allelic effects of the parental lines [54].

Results Variation in stigma position among the studied tomato populations

The three populations studied for association mapping presented wide SP phenotypic variation. In TRA, the SP ranged from 1 to 4; the accessions showing the highest SP belonged to the flat_big, oxheart, and bell pepper types (Table 1; Fig. 1; Additional file 1: Table S3). High SP variation was also detected in the CER, with two *S. pimpinellifolium*, two *S. lycopersicum* var. *cerasiforme* and one admixed accession expressing the highest value (Table 1; Additional file 1: Table S4). Additionally, the MAG population widely varied in terms of the trait, ranging from inserted to completely exserted SP in several lines in both years of observation (Table 1; Additional file 1: Table S5).

The distribution of SP values differed depending on the population and the specific trial. The frequency of accessions with inserted or flush stigmas was greater in the TRA field trials, with the highest percentage of inserted-SP accessions in the ESP trial (Fig. 2a). In contrast, the highest percentage of exserted-SP accessions was found in CER (25.8%) and MAG_H (36.5%), the two populations carrying a relatively high representation of the wild germplasm (Fig. 2a).

To study the relationships between SP and other fertility-related phenotypes, data were retrieved for the TRA population grown in the ITA field (Additional file 1: Table S6; [35]). Strong correlations were detected among these

Table 1 Mean and range of stigma position scores and accessions with the highest values recorded in the traditom, *S. lycopersicum* var. *cerasiforme* and MAGIC populations

Population	Field	No. of accessions	SP score			Genotypes with highest SP scores	
			Mean	Min	Max	Code	Name and origin
TRA	ESP	217	1.67	1	4	TRBA1940	Cor de bou, Spain
						TRVI1830	Bell pepper, Italy
	FRA	217	1.66	1	3	TRBA1930	Cor de bou, Spain
						TRCA0910	Montserrat, Spain
						TRMO0760	Poivron Jaune, France
						TRVI1810	Bell pepper, Italy
						TRVI1830	Bell pepper, Italy
	ITA	217	1.73	1	4	TRBA1930	Cor de bou, Spain
						TRMO0510	Marmande, France
CER		136	1.94	1	4	ECU1007	*S.l. cerasiforme*, Ecuador
						LA1245	*S. pimpinellifolium*, Ecuador
						LA1582	*S. pimpinellifolium*, Peru
						Mex-114	*S.l. cerasiforme*, Mexico
						Mixture	*S. pimpinellifolium*, nd
MAG	MAG_N	255	0.42	1	3	-	21 lines scored 3
	MAG_H	255	2.19	1	4	-	24 lines scored 4

SP stigma position, TRA Traditom core collection, CER *S. lycopersicum* var. *cerasiforme* population, MAG MAGIC population, ESP Spain, FRA France, ITA Italy, MAG_N MAGIC population under normal conditions, MAG_H MAGIC population under high temperatures

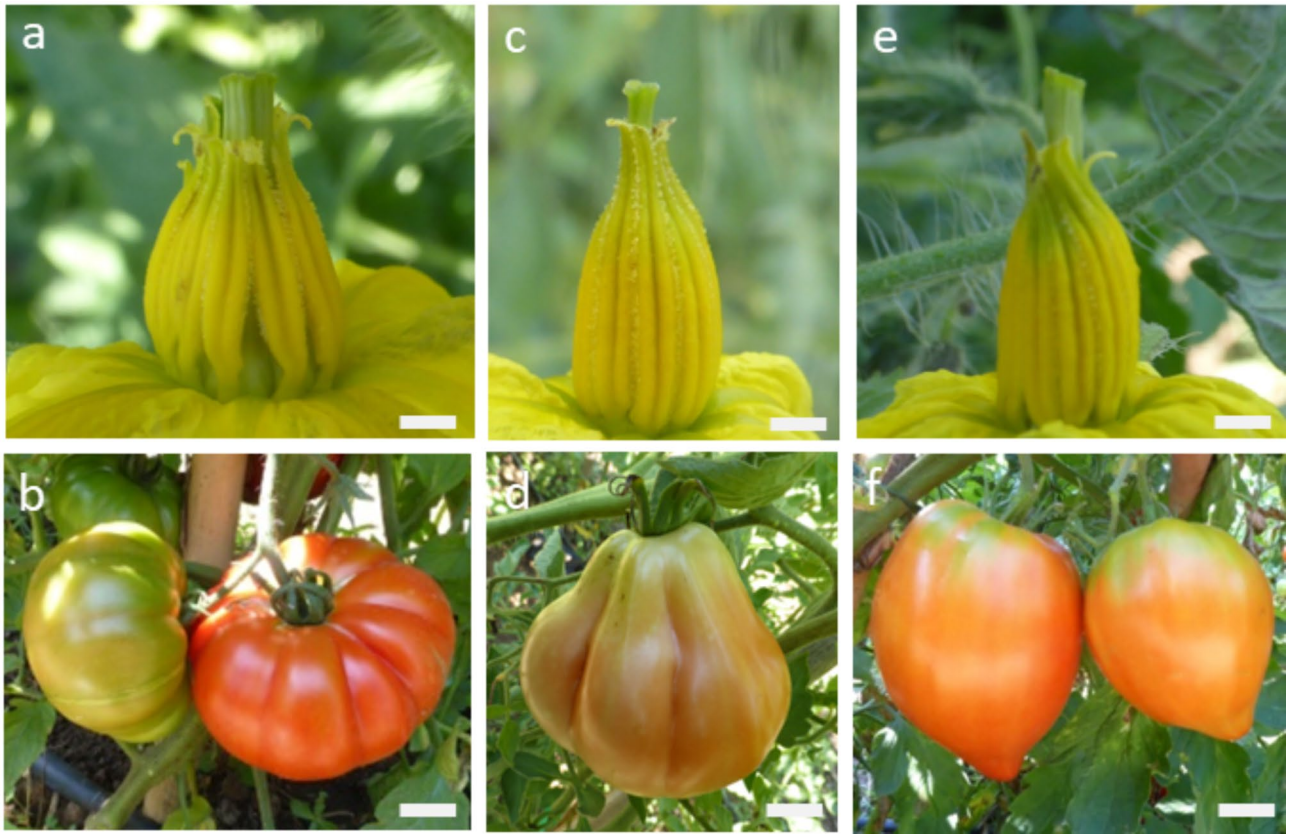

Fig. 1 Exserted stigma phenotypes and representative fruits of Traditom tomato accessions cultivated in Italy. The accessions belong to the flat_big (**a**, **b**), obovoid (**c**, **d**) and oxheart (**e**, **f**) fruit typologies. The scale bar is 2 mm in a, c, and e and 2 cm in b, d, and f

traits and among them and SP (Fig. 2b). The SP score under stressed conditions presented the greatest number of relationships, showing positive correlations with FW, FASC, and SxF and negative with SI and FN (Fig. 2b).

To describe SP variability among different tomatoes, TRA accessions were grouped into 12 typologies based on a combination of fruit shape and size (Additional File 1: Table S7). The nonparametric test, independently carried out for each field, always revealed differences among typologies (Fig. 2c; Additional file 1: Table S7). Four typologies emerged for showing exserted SP: flat_big, obovoid, oxheart and bell pepper (Fig. 2c).

Differences between normal and stress conditions were not significant in the ESP but were highly significant in the other TRA fields, with the score in H being higher than that in N (not shown). To investigate the SP stability in more detail, the ΔSP was calculated. In the TRA field, most accessions were not affected by temperature variation; when fluctuations were observed, most unstable accessions presented increased stigma exsertion under increasing temperature (Fig. 2d). Compared with TRA, the number of genotypes with SP fluctuations was greater in CER and MAG, with a proportion of genotypes showing a positive ΔSP close to or greater than 50% (Fig. 2d).

In total, 101 TRA accessions presented a positive ΔSP in at least one environment (Additional file 1: Table S3); 36 were reported in ESP, 49 in FRA and 48 in ITA (Fig. 2e). Four accessions (three with flat_medium fruit and one with round_medium fruit) presented this phenotype in all environments and 24 in two environments. To assess the extent of within-typology SP variability, the mean values of ITA_N and ITA_H were plotted for those tomato types showing, on average, exserted SP. In all types, accessions with strongly exserted SP were found, at least under high temperatures, together with accessions with more inserted stigmas; the bell_pepper type included only accessions with stably exserted SP (Additional file 2: Fig. S2).

Genotyping and GWAS analysis in the traditom (TRA) population

In TRA, the comparison of QQ plots obtained after GWAS carried out with the GLM + Q and the MLM + Q + K model revealed a lower inflation for the latter model (Additional file 2: Fig. S3); thus, MLM was retained to define SP-related QTLs. MLM analysis revealed 22 markers significantly associated with SP, whereas no significant position was detected for ΔSP. After pooling positions in LD, 12 QTLs remained,

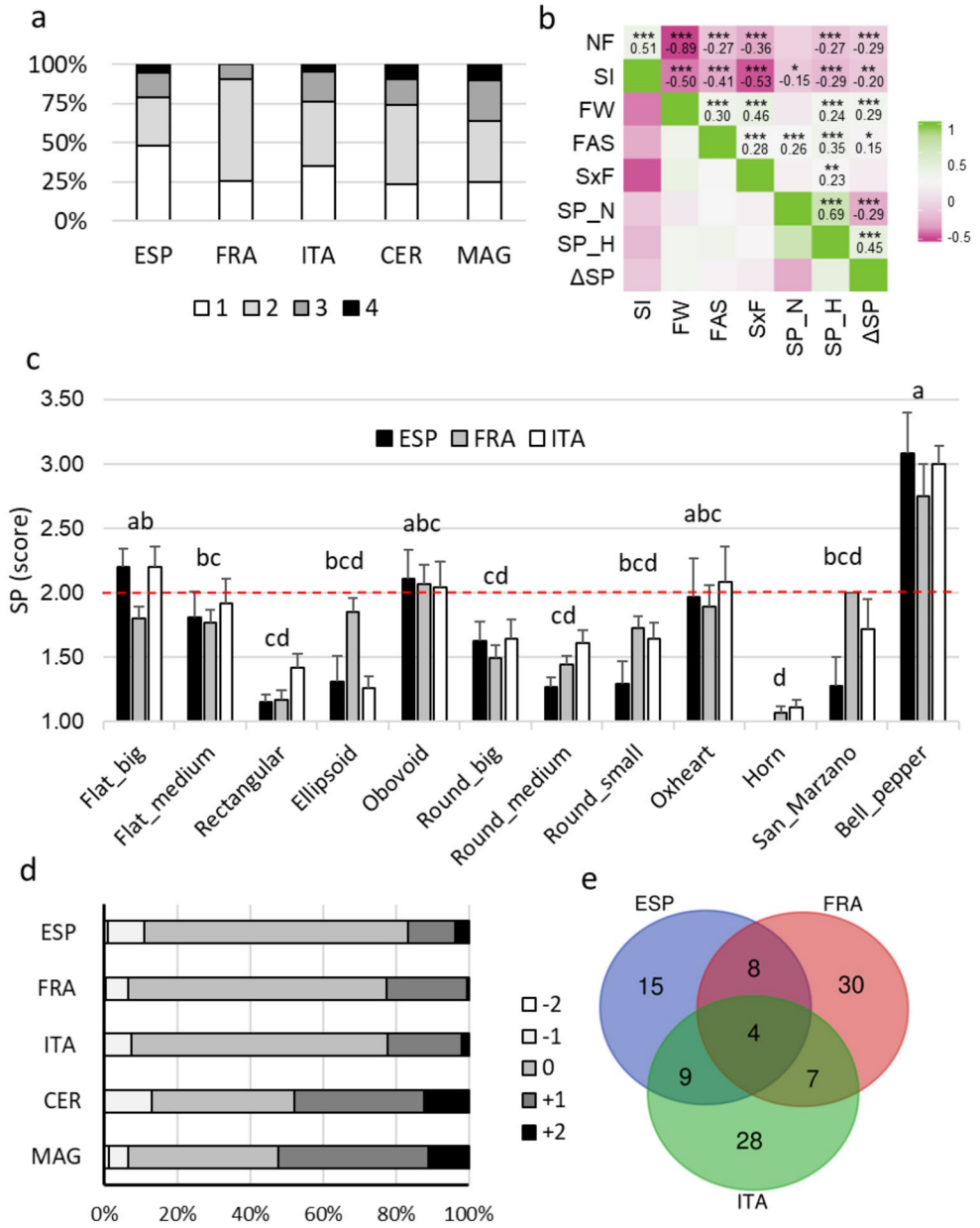

Fig. 2 Phenotyping of stigma position (SP) in the studied populations. (**a**) Mean SP distribution in the Traditom (TRA) population grown in Spain (ESP), France (FRA) and Italy (ITA), in the *S. lycopersicum* var. *cerasiforme* (CER), and in MAGIC (MAG) populations. MAG represents only values from the stressed field (MAG_H). SP was scored as follows: 1, stigma inserted; 2, stigma flush; 3, stigma slightly exserted (< 2 mm); and 4, stigma highly exserted (> 2 mm). (**b**) Correlation analysis of fertility-related traits detected in the TRA collection grown in the ITA trial. Magenta or green cells indicate a negative or positive correlation between traits; *, ** and *** indicate correlations with statistical significance for $p < 0.05$, 0.01 and 0.001, respectively; the significant correlation coefficients are reported within each cell. (**c**) SP phenotyping in the TRA collection considering the mean of two observations during the growing season in the ESP, FRA, and ITA fields, with accessions grouped into 12 typology classes. The dotted line indicates the value of the score for the flush stigma. Typologies indicated by different lowercase letters differed in all three fields after the Kruskal–Wallis test and Dwass, Steel, and Critchlow–Fligner multiple comparison analysis. (**d**) Fluctuations in SP values under increasing temperatures, shown as the percentage of TRA accessions having a negative, null or positive ΔSP in the ESP, FRA and ITA trials and in the CER and MAG populations. (**e**) Number of TRA accessions showing a positive ΔSP and degree of overlap among the ESP, FRA and ITA trials

distributed on nine chromosomes (Fig. 3; Additional file 1: Table S8; Additional file 2: Fig. S4). Three positions were detected in more than one environment. The R^2 value ranged from 7.34 to 14.91; the highest value was detected for marker SL4.0ch04:06040838, which was significant in two fields and three datasets (Fig. 3; Additional file 1: Table S8). Among the 16 QTL effects estimated, four presented an additive mode of inheritance, and seven presented a dominant mode of inheritance; five QTLs showed departures from both models

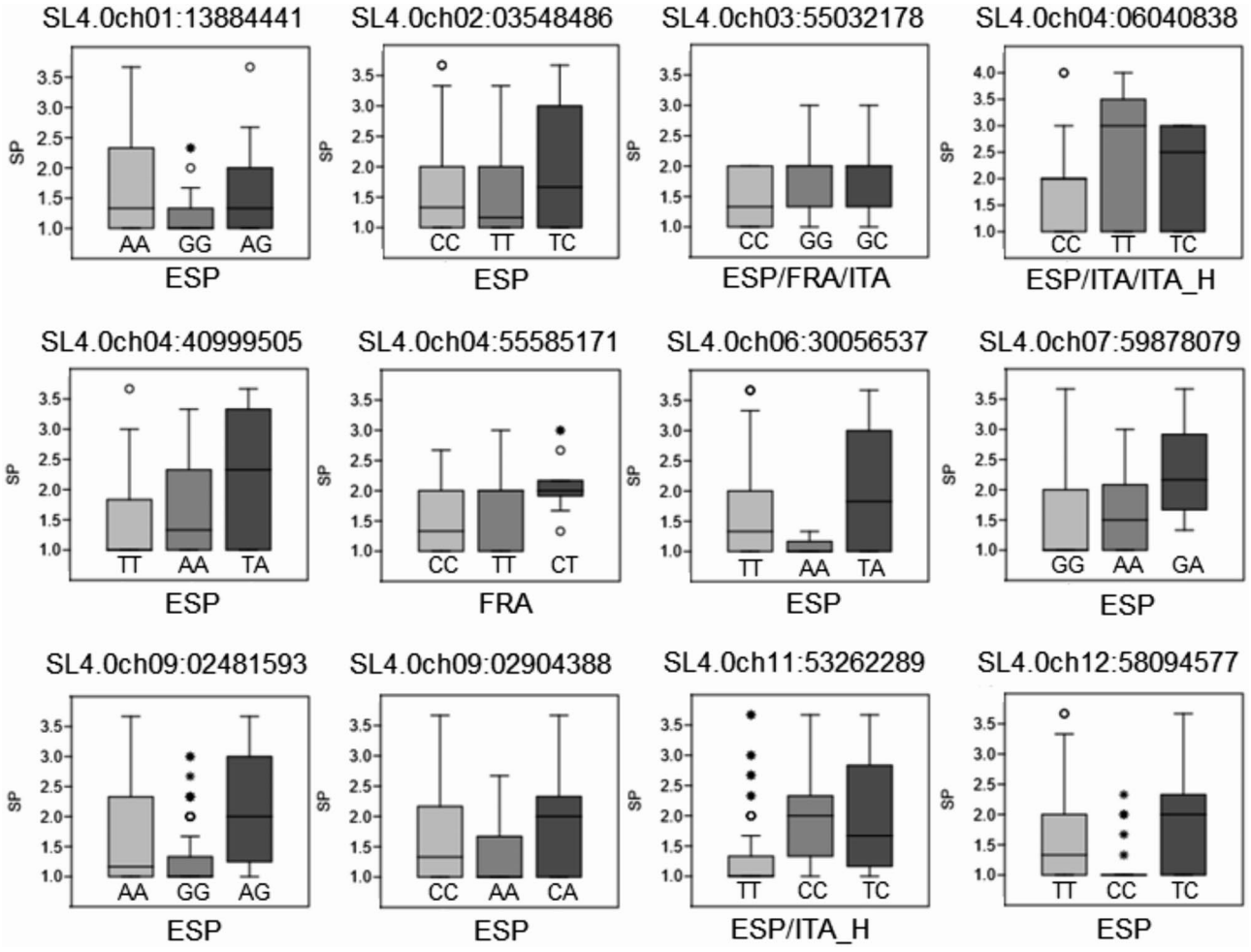

Fig. 3 Box plots showing genotypic differences for the 12 QTLs identified in the Traditom core collection (TRA). The TRA was analyzed at three locations (Spain, ESP; France, FRA; Italy, ITA) and at two developmental times corresponding to normal (N) and heat (H) conditions. Each graph reports the marker name, the stigma position (SP) distribution for all genotypes (the reference allele is reported first, *light gray bar*) and the condition(s) where the association was found. For markers significant in more than one condition, only the condition with highest R^2 is shown. Association details and statistics are reported in Additional file 1: Table S8

(Additional file 1: Table S8). Approximately half of the QTLs presented a greater SP in heterozygous individuals, indicating the presence of overdominant effects (Fig. 3; Additional file 1: Table S8).

Single chromosome LD decay at the TRA fixed baseline of 0.16 ranged between 36 and 1,286 kbp (Additional file 1: Table S9; Additional file 2: Fig. S7); the CIs estimated for each detected QTL ranged from 56 to 2,554 kbp (Additional file 1: Table S8).

Genotyping and GWAS analysis of the *S. lycopersicum* var. *cerasiforme* (CER) population

Also in CER, the analysis of two GWAS models through QQ plot comparison indicated that MLM + Q + K gave a more reliable set of associations (Additional file 2: Fig. S5). MLM yielded 17 significant SNP loci, that, after compacting the loci in LD, merged into 13 positions. Ten associations were identified in N growth conditions, sometimes supported by the analysis with mean values, two were reported only in H growth conditions, and one in all datasets (Fig. 4; Additional file 1: Table S8). R^2 ranged from 9.09 to 23.76; the highest value was detected for the QTL on the long arm of chromosome 11 (SL4.0ch11:53426830). Notably, all significant SNPs showed an additive effect; in most cases, the highest score was found in heterozygous genotypes (Additional file 1: Table S8).

Single chromosome LD decay at the CER fixed baseline of 0.25 ranged between 36 and 104 kbp (Additional file 1: Table S9; Additional file 2: Fig. S2); the CIs estimated for each detected QTL ranged from 33 to 209 kbp (Additional file 1: Table S8).

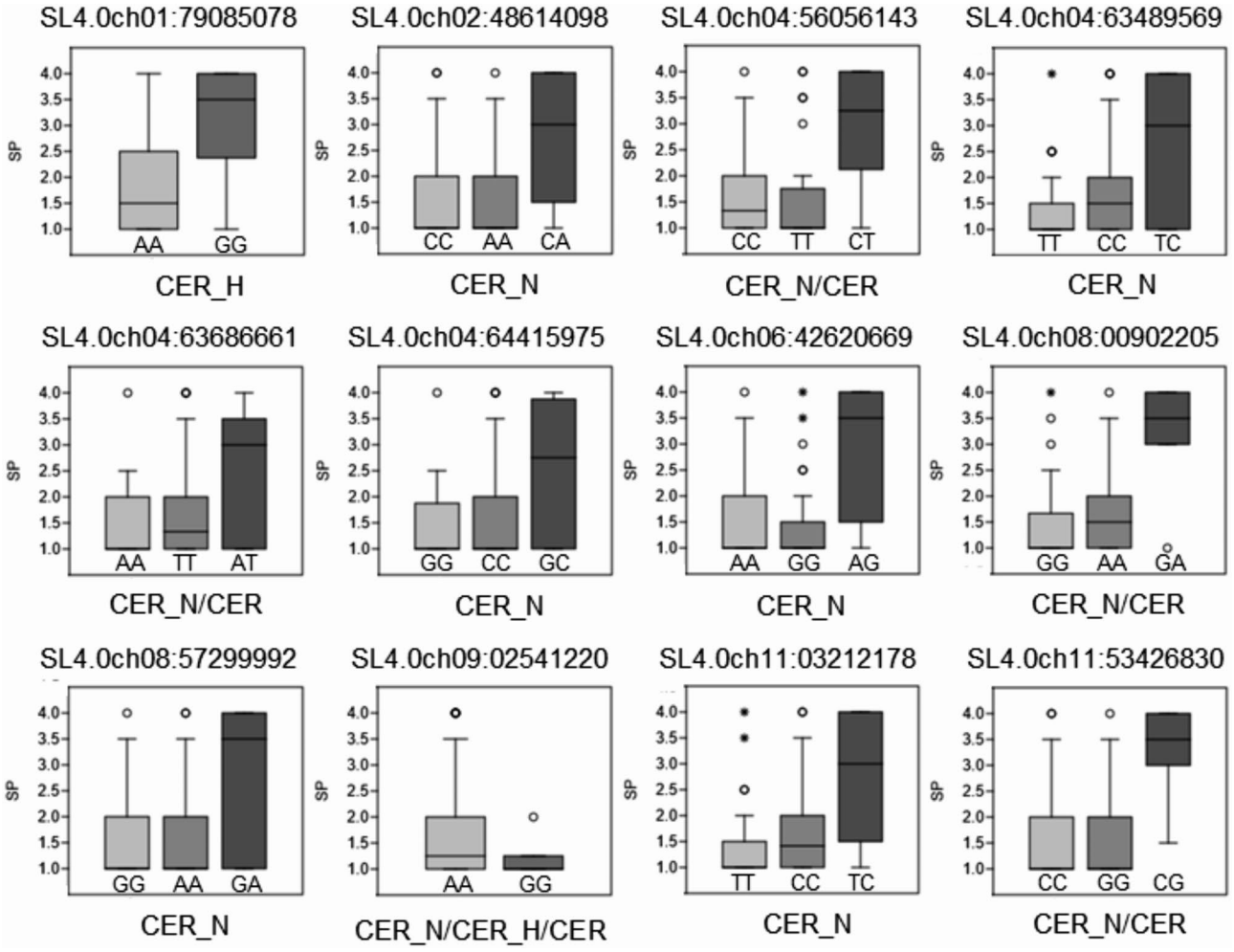

Fig. 4 Box plots showing genotypic differences for the QTLs identified in the *S. lycopersicum* var. *cerasiforme* (CER) population. The CER was analyzed at two developmental times corresponding to normal (CER_N) and heat (CER_H) conditions. Each graph reports the marker name, the stigma position (SP) distribution for all genotypes (the reference allele is reported first, *light gray bar*) and the conditions where the association was found. For markers significant in more than one condition, only the condition with highest R^2 is shown. Marker SL4.0ch01:78591972 on the long arm of chromosome 1 is not shown, because it presented the same distribution of the distally linked marker SL4.0ch01:79085078. Association details and statistics are reported in Additional file 1: Table S8

Genotyping and association analysis in the MAGIC (MAG) population

Using the datasets collected for the MAG population, 12 significant associations spread across seven chromosomes were found (Fig. 5; Additional file 1: Table S10). R^2 ranged from 4.61 to 12.82, with the highest value found for SL4.0ch02:48342204; this, together with SL4.0ch07:63560412, was the only QTL that emerged under both growth conditions (Additional file 1: Table S10). After QTL detection, the allelic effect of the eight parental lines was estimated to determine which parental alleles were in the opposite/same direction (Fig. 5; Additional file 1: Table S10).

After the significant positions detected in the different analyses were merged, the whole landscape of SNPs associated with SP was reported (Fig. 6). Several colocalizations were found, both among the QTLs first reported in this work and among them and previously reported positions.

For instance, marker SL4.0ch02:48614098, detected in CER, and marker SL4.0ch02:48342204, associated with SP in MAG, fell within a < 0.5 Mbp window, also containing the known gene *Style2.1*. On the short arm of chromosome 9, three markers from both the TRA and CER analyses colocalized in a window of about 400 kbp, as did markers from all the three analyses on the long arm of chromosome 11 (Fig. 6).

The CIs estimated for QTLs detected in MAG ranged from 0.07 to 4.00 Mbp (Additional file 1: Table S10).

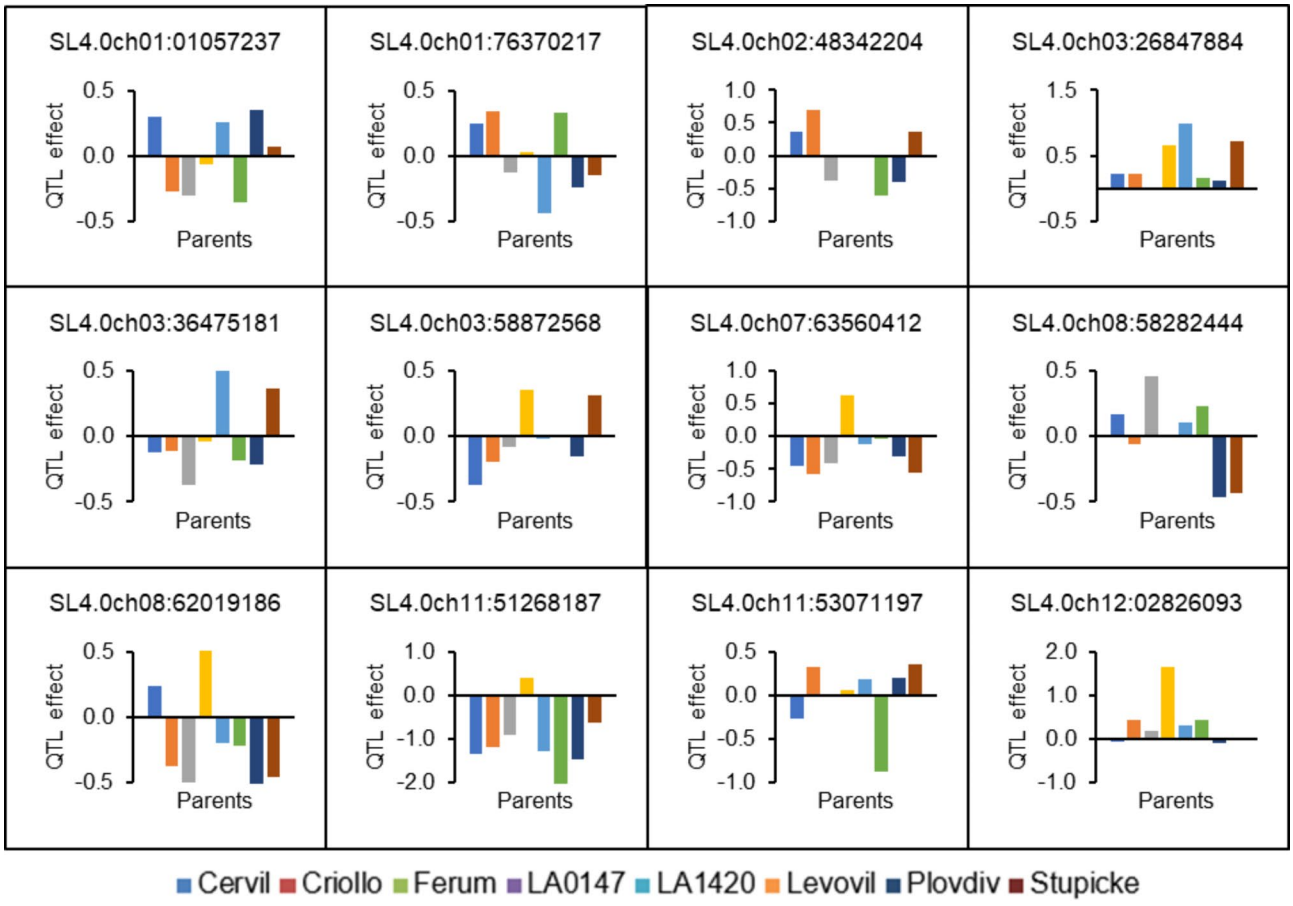

Fig. 5 Allelic effects estimated for 12 QTLs involved in stigma position detected in the MAGIC population. QTL effects were mean centered to facilitate the visualization of differences between parents

Validation of selected QTLs with a segregant interspecific population (SIP)

To validate the GWAS results, a segregant interspecific F_2 population (SIP) was created after crossing *S. lycopersicum* with a *S. pimpinellifolium* genotype contrasting for SP. When phenotyped, the cultivated and wild parents stably presented the expected SP phenotype; F_1 progeny plants presented intermediate values, whereas F_2 progeny plants showed an approximately normal segregation (Additional file 2: Fig. S6).

We attempted to validate five QTLs selected including two known (*qsp3/qsl3*, *sty8.1*) and three previously unreported positions (hereafter named *se1.1*, *se11.1*, and *se12.1*; Table 2). For *se11.1* and *se12.1*, we assessed the QTL marker itself, whereas for the other positions, we addressed markers in proximity to the selected QTL. All the tested markers showed Mendelian F_2 segregation; three of them, *se1.1*, *sty8.1* and *se11.1*, were significantly correlated with the SIP_H values. In addition, *se11.1* was also correlated with SIP_N (Table 2; Additional file 2: Fig. S6).

The CIs calculated for the validated QTLs included 36, 22 and 30 annotated genes for *se1.1*, *sty8.1* and *se11.1*, respectively; such intervals revealed 2/41, 3/26 and 4/120 variants with high/moderate predicted impact between the SIP wild parent LA1589 and the reference genome (Additional file 1: Tables S11, S12, and S13). Within QTL regions, candidates were prioritized based on reported roles in the control of pistil development, organ-specific expression or differential expression reported in previous works.

Among the genes filtered for *se1.1*, we identified several transcription factors (TFs), including a bHLH (*Solyc01g090790*, *SlbHLH005*, according to [55]), a homeobox HD-ZIP (*Solyc01g090460*), a MYB (Solyc01g090530), a GATA (*Solyc01g090760*), an ethylene-responsive (*Solyc01g090560*) and a squamosa binding protein (SBP)-like (*Solyc01g090730*) member (Additional file 1: Table S11). In addition, genes involved in cell wall metabolism, such as the elongation factor *Solyc01g090690*, and in stress response, such as the DNAj *Solyc01g090550*, were detected (Additional file 1: Table S11). Outside the CI, but tightly linked to its distal end, we found two adjacent expansin genes, *Solyc01g090810* and *Solyc01g090820*, annotated as *SlEXPB8* and *SlEXPB6* by [56] (Additional file 1: Table S11).

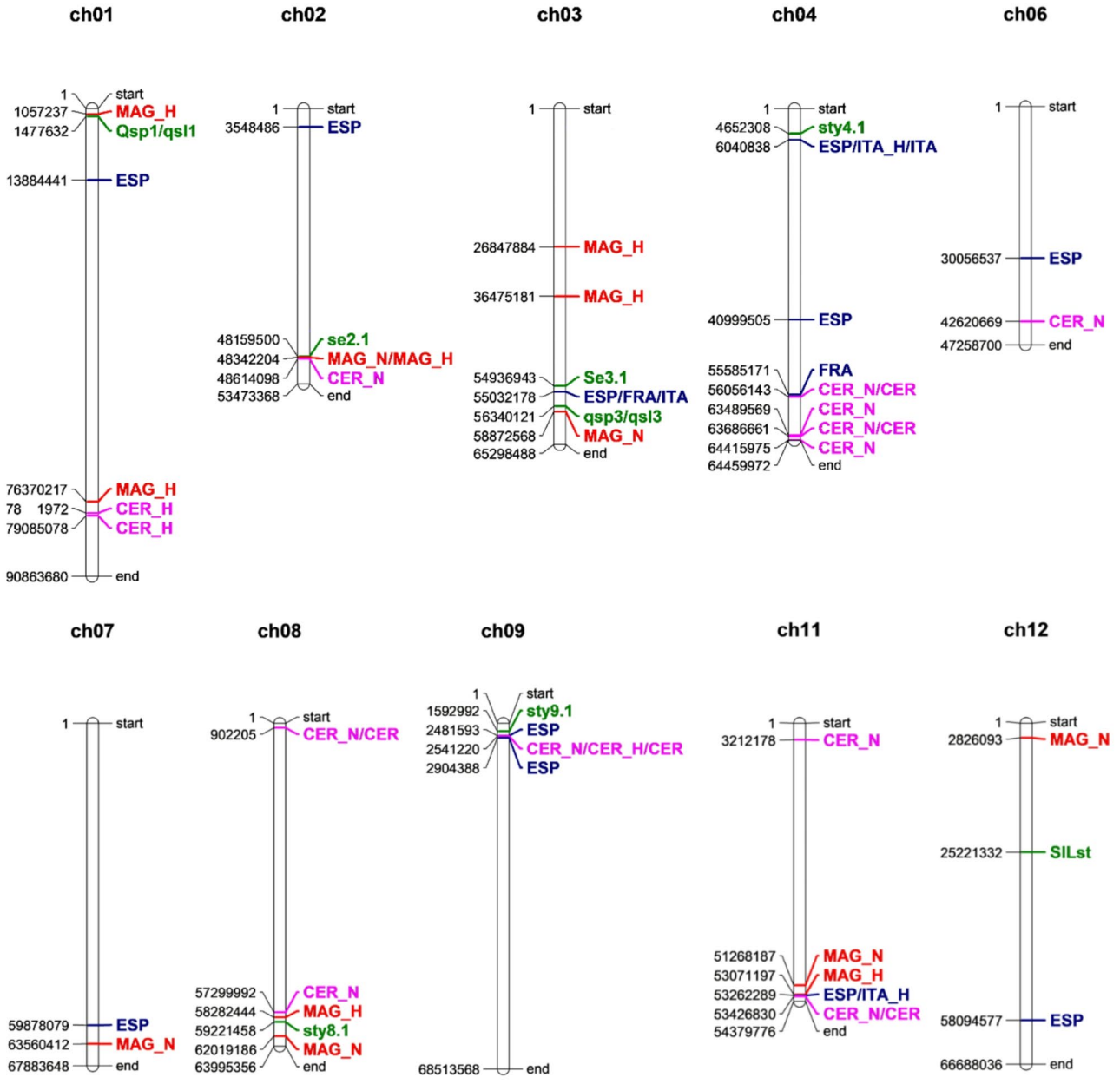

Fig. 6 Physical map of the ten tomato chromosomes hosting significant markers associated with stigma position (SP). Markers emerged in the analysis of Traditom core collection (TRA) data scored in Spain (ESP), France (FRA) and Italy (ITA), of the *S. lycopersicum* var. *cerasiforme* (CER) collection and of the MAGIC (MAG) population. All experiments included data collected under normal (N) and heat stress (H) conditions. The position in bp (SL4.0) for each marker/gene is shown on the left side of each chromosome. Significantly associated traits are shown on the right in *blue* for TRA, *pink* for CER and *red* for MAG. The previously described QTL/genes are shown in *green*; for extended names, refer to the text

Genes filtered in the *sty8.1* CI included a GAGA (*Solyc08g076230*, *SlBPC6* according to [57]), a homeobox (*Solyc08g076370*, *ROC3*), an AP2-like (*Solyc08g076380*) and a zinc-finger (*Solyc08g076390*) TF (Additional file 1: Table S12). In addition, a plastid glycolate-glycerate translocator 1 (PLGG1, *Solyc08g076290*) involved in plant biomass production [58], and genes involved in abiotic stress responses (*Solyc08g076310*) and fruit ripening (*Solyc08g076320* [59]), were reported (Additional file 1: Table S12).

In the *se11.1* CI, we found a YABBY (*Solyc11g071810*) and a MYB (*Solyc11g072060*) TF. Genes belonging to families involved in stress response, such as two DNAj [*Solyc11g071830*, also named *HEAT SHOCK FACTOR* (*HSF*) *40*, and *Solyc11g071930*] and a SUN (*Solyc11g071840*, *SUN31*) gene, have also been reported. Finally, we found a protein kinase domain

Table 2 QTLs and markers chosen for validation and results of marker/trait correlation

Targeted QTL(s)			Marker position		Spearman's coefficient with the trait in validation:	
Name	Marker name/position	Related SP trait(s)	Position (SL4.0)	Segregation (X^2 for 1:2:1)	correlation	
					SIP_N	SIP_H
se1.1	SL4.0ch01:76370217	MAG_H	76,660,779	2.06	0.134	0.304**
qsp3/qsl3	SL4.0ch03:58872568	MAG_N	61,254,126	3.56	0.144	0.136
sty8.1	SL4.0ch08:58282444	MAG_H	58,081,192	0.56	0.148	0.243*
se11.1	SL4.0ch11:53426830	CER_N, CER	53,426,732	1.83	0.223*	0.218*
se12.1	SL4.0ch12:58094577	ESP	58,094,577	2.67	-0.077	0.024

SIP segregating interspecific population, MAG MAGIC population, CER *S. lycopersicum* var. *cerasiforme* population. N and H represent normal and high-temperature conditions, respectively. * and ** indicate significant correlations for $p \leq 0.05$ and $p \leq 0.01$, respectively

(*Solyc11g071820*) and a self-incompatibility protein gene (*Solyc11g071900*) associated with fruit traits [8, 60], and *Solyc11g071940*, annotated as the *Cell Size Regulator* (*CSR-D*) locus, whose mutation underlies the fruit weight QTL *fw11.3* [61].

The *se11.1* CI spanned the region that included the *FASCIATED* (*FAS*) gene (*Solyc11g071810*), whose mutation causes an increase in fruit locule number in tomato [62], a phenotype positively correlated with exserted SP. To assess whether the significant QTL markers in the *se11.1* position were specifically caused by the *fas* mutation, we subsampled 147 TRA genotypes that showed no phenotypic evidence of fasciation; the allelic effect of marker SL4.0ch11:53262289 remained significant in the ESP and ESP_H datasets (Additional file 2: Fig. S9b). Similarly, in the CER population, we subsampled 77 accessions with the wild-type *FAS* allele [63]; in these genotypes, the allelic effect of marker SL4.0ch11:53426830 was also maintained, showing strong overdominance, as in the whole CER population (Additional file 2: Fig. S9d).

Discussion

Despite its importance for the mating system and fertility, relatively few studies have analyzed the control of tomato flower morphology compared with studies addressing fruit proximal traits and primary metabolites. In the past, authors reporting SP-related QTLs have studied mainly plant materials obtained from biparental crosses between wild and cultivated genotypes [5, 10, 13, 16, 17]. Here, we analyzed SP associations in four populations differing in their relative composition of wild and cultivated germplasms. The TRA represented the variation in European landraces, which are generally characterized by large fruits (87% of the accessions had a mean fruit weight > 50 g; [35]). The CER sampled small-fruited tomatoes, which represented spontaneous *S. lycopersicum* var. *cerasiforme*, *S. pimpinellifolium* and admixed types (98% of the accessions had a mean fruit weight < 50 g; [36]). Finally, MAG was derived from a multiparental cross of eight founders representing both cherry and large-fruited types [33].

In addition, the choice of two association analysis strategies represented a complementary approach; with GWAS, we expect to detect major alleles in populations with different evolutionary histories, whereas with MAGIC, we exploit the variability of the founder genotypes, emphasizing alleles with minor frequency in the whole tomato collection. Thus, this study aimed to maximize the possibility of QTL detection and to strengthen the reliability of QTLs that were reported in more than one analysis. For validation, we adopted an interspecific population because four out of the five targeted QTLs emerged from materials that include wild germplasm.

Stigma position widely varied across the studied populations

All the SP classes were found in each of the populations studied. When the TRA accessions were grouped into fruit typology classes, the flat_big, obovoid, oxheart and bell_pepper genotypes presented the highest SP values. These types are characterized by multilocular and large fruits. A greater proportion of accessions with exserted SP was found in CER and MAG; in the latter, the distribution was more even, which was expected, as three of the founders had exserted SP (Additional file 1: Table S5). Thus, accessions with exserted stigmas are found in both small-fruited wild genotypes and large-fruited cultivated typologies; the set of mutations controlling its variation is likely to only partially overlap in the two panels.

In the TRA core collection, that is essentially composed of cultivated germplasm, SP was negatively correlated with FN and SI and positively correlated with FW, FASC and SxF. This confirmed that in domesticated tomatoes stigma exsertion is more common in varieties with large, fasciated, and flattened fruits, as seen in the fruit typology analysis.

Stigma position responds to environmental variation in a genotype-specific manner

Environmental stress affects tomato reproductive development [64–66]. One of the issues of tomato growth at relatively high temperatures is the induction of stigma exsertion, which hampers self-pollination and fruit set

[20, 67]. Monitoring SP trends under normal and heat growth conditions revealed that SP sensitivity to high temperatures is genotype specific. In fact, we detected accessions with stable SPs (inserted or exserted) and others with variable SPs under different conditions.

The analysis revealed that the pistil length of most of the temperature-sensitive accessions increased with increasing temperature, suggesting that heat stress has an inductive effect on style elongation. This result broadens the number of SP-controlling candidates to genes related to abiotic stresses.

Association analysis of stigma position in different populations revealed new significant sites while confirming known QTLs

Three genes involved in the control of SP variation have been cloned in tomato. *Se2.1* is considered the major determinant in the evolution from allogamy to autogamy. Consequently, the *se2.1* mutation is essentially fixed in the cultivated tomato [6]. As a major gene, *Se2.1* has emerged in other studies, irrespective of the temperature regime ([10, 17]; this work). *Se3.1* was subsequently identified in a tomato GWAS [14], confirming the SP-related structural variant emerged in the analysis of bulked transcripts of genotypes with contrasting SPs ([38]; A. Riccini and A. Mazzucato, unpublished results). The *se3.1* variation was associated with the shift from flush to inserted stigmas, which occurred in the domesticated germplasm [14]. Here, we also detected a significant marker near *Se3.1* in all environments considered in the TRA analysis, a population which is mainly composed of cultivated germplasm. Finally, *SlLst*, a mutant that exerts SP at high temperatures, is linked to an EIN4-like ethylene receptor, involving this hormone in the SP phenotype [15]. We did not find a position linked to this genetic determinant, possibly because it represents a variant that is not diffused in the tomato germplasm.

Other previously described QTLs were confirmed by our study, such as *qsp1*/*qsl1* [10], which is coincident with a marker found in MAG_H, and *qSP3*, which colocalizes with a MAG_N marker. In addition, *sty4.1*, *sty8.1* and *sty9.1* [5] were confirmed by our analysis. Among the known QTLs or genes involved in SP control, we did not find significant markers near only *se5.1* [16] or *SlLst* [15].

Among the novel QTLs revealed by this study, the positions detected in more than one population deserve more attention. A novel QTL was found in the long arm of chromosome 1, indicated by three markers in MAG_H and CER_H, which were located within 2.2 Mbp. These positions span the overlapping region of the introgression lines IL1-1 and IL1-2, where an exserted SP has been reported [68], thus supporting the existence of a QTL in the region. Four markers from six different analyses, spanning approximately 2 Mbp, revealed an unreported QTL on the long arm of chromosome 11; this position was detected under both normal and heat conditions and yielded some of the highest R^2 values in TRA and CER.

Several of the QTLs described presented the highest genotypic mean value in heterozygotes, in accordance with reports of a high frequency of overdominance and epistatic effects in reproductive traits [69]. More specifically, positive heterosis was reported for style length in six tomato hybrids involving a wild species in the pedigree [70], indicating that epistatic and overdominant effects may play an important role in SP control.

QTLs on chromosomes 1, 8 and 11 were validated in an independent biparental population

To validate the GWAS results, five QTLs were tested on an interspecific F_2 population segregating SP. Two markers failed to produce a significant relationship, the one used for *qsp3/qsl3*, probably because it was too far from the QTL and *sty12.1*, probably because the QTL was found only in the TRA population, and the relative polymorphism may not segregate in the biparental validation progeny.

In contrast, *se1.1* was validated in the SIP population; among the genes contained in the CI, we identified TFs potentially involved in SP control, such as *SlbHLH005*, which is similar to *SPATULA* (*SPT*) and *UNE10* [55], two Arabidopsis genes involved in carpel development [71]. This gene presents variants with high predicted impact. Two TFs mentioned as miRNA targets in heat-stressed pistils [20] were also found in this CI, the HD-ZIP *Solyc01g090460* and *R2R3MYB104*; both present allelic variants with low and moderate predicted impact (Additional file 1: Table S11). Two genes involved in cell wall metabolism were found distal to the CI, including *EXPB6*, a pistil-specific gene expansin described in tomato fruit set [72] and presenting variants with high predicted impact. Expansin family members have previously been shown to be involved in style elongation as SPT interactors [73].

The marker tested on chromosome 8 was estimated to be approximately 1 Mb from *sty8.1*, a QTL previously reported with a very high R^2 value [5]. The strong overdominance reported for this locus in the CER population supports the hypothesis that other factors apparently modify the effect of the QTL on the phenotype [5]. In this CI, the gene encoding the homeobox-leucine zipper protein ROC3 was found; this gene presented a variant with moderate impact, also reported between *S. pimpinellifolium* LA1589, the wild parent of the SIP F_2, and the reference genome. *ROC3* has a pistil-specific paralog on chromosome 4 [72]. *SlBPC6* is a member of the Barley B Recombinant/Basic PentaCysteine (BBR/BPC) gene family. BBR/BPC TFs are reported to control flower development, size of the stem cell niche and seed development

through transcriptional regulation of homeotic transcription factor genes [74]. The spatial expression of *SlBPC6* and its differential transcription under several abiotic stresses [57] make it a plausible candidate to control pistil development.

The QTL found on chromosome 11 was positively validated, with a significant effect under both normal and stress conditions. The calculated CI spans 30 coding sequences, including genes related to stress response, such as *HSF40*, which is involved in the response to heat, cold, and plant hormones. HSF40 is a pistil- and stamen-specific protein and is regulated during the reproductive phase [75]. HSFs are candidate SP-controlling genes, together with genes involved in the metabolic pathways of abscisic and salicylic acid, ethylene, oxidative bursts, and calmodulin, which are known to play significant roles in the heat stress response [76–78]. Because herkogamy can rapidly evolve in response to environmental changes, such as variations in pollinator communities or abiotic factors [79], it is plausible that stress-related genes are involved in SP determination. In addition, the existence of several low- and moderate-impact variants, also detected in comparison with LA1589, makes it a good candidate for controlling SP in tomato.

Among the genes involved in cell wall remodeling, the region contained SUN31 (*Solyc11g071840*), encoding a calmodulin-binding protein expressed during flower and fruit development [60, 80]. In parallel with the other CIs, in *se11.1* we reported genes related to hormone action, such as the IAA biosynthetic gene *YABBY2b*. *YABBY2b* is involved in the inversion, including the *CLAVATA3/ESR-related* gene (*Solyc11g071380*), which is responsible for the *fas* phenotype [81]. *FAS* is a major gene in the control of locule number in tomato; knockout mutations confer fasciation and, generally, a flat fruit shape [82]. Accessions with fasciated fruits are frequent in TRA; such a phenotype is associated with SP because stigma exsertion is more frequent in such fruit types. The involvement of YABBY TFs in style elongation of *Mimulus lewisii* [83] supports a direct control of FAS on SP. However, the mechanisms underlying the correlation between YABBY genes and style exsertion remain to be elucidated because loss-of-function mutants present a reduction in style length in *Mimulus* [83] but an increase in style length in tomato [81]. The analysis of subsamples of individuals not carrying the *fas* mutation in TRA and CER confirmed a significant QTL on the long arm of chromosome 11, indicating that at least a second gene is involved in SP regulation in this region.

Dissecting stigma position control aids in breeding more adapted and resilient tomato varieties

The identification of QTLs impacting on SP will help the selection of tomato varieties with improved performance. Enhancing the stability of stigma insertion is important to guarantee sufficient pollination, that in turn impacts fruit set and fruit size. In fields for seed production of open pollinated varieties, or for the multiplication of F_1 hybrid parental lines, inserted SP protects from accidental crosses, that may lower the uniformity and quality of seed stocks.

Such breeding goals will acquire more importance in the future, because traditional varieties, often affected by a flush or exserted stigma, are rediscovered by consumers in the fresh tomato market and will need improvement for SP. Moreover, table tomatoes often belong to the flattened-ribbed typology [84] that, depending on the *fas* mutation, facilitates SP exsertion. Breeding fasciated tomatoes with inserted SP will be a future goal. Most importantly, it will be crucial to identify those QTLs that underly the sensitivity of SP to increased temperatures, in the view of breeding genotypes more resilient to climatic changes [19].

To achieve these needs, the QTLs described here should be further validated; possibly, flanking markers should be identified and the genetic windows narrowed by fine mapping. This will prioritize candidates and drive functional analysis. Notably, tomatoes showing extremes in SP exsertion are often not listed among model genotypes, being wild, semi-wild or traditional accession; this could require developing tailored regeneration and transformation protocols.

Conclusions

This work demonstrated that the SP phenotype is controlled by different key genes in tomato. The loss of exsertion occurred with domestication through recessive mutations (i.e., *se2.1*) and was later associated with genetic changes that increased stigma insertion in modern varieties (i.e., *se3.1*). In addition, single mutations, which directly or indirectly affect style elongation, and genes responsible for stress sensitivity are likely to contribute to the phenotype in a genotype-specific manner (i.e., *SlLst*). This study identified both known and novel loci that play a role in these pathways. Future research will explore the underlying genes as well as the mechanisms involved in herkogamy regulation.

Abbreviations

ARF	Auxin response factor
CAPS	Cleaved amplified polymorphic sequence
CER	S. lycopersicum var. cerasiforme population
CI	Confidence interval
ESP	Experimental trail carried out in Spain
FAS	Fasciated gene
FASC	Fasciation
FN	Fruit number
FRA	Experimental trial carried out in France
FW	Fruit weight
GA	Gibberellin
GLM	General linear model

GWAS	Genome-wide association study
H	High-temperature growth conditions
HLH	Helix-loop-helix
HSF	Heat shock factor
IAA	Indol-acetic acid
ITA	Experimental trial carried out in Italy
LD	Linkage disequilibrium
MAG	MAGIC population
MLM	Mixed linear model
N	Normal growth conditions
PCA	Principal component analysis
QTL	Quantitative trait locus
S-ADEN	S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase gene
SI	Shape index
SBP	Squamosa binding protein
SIP	Segregant interspecific population
SNP	Single-nucleotide polymorphism
SP	Stigma position
SPT	Spatula gene
SxF	Seeds per fruit
TF	Transcription factor
TRA	Traditom core collection
VCF	Variant call format

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06449-2.

Supplementary Material 1: Table S1. Seed source and geographic origin for accessions included in the study. Table S2. Markers used for the validation of QTLs associated with stigma position (SP). Table S3. Phenotypic data collected for the stigma position (SP) in the Traditom core collection. Table S4. Phenotypic data collected for the stigma position (SP) of the ***S. lycopersicum*** var. ***cerasiforme*** population. Table S5. Phenotypic data collected for stigma position (SP) in parents and lines of the MAGIC population. Table S6. Fruit phenotypic data collected from the Traditom core collection grown in Italy. Table S7. Classification of the Traditom core collection into fruit typology classes. Table S8. SNPs significantly associated with stigma position (SP) in the Traditom core collection (TRA) and in the ***S. lycopersicum*** var. ***cerasiforme*** (CER) populations. Table S9. LD decay at fixed baselines for each chromosome. Table S10. Significant SNPs associated with stigma position (SP) in the MAGIC population. Table S11. Genes annotated within the ***se1.1*** confidence interval. Table S12. Genes annotated within the ***sty8.1*** confidence interval. Table S13. Genes annotated within the ***se11.1*** confidence interval.

Supplementary Material 2: Figure S1. Temperatures registered in the experimental fields. Figure S2. Stigma position (SP) registered within selected typologies of the Traditom core collection. Figure S3. QQ plots detected for the GWAS analysis in the Traditom core collection. Figure S4. Manhattan plots detected for the GWAS analysis in the Traditom core collection. Figure S5. QQ and Manhattan plots detected for the GWAS analysis in the ***S. lycopersicum*** var. ***cerasiforme*** population. Figure S6. Validation of QTLs associated with stigma position (SP). Figure S7. LD decay inferred using locally weighted scatterplot smoothing in the Traditom core collection. Figure S8. LD decay inferred using locally weighted scatterplot smoothing in the ***S. lycopersicum*** var. ***cerasiforme*** population. Figure S9. Genotypic means for chromosome 11 SNPs associated with the stigma position (SP) in whole collections and in filtered subsamples with wild type allele at the ***FAS*** gene.

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Author contributions
A.M., A.G., J.P., A.J.M., and M.C. conceptualized and coordinated the research; A.G. coordinated the Traditom and Harnesstom projects; A.R., I.D., Y.C., S.S., M.R.F. performed the cultivation and phenotyping of the populations; A.M., A.R., F.O., B.F., S.S. curated phenotypic data; A.M., A.R., F.O., B.F., F.B. performed GWAS and bioinformatic analyses; B.F. and F.O. performed validation analysis; A.R., F.O., B.F., A.M., M.C. prepared figures and drafted the manuscript. All authors reviewed the manuscript.

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Data availability
The authors confirm that all the data from this study are available in the manuscript and its Supplementary Information. Genotypic data were derived from previous studies [33, 36, 41]. Data access for all the databases used is open.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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