Coding SNPs analysis highlights genetic relationships and evolution pattern in eggplant complexes

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Abstract

Brinjal (Solanum melongena), scarlet (S. aethiopicum) and gboma (S. macrocarpon) eggplants are three Old World domesticates. The genomic DNA of a collection of accessions belonging to the three cultivated species, along with a representation of various wild relatives, was characterized for the presence of single nucleotide polymorphisms (SNPs) using a genotype-by-sequencing approach. A total of 210 million useful reads were produced and were successfully aligned to the reference eggplant genome sequence. Out of the 75,399 polymorphic sites identified among the 76 entries in study, 12,859 were associated with coding sequence. A genetic relationships analysis, supported by the output of the FastSTRUCTURE software, identified four major sub-groups as present in the germplasm panel. The first of these clustered S. aethiopicum with its wild ancestor S. anguiv; the second, S. melongena, its wild progenitor S. insanum, and its relatives S. incanum, S. lichtensteinii and S. linneanum; the third, S. macrocarpon and its wild ancestor S. dasyphyllum; and the fourth, the New World species S. sisymbriifolium, S. torvum and S. elaeagnifolium. By applying a hierarchical FastSTRUCTURE analysis on partitioned data, it was also possible to resolve the ambiguous membership of the accessions of S. campylacanthum, S. violaceum, S. lidi, S. vespertilio and S. tomentosum, as well as to genetically differentiate the three species of New World Origin. A principal coordinates analysis performed both on the entire germplasm panel and also separately on the entries belonging to sub-groups revealed a clear separation among species, although not between each of the domesticates and their respective wild ancestors. There was no clear differentiation between either distinct cultivar groups or different geographical provenance. Adopting various approaches to analyze SNP variation provided support for interpretation of results. The genotyping-by-sequencing approach showed to be highly efficient for both quantifying genetic diversity and establishing genetic relationships among and within cultivated eggplants and their wild relatives. The relevance of these results to the evolution of eggplants, as well as to their genetic improvement, is discussed.
Eggplant, also known as brinjal eggplant or aubergine (Solanum melongena L., Solanaceae, 2n = 2x = 24), is cultivated worldwide and is one of the most important vegetable crops, being the second most important solanaceous crop grown for its fruit after tomato (S. lycopersicum L.) [1]. The bulk of eggplant production is concentrated in China, India, Iran, Egypt and Turkey, with Italy and Spain representing the most important European Union producers [1]. Because of its importance as a staple vegetable food in many countries from tropical and sub-tropical regions, eggplant is included with 34 other food crops in the Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture [2]. Eggplant berries are a source of dietary minerals as well as vitamins and other health-promoting metabolites such as anthocyanins and chlorogenic acid, with nutraceutical and anti-oxidant properties [3–6].

Brinjal eggplant selection and breeding over the years have been mainly focused on the improvement of fruit traits [7], such as size, weight, color, and shape [8,9], reduced prickliness, yield potential [10], and more recently organoleptic, nutritional and bioactive properties [11–14]. This has resulted in the development of a large number of eggplant varieties, whose fruit shape varies from flattened to elongated. However, like in many other domesticates, anthropogenic selection has resulted in a drastic reduction of the genetic variation across eggplant genome, due to both the genetic bottleneck resulting from the sampling process of a limited number of wild plants chosen for domestication [15] and the migration of a limited number of genotypes from the primary to the secondary centers of domestication [16]. The eggplant inter-fertile cultivated species as well as crop wild relatives (CWRs) are a source of variation for many traits of interest and represent an obvious target to aid eggplant improvement; however, to date, their potential use has largely remained unexploited [17].

Unlike tomato (S. lycopersicum), potato (S. tuberosum L.) and pepper (Capsicum spp.), eggplant is native to the Old World and was independently domesticated from S. insanum L. in the Indian subcontinent and in China [16,18], with a possible additional and independent center of domestication in the Philippines [19]. Besides S. melongena, two other eggplant species are commonly grown in sub-Saharan Africa [20], the scarlet eggplant (S. aethiopicum L.) and the gboma eggplant (S. macrocarpon L.). Both species can be inter-crossed with brinjal eggplant producing hybrids with intermediate fertility [21]. The three cultivated eggplants belong to the Leptostemonum clade and to a species-rich subclade composed exclusively of Old World taxa (the Old World clade sensu [22,23]) from Africa, Australia, and Asia (including Eurasia and the Middle East).

Scarlet eggplant (S. aethiopicum L.) is an important vegetable in Central and West Africa, but it is also cultivated in the Caribbean and Brazil as well as in some areas of South Italy [24]. It is a hypervariable species and includes hundreds of local varieties [25] clustered in four main cultivar groups: Aculeatum, Gilo, Kumba and Shum, which are completely inter-fertile [21]. The four cultivar groups are differently exploited, since Aculeatum is used as ornamental, Gilo for its fruits, Kumba for both fruits and leaves, while Shum for its leaves [25,26]. Gboma eggplant (S. macrocarpon L.) is less widespread in cultivation, although the species represents a major vegetable in some countries like Benin and in the rain forest regions of Coastal Africa and Congo River [27]. It is also a morphologically variable species and it is grown for its fruits, leaves or both [25,26]. The high variability within both scarlet and gboma eggplants has been recently confirmed by Plazas et al. [21], whom by applying conventional descriptors as well as the high-throughput Tomato Analyzer phenomics tool characterized a wide set of accessions of both cultivated species as well as from the scarlet eggplant wild ancestor S. anguivi Lam., S. aethiopicum-S. anguivi intermediate forms, and the gboma eggplant wild ancestor S. dasyphyllum Schumach. & Thonn. Each of the three cultivated eggplants together with their wild
ancestors and the closest wild relatives are commonly referred to as the brinjal, scarlet and gboma eggplant complexes [10,18,21,26].

Wild relatives of cultivated eggplants, which are well adapted to grow in a wide range of conditions, from desert to swampy areas and environments with wide ranges of temperatures, are a source of useful traits for eggplants breeding. Unfortunately the latter remain largely unexploited and a limited number of reports on the use of the variation available in the wild species has been reported [17,20,28] while, to our knowledge, no modern commercial varieties of eggplants carry introgression from wild species.

In brinjal eggplant and related species the delimitation of biologically meaningful genepools is challenging due to limited crossability data reported in literature [29], as well as to the extremely large number of potential genepool members. By taking into account both relatedness, as measured by phylogenetic analyses and available data on crossability, recently Syfert et al. [30] suggested the inclusion of one species (S. insanum) in the primary genepool (GP1), forty-eight species with which eggplant can be inter-crossed with varying degrees of difficulty in GP2, and three wild and weedy species native to the New World in GP3, i.e. S. sisymbriifolium Lam., S. torvum Sw. and S. viarum Dunal, with which only highly sterile hybrids can be obtained through embryo rescue or are not obtainable.

The great advances in next generation sequencing (NGS) technologies, with rapid increases in data volumes and quality combined with reducing costs, have provided breeders with a wide array of genomic tools which facilitate the characterization of germplasm collections and allow to gain a better understanding of how the genome contributes to the diversity detected at phenotypic level [31]. Single nucleotide polymorphisms (SNPs) represent the most frequent type of genetic polymorphism and have become the marker of choice for many applications in plant biology, conservation and breeding [32].

Here we report a genotype by sequencing (GBS) approach based on reducing genome complexity to detect SNPs polymorphisms in a set of seventy-six accessions of species belonging to the brinjal, gboma and scarlet eggplant complexes, which include taxa included in the S. melongena primary, secondary and tertiary genepools. Our main goal was to assess, using a high-throughput genotyping technique, the genetic relationships within and between the genepools of the brinjal eggplant (S. melongena) and the two other cultivated eggplants, namely the scarlet (S. aethiopicum) and gboma (S. macrocarpon) eggplants. Apart from cultivated accessions, we also included in the study accessions of close wild relatives of the three crops, as well more distant species from the tertiary genepool species. The information obtained will be of great relevance for clarifying the relationships among cultivated and wild eggplants and will be useful to breeders using wild species for eggplant breeding

### Material and methods

#### Plant materials

A total of 76 accessions, including 16 entries of S. melongena from Asian and European origin, 30 of S. aethiopicum belonging to the four varietal groups (Aculeatum, Gilo, Kumba and Shum) plus intermediate forms between S. aethiopicum and S. anguivi, five of S. macrocarpon, and 25 accessions of 14 wild species were used for the present study (Table 1). Among the 16 entries of brinjal eggplant, two of them are doubled haploids (S. melongena_10 and S. melongena_12) obtained by anther culture [33]. Also, four brinjal eggplant entries come from two original sources (entries S. melongena_1 and S. melongena_2 from the original source MEL1; and accessions S. melongena_6 and S. melongena_7 from the original source MEL5) (Table 1). Among the wild relatives are included the putative ancestors of brinjal eggplant (S. insanum), scarlet eggplant (S. anguivi), and gboma eggplant (S. dasyphyllum) [34–36], as well as eight
Table 1. Plant materials used including taxon, accession name, accession code used in the present work, country of origin and fruit shape and predominant colour.

| Taxon and accession | Code | Country of origin | Fruit shape | Predominant fruit colour |
|---------------------|------|-------------------|-------------|--------------------------|
| *S. aethiopicum* L. gr. Aculeatum | MM457 | S. aethiopicum aculeatum_1 | Japan | 1 | 1.3 |
|                      | UPV29803 | S. aethiopicum aculeatum_2 | China | 1 | 1.2 |
|                      | RNL0187 | S. aethiopicum aculeatum_3 | Burkina Faso | 1 | 1.2 |
|                      | MM1483 | S. aethiopicum aculeatum_4 | Ghana | 1 | 1.3 |
| *S. aethiopicum* L. gr. Gilo | BBS151A | S. aethiopicum gilo_1 | Ivory Coast | 7 | 1.1 |
|                      | IVIA026 | S. aethiopicum gilo_2 | Unknown | 7 | 1.2 |
|                      | RARE_PLANTS_GILO | S. aethiopicum gilo_3 | Unknown | 3 | 1.3 |
|                      | RNL0252 | S. aethiopicum gilo_4 | Ghana | 3 | 1.2 |
|                      | UPV29014 | S. aethiopicum gilo_5 | Unknown | 5 | 1.2 |
|                      | RNL0395 | S. aethiopicum gilo_6 | Liberia | 3 | 1.1 |
|                      | RNL0288 | S. aethiopicum gilo_7 | Ghana | 5 | 2 |
|                      | BBS181A | S. aethiopicum gilo_8 | Ivory Coast | 1 | 1.3 |
|                      | BBS147G | S. aethiopicum gilo_9 | Ivory Coast | 1 | 1.3 |
|                      | BBS140B | S. aethiopicum gilo_10 | Ivory Coast | 3 | 1.2 |
|                      | BBS159B | S. aethiopicum gilo_11 | Ivory Coast | 5 | 1.1 |
|                      | BBS142A | S. aethiopicum gilo_12 | Ivory Coast | 5 | 1.2 |
|                      | AN05 | S. aethiopicum gilo_13 | Angola | 3 | 1.1 |
| *S. aethiopicum* L. gr. Kumba | INRA_4 | S. aethiopicum kumba_1 | Senegal | 1 | 1.1 |
|                      | MM1207 | S. aethiopicum kumba_2 | Mali | 1 | 1.1 |
|                      | BBS111 | S. aethiopicum kumba_3 | Ivory Coast | 1 | 2 |
|                      | BBS110 | S. aethiopicum kumba_4 | Ivory Coast | 1 | 1.1 |
| *S. aethiopicum* L. gr. Shum | RNL0022 | S. aethiopicum shum_1 | Benin | 3 | 1.3 |
|                      | RNL_0340 | S. aethiopicum shum_2 | Zimbabwe | 1 | 1.2 |
| *S. aethiopicum* L.-*S. anguivi* Lam. intermediate | BBS116 | S. aethiopicum-anguivi_1 | Ivory Coast | 3 | 1.3 |
|                      | BBS192E | S. aethiopicum-anguivi_2 | Ivory Coast | 5 | 1.2 |
|                      | BBS148D | S. aethiopicum-anguivi_3 | Ivory Coast | 3 | 1.1 |
|                      | BBS131C | S. aethiopicum-anguivi_4 | Ivory Coast | 3 | 1.1 |
|                      | BBS184 | S. aethiopicum-anguivi_5 | Ivory Coast | 3 | 1.1 |
|                      | BBS180A | S. aethiopicum-anguivi_6 | Ivory Coast | 5 | 1.1 |
|                      | BBS114 | S. aethiopicum-anguivi_7 | Ivory Coast | 5 | 1.2 |
| *S. anguivi* Lam. | ANG1 | S. anguivi_1 | Ivory Coast | 3 | 1.1 |
|                      | ANG2 | S. anguivi_2 | Ivory Coast | 3 | 1.3 |
| *S. campylacanthum* Hochst. ex A. Rich | CAM5 | S. campylacanthum_1 | Tanzania | 3 | 1.2 |
|                      | CAM6 | S. campylacanthum_2 | Kenya | 3 | 1.2 |
|                      | CAM8 | S. campylacanthum_3 | Tanzania | 3 | 1.2 |
| *S. dasypyllum* Schumach. & Thonn. | DAS1 | S. dasypyllum_1 | Uganda | 1 | 1.2 |
| *S. elaeagnifolium* Cav. | ELE1 | S. elaeagnifolium_1 | Senegal | 3 | 1.2 |

(Continued)
Table 1. (Continued)

| Taxon and accession | Code                  | Country of origin | Fruit shape | Predominant fruit colour |
|---------------------|-----------------------|-------------------|-------------|--------------------------|
| ELE2                | S. elaeagnifolium_2   | Greece            | 3           | 1.2                      |
| S. inanum L.        | MM577                 | Israel            | 5           | 1.2                      |
| INS1                | S. insanum_1          | Sri Lanka         | 5           | 1.2                      |
| INS2                | S. insanum_2          | Sri Lanka         | 3           | 1.2                      |
| INS3                | S. insanum_3          | Japan             | 3           | 1.2                      |
| S. lichtensteinii Wild. | LIC1                 | South Africa      | 3           | 1.3                      |
| S. lidi Sunding     | LID1                  | Spain             | 3           | 1.3                      |
| INS1                | S. inanum_1          | Sri Lanka         | 5           | 1.2                      |
| INS2                | S. inanum_2          | Sri Lanka         | 3           | 1.2                      |
| INS3                | S. inanum_3          | Japan             | 3           | 1.2                      |
| S. linnaeanum Hepper & P.-M.L. Jaeger | LIN1 | Spain | 3 | 1.3 |
| LIN3                | S. linnaeanum_2       | Tunisia           | 3           | 1.3                      |
| S. macrocarpon L.   | MM1558                | Malaysia          | 1           | 2                        |
| BBS168              | S. macrocarpon_2      | Ivory Coast       | 1           | 2                        |
| BBS117              | S. macrocarpon_3      | Ivory Coast       | 1           | 1.3                      |
| BBS171B             | S. macrocarpon_4      | Ivory Coast       | 1           | 2                        |
| BBS178              | S. macrocarpon_5      | Ivory Coast       | 5           | 1.2                      |
| S. melongena L.     | MEL1_2                | Ivory Coast       | 5           | 2                        |
| MEL1_3              | S. melongena_2        | Ivory Coast       | 5           | 2                        |
| MEL2                | S. melongena_3        | Ivory Coast       | 5           | 7                        |
| MEL3                | S. melongena_4        | Ivory Coast       | 7           | 1.2                      |
| MEL4                | S. melongena_5        | Sri Lanka         | 3           | 7                        |
| MEL5_2              | S. melongena_6        | Sri Lanka         | 7           | 7                        |
| MEL5_5              | S. melongena_7        | Sri Lanka         | 7           | 7                        |
| MEL6                | S. melongena_8        | Sri Lanka         | 7           | 7                        |
| AN-S-26             | S. melongena_9        | Spain             | 5           | 7                        |
| DH_AN-S-26          | S. melongena_10       | Spain             | 5           | 7                        |
| MM1597              | S. melongena_11       | India             | 9           | 1.2                      |
| DH_ECAVI            | S. melongena_12       | Breeding line     | 7           | 8                        |
| H15                 | S. melongena_13       | Spain             | 5           | 7                        |
| A0413               | S. melongena_14       | Unknown           | 1           | 2                        |
| ASI-S-1             | S. melongena_15       | China             | 1           | 8                        |
| IVIA371             | S. melongena_16       | Spain             | 5           | 7                        |
| S. sisymbrifolium Lam. | SIS1                  | Unknown           | 3           | 1.2                      |
| SIS2                | S. sisymbrifolium_2   | Unknown           | 5           | 1.3                      |
| S. tomentosum L.    | TOM1                  | South Africa      | 3           | 1.3                      |
| S. torvum Sw.       | TOR2                  | Sri Lanka         | 3           | 1.2                      |
| TOR3                | S. torvum_2           | Unknown           | 3           | 1.3                      |

(Continued)
other wild species from Old World origin (S. campylacanthum Hochst. ex A. Rich, S. incanum L., S. lichtensteinitii Wild., S. lidi Sunding, S. linnaeanum Hepper & P.-M.L. Jaeger, S. tomentosum L., S. vespertilio Aiton, and S. violaceum Ortega), and three native to the New World (S. elaeagnifolium Cav., S. sisymbriifolium, and S. torvum) [30,37]. All these materials are conserved in the germplasm collection maintained at Universitat Politècnica de València (Valencia, Spain).

Library construction and sequencing

DNA was extracted following a modified CTAB method [38] as indicated elsewhere [39]. Library construction (11/2015) was performed as proposed in Peterson et al. [40] and modified as in Acquadro et al. [41], by using a HindIII-MseI enzyme combination and adding a final biotin/streptavidin-coated beads based purification step. Quality, quantity and reproducibility of libraries were assessed on a Bioanalyzer instrument (DNA High Sensitivity chip) as well as qPCR. On the basis of the quantitation, DNA libraries were pooled and sequenced on Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA), following the manufacturer protocol using I00SE chemistry.

Sequence analysis

Raw reads were analyzed with Scythe (https://github.com/vsbuffalo/scythe) for filtering out contaminant substrings and Sickle (https://github.com/najoshi/sickle), which allows to remove reads with poor quality ends (Q<30). Illumina reads were de-multiplexed on the basis of the Illumina TruSeq index. Alignment to the reference eggplant genome [42,43] was carried out using BWA aligner [44] (i.e., mem command) with default parameters and avoiding multiple-mapping reads. SNP mining was conducted by adopting a Samtools-based pipeline [45]. Homozygous/heterozygous SNP/Indel calls were considered only with phred-scaled genotype likelihood equal zero. A catalog of candidate high quality SNPs was produced. Relationships among the genotypes were computed using: i) whole genome, and ii) coding (within exons) SNP/indel datasets. The proportion of heterozygous SNPs for each genotype was estimated by the ratio of total number of heterozygous SNPs and all the detected SNPs (excluding missing SNPs) as well as the ratio of the number of heterozygous SNPs in coding regions and all the detected SNPs in coding regions.

Genetic relationships analysis and population structure

SNP data were coded according to the number of occurring polymorphisms, being assigned a 0 if they showed the homozygous reference type, a 1 if the variant occurred in one

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**Table 1.** (Continued)

| Taxon and accession | Code          | Country of origin | Fruit shape | Predominant fruit colour |
|---------------------|---------------|-------------------|-------------|--------------------------|
| S. vespertilio Aiton| VES2 S. vespertilio_1 | Spain             | 3           | 1.3                      |
| S. violaceum Ortega| VIO1 S. violaceum_1 | Sri Lanka         | 3           | 1.2                      |

* Fruit shape according to the following scale: 1 = broader than long; 3 = as long as broad; 5 = slightly longer than broad; 7 = twice as long as broad; 8 = three times as long as broad; 9 = several times as long as broad.
* Fruit predominant colour when the fruit is physiologically immature according to the following categories, in which the green colour (1) has been subdivided into three subcategories: 1.1 = clear green; 1.2 = intermediate green; 1.3 = dark green; 2 = milk white; 3 = deep yellow; 4 = fire red; 5 = scarlet red; 6 = lilac grey; 7 = purple; 8 = purple black; 9 = black.

https://doi.org/10.1371/journal.pone.0180774.t001
chromosome and a 2 if the variant was present in both chromosomes. Genetic similarities between pairs of entries were quantified by the Dice similarity index [46] as \( \frac{2m^+}{2m^+ + m^-} \), where \( m^+ \) is the number of matches (1–1 and 2–2) and \( m^- \) is the number of mismatches (0–1, 0–2 and 1–2). Genetic relationships were described by using both the unweighted pair-group arithmetic mean (UPGMA) method with 1,000 bootstraps, and principal coordinate analysis (PCoA) by means of Past 3.14 software [47]. A co-phenetic matrix was also produced using the hierarchical cluster system, by means of the COPH (cophenetic values) routine, and correlated with the original distance matrix, in order to test for associations between clusters and the similarity matrix.

FastSTRUCTURE [48] was used to estimate the number of sub-populations in the panel, applying the admixture model for the ancestry of individuals and correlated allele frequencies. A hierarchical FastSTRUCTURE analysis [49] was also applied on accessions which clustered in sub-group 1 and subgroup 4 following UPGMA analysis as well as on the set of all the remaining. The program was run with default setting using simple prior to obtain a reasonable range of values for the number of populations (K), FastSTRUCTURE was executed for multiple values of K (K = 1–9). The script chooseK.py [48] was then used to infer the most likely number of populations.

Results and discussion

Sequencing and SNPs identification

A total of 225 million single reads were produced. About 94% of raw reads contained the expected restriction site overhang, along with discriminating inline barcodes. The average number of successfully de-multiplexed reads per sample was 2.7 M, with a standard deviation of 1.5 M (S1 Fig). Sequences were trimmed and quality cleaned to 210 million of useful reads (6.2% discarded). The latter were then aligned to the recently produced reference eggplant genome [42,43] and close to 100% of reads were successfully mapped to single regions (no multiple mapping was permitted). Mapped sequences showed an extensive coverage alongside the 12 chromosomes (data not shown).

In all, 75,399 polymorphic sites were identified among the 76 lines in study. Overall, all the S. melongena accessions, together with the three S. insanum accessions, showed a reduced level of polymorphism (on average 2.47 and 4.75% respectively) when aligned to the reference genome. On the other hand the frequency of polymorphic SNPs ranged from 10.62 to 24.32% in the other entries (S1 Table).

Solanum melongena is a largely autogamous species [20], thus its low level of heterozygosity (on average 1.66%) is coherent with the expectation that germplasm accessions and non-hybrid varieties should be highly homozygous (Fig 1, S1 Table). Interestingly, the two S. melongena varieties (S. melongena_10, S. melongena_12, Fig 1, S1 Table), which are the result of diploidization of haploid plants obtained through anther culture, displayed some heterozygosity (<0.5%). This might be due to somaclonal variation, which is manifested as cytological abnormalities, sequence change, and gene activation and silencing which occur through the ‘in vitro’ culture process and that provides evidence that DNA modifications occur more frequently in ‘in vitro’ cultivated than in seed-grown plants [50]. However, it might be also a consequence of SNPs mapping on paralog genes since, similarly to tomato, potato and pepper, also eggplant the genome carries signs of the “T” triplication occurred during Solanaceae evolution [42], or being the results of some mapping artifacts. This would suggest that the heterozygosity detected in the rest of S. melongena accessions would be overestimated by almost one third.

The two other cultivated eggplant species (i.e., S. aethiopicum and S. macrocarpon) showed, on average, higher heterozygosity than S. melongena, ranging from 4.52 to 9.53% (Fig 1, S1 Table). This might be a consequence of their higher allogamy and the more limited breeding
efforts for stabilizing phenotypic and yield-related traits. Low heterozygosity was also observed in the wild *S. insanum*, *S. lichtensteinii* and *S. linnaeunum* (< 3.5%), while higher values, over 10%, were observed in the wild species *S. campylacanthum*, *S. anguivi* and *S. violaceum*.

Some missing data were observed in *S. melongena* and *S. aethiopicum* (ranging from 2.71% to 9.87%), some accessions showed a medium-high level of missing data (e.g., *S. macrocarpon* 19% on average), while others showed a surprisingly high number of missing data (up to 54.5%, 54.43 and 36.88, in *Solanum torvum*, *S. sisymbriifolium* and *S. elaeagnifolium*, respectively). This might be explained by the fact that these latter species are native to the New World [30,37] and in consequence have a more distant common ancestor, and greater evolutionary divergence. Missing data were distributed on the different eggplant chromosomes; however, by adopting a five million bases sliding window analysis, some hot spot regions were highlighted (Fig 1). The filtering of the whole SNP dataset for the sites present in CDS regions granted 12,859 SNPs. The latter were used for all the subsequent analyses, since the relative number of missing data was lower in the coding dataset (3% on average) than in the whole dataset (10% on average, Fig 1, S1 Table). As an example the percentage of missing data of South American accessions (*S. elaeagnifolium*, *S. sisymbriifolium* and *S. torvum*–S1 Table) was lowered from about 46.1% to 15.1%, thereby increasing the resolution power of our analyses.

### Genetic relationships analysis and population structure

The UPGMA-based dendrogram and the output of FastSTRUCTURE [48] analysis (Fig 2) show the genetic relationships between the 76 accessions. Both, as well as the K analysis (Fig 2...
box), suggest a population structure comprising four sub-groups. Each entry was fingerprinted and the co-phenetic correlation coefficient (r-value) between the Dice data matrix and the co-phenetic matrix was 0.978, highlighting a very good fit between the dendrogram clusters and the similarity matrices from which they were derived, indicating that the UPGMA method is suitable for the interpretation of our data. The fact that the sister entries S. melongena_1 and S. melongena_2, which are derivatives from the original source MEL1 cluster together in the dendrogram, and the same occurs for accessions S. melongena_6 and S. melongena_7, which derive from MEL5 provide a confirmation that the analysis is congruent.

According to the level of membership provided by FastSTRUCTURE [48], sub-group 1 (blue) includes all the accessions of scarlet eggplant (S. aethiopicum) and S. anguivi, which on the basis of previous studies has been reported to be its wild ancestor [30,34,51]. Sub-group 2 (orange) includes members of the brinjal eggplant complex [52,53], among which the most genetically related accessions of S. melongena and its wild progenitor S. insanum, the accession of S. incanum, and the two of both S. lichensteinii and S. linnaeanum. Sub-group 3 (yellow) includes five accessions of gboma eggplant (S. macrocarpon) and the one S. dasyphyllum, which is its wild progenitor [30,35]. Sub-group 4 (grey) includes the accessions of the New World species, which form part of the tertiary gene pool of brinjal eggplant [30]. Finally, the remaining accessions of S. campylacanthum, S. violaceum, S. lidii, S. vespertilio and S. tomentosum had ambiguous membership and were thus classified as admixed, as their level of membership to a single group was lower than 70% (Fig 2). With the goal to provide insight into the complex relationships of the germplasm used, and to detect additional sub-population structure, a hierarchical FastSTRUCTURE analysis was applied by running STRUCTURE on partitioned data, i.e. on accessions which clustered in sub-group 1 and sub-group 4 following UPGMA analysis, as well as on the remaining materials (S2 Fig). The hierarchical FastSTRUCTURE analysis for the scarlet eggplant complex revealed that the optimal number of populations was obtained at K = 2, and that the accessions of S. aethiopicum and S. anguivi, included in the UPGMA subgroup 1, share a common gene pool. For the brinjal eggplant and gboma eggplant complexes group, the hierarchical FastSTRUCTURE analysis suggests that four populations are present. In this set of accessions K = 2 separates the brinjal eggplant S. melongena and its close relatives S. insanum, S. incanum, S. lichensteinii and S. linnaeanum [18] from the gboma eggplant S. macrocarpon and its wild ancestor S. dasyphyllum [35] together with the Canary Islands endemics S. lidii and S. vespertilio and the related South African S. tomentosum [23,54,55], while S. campylacanthum and S. violaceum appear as an admixture (S2 Fig). At K = 3 the S. lidii, S. vespertilio, S. tomentosum and S. violaceum are separated from the gboma eggplant. Finally, at the optimal K = 4, S. campylacanthum accessions group separately, while S. incanum, S. lichensteinii and S. linnaeanum appear as an admixture of S. melongena/S. insanum and S. campylacanthum (S2 Fig). This might be a consequence of gene flow among them or the result of the recent speciation from a common ancestor or both. These species are phylogenetically closely related but are present are distributed in different geographical areas [18]; this suggests that presumably they evolved from a common ancestor for adaptation to different niches, which might difficult gene flow. The hierarchical FastSTRUCTURE analysis of the New World species recognized at K = 2 two populations, one of which included S. elaeagnifolium while the other both S. syzgiumfruticosum and S. torvum. However, at the optimal K = 3, the latter was further splitted in two genetically differentiated genepools, each including one of the two species.

**PCoA analyses**

The whole data set was also subjected to PCoA analysis (Fig 3) which, on the whole, confirmed the grouping of genotypes based on UPGMA and FastSTRUCTURE [48] clustering. Because a
Fig 2. The genetic architecture of the full germplasm panel: Consensus UPGMA dendrogram and FastSTRUCTURE output at K = 4. Bootstrap values (%) for the main nodes are reported in red. Each entry is represented by a vertical line.
limited number of samples of each of the wild relatives was included in our study, the PCoA analysis did not allow to highlight the within-species diversity as it did in the cultivated species; however, it made possible some additional inferences. The first two axes explained 71.4% of the genetic variation. The first axis, explaining 57.6% of the genetic variation, clearly separated cultivated scarlet eggplant *S. aethiopicum* and its wild ancestor *S. anguivi* from all the other accessions, with no evident separate clustering of the two species. The latter are fully inter-fer-tile [34,51] and the identification of intermediate forms [27,29] suggests occurrence of genetic flow between them.

The second axis, explaining 13.8% of the genetic variation, clearly split the entries of *S. sisymbriifolium*, *S. torvum* and *S. elaeagnifolium*, which clustered in the previously described group 4, from the ones of sub-clusters 2 and 3 as well as the entries classified as admixed, i.e. brinjal and gboma eggplants, their respective progenitors *S. insanum* and *S. dasyphyllum* together with other Old World wild species, as well as the entries classified as admixed.

Both *S. sisymbriifolium* and *S. torvum* are native of South and Central America and, together with *S. viarum*, were classified in GP3 by Syfert et al. [30]. They have been also reported to be, within subgenus *Leptostemonum* (Dun.) Bitt., phylogenetically far away from the cultivated eggplants and the other Old World species [23,30,54,55]. *Solanum elaeagnifolium* is also a New World species [37] which was not included in the study of Syfert et al. [30], and whose origin is attributable to GP3 on the basis of the present results.

Both *S. sisymbriifolium* and *S. torvum* are of interest for eggplant breeding, as they are tolerant or resistant to many diseases [20]. Their high phylogenetic distance to cultivated eggplants is confirmed by the many ineffective attempts to hybridize them with *S. melongena* [29,56–58]. No sexual hybrids have ever been reported between *S. melongena* and *S. sisymbriifolium*, while interspecific hybrids obtained through embryo rescue of the cross *S. melongena* × *S. torvum* were highly sterile and no backcrosses have been reported to date [17]. Furthermore, although tetraploid somatic hybrids between either *S. sisymbriifolium* or *S. torvum* with *S. melongena* were obtained, they did not produce sexual offspring [59,60].

On the basis of PCoA analysis, the cultivated species which appears genetically closer to the cultivated eggplant is gboma eggplant (*S. macrocarpon*), clustering together with *S. dasyphyllum*, which has been reported by many authors to be its wild ancestor [23,35,52,54,61] (Fig 3). This seems to indicate that gboma eggplant, might be genetically closer to *S. melongena* than the cultivated scarlet eggplant (*S. aethiopicum*), which is included in section Oliganthes (Dunn.) Bit. [34,62]. However contrasting results have been reported in literature. Based on chloroplast DNA RFLPs [63], ISSRs [64], AFLPs and nrITS sequences [19] it was previously reported that *S. aethiopicum* is closer to *S. melongena* than *S. macrocarpon*; otherwise Sakata and Lester [65], in a study based on chloroplast DNA RFLPs, and Vorontsova et al. [23] using ITS, waxy and trnT-F regions sequences obtained opposite results. Interestingly, Furini and Wunder [66] using AFLPs as well as Levin et al. [54], Weese and Bohs [53] and Särkinen et al. [55] using several nuclear and plastid DNA sequences found that *S. aethiopicum* and *S. macrocarpon* were phylogenetically closer among them than to *S. melongena*. Studies based on the species inter-fertility highlighted that interspecific hybrids between *S. melongena* and *S. aethiopicum* as well as backcrosses could be easily obtained [17,67,68]; on the other hand, although hybrids between *S. melongena* and *S. macrocarpon* were obtained [56,67,69], in most cases they were high sterile and only the backcross of a tetraploid hybrid between the two species with *S. melongena* was successful [69]. The difficulty in obtaining the hybrids between these two
species, despite being phylogenetically close [23,65], might be caused by some chromosomal rearrangement or other hybridization barriers. At last, Kouassi et al. [58] reported that the backcrosses towards *S. melongena* of the hybrid between *S. dasyphyllum* (wild ancestor of *S. macrocarpon*) and *S. melongena* was successful. A clarification is provided by our data obtained from *FastSTRUCTURE* analysis (Fig 2) which highlights that the three cultivated species belong to clearly separate groups, suggesting that *S. macrocarpon* should be excluded from section *Melongena* (Mill.) as proposed by Sakata et al. [63].

PCoA analysis also showed that *S. campylacanthum*, *S. incanum*, *S. insanum*, *S. lichtensteinii* and *S. linnaeanum*, which form part of the "brinjal eggplant" complex [52,53], cluster in proximity with eggplant (Fig 3). Among them, *S. campylacanthum* appears to be the most genetically differentiated from the others. This is in agreement with previous AFLP, nuclear and chloroplast DNA sequence results [23,53,61]. Indeed, interspecific hybrids were obtained between *S. campylacanthum* and *S. melongena*, but the number of seeded fruits and seeds per fruit was lower in respect to the ones obtained following crosses with other species within the “common eggplant” complex [52,58,70]. *Solanum linnaeanum* and the accession of *S. lichtensteinii* cluster together and close to *S. melongena*. This result confirms that the two species are genetically related [23,53] and supports the hypothesis that *S. linnaeanum* and *S. lichtensteinii* are of South African origin and share a common ancestor, although the former grows in several tropical and subtropical areas of the world [18,23].
Solanum linnaceanum and S. lichtensteinii produce hybrids with moderate or high fertility when crossed with eggplant [18], which can be also backcrossed with relative ease [17,19,23,58,28]. However our data show that they are genetically more distant from S. melongena than S. incanum or S. insanum [19,23,53,65,66]. Solanum incanum was suggested to be eggplant’s pre-domestication ancestor and is being used in eggplant breeding programs as a source of variation for phenolics content and resistance to drought [18]. Recent morphological and molecular work has shown that species-level differences exist between S. incanum and S. melongena and, on the basis of new evidence, S. insanum is considered the eggplant wild progenitor [36]. The two species are also fully inter-fertile and their hybrid produce many fruits and seeds [29]. It is also not surprising that, since frequent genetic flow occurs between both species in the indo-birmanian region [71,72], in our PCoA analysis the S. insanum accessions appear intermingled with the ones of S. melongena.

Our data show that the three species S. lidii, S. tomentosum, S. vespertilio cluster into proximity to each other and S. violaceum a little more apart (Fig 3). Solanum lidii and S. vespertilio are endemic to the Canary Islands (Spain) and are genetically similar sister species, which were found to cluster together in previous molecular studies [23,54,55,73,74]. In several molecular studies S. tomentosum was also found to cluster close to S. lidii and S. vespertilio [23,54,55,73], thus our results confirm that the three species are close relatives. Solanum violaceum clusters with these three taxa in both the FastSTRUCTURE and PCoA analyses in spite of having a native distribution in India and Southeast Asia [19].

Within-groups PCoA analyses

In order to gain a better landscape of the genetic relationships among the species in study, PCoA analyses were also separately performed on entries clustering in the sub-groups 1, 2 and 4, following FastSTRUCTURE [48] analysis (Fig 4A, 4B and 4C). The separate PCoA of entries grouped in sub-group 1 (Fig 4A) confirmed that the different S. aethiopicum varietal types are partially intermingled and show a high within varietal type genetic diversity; furthermore, the absence of an evident genetic differentiation with their wild ancestor S. anguivi was confirmed. As observed by Sunseri et al. [24] in a molecular characterization based on AFLP and SSR markers, the different cultivar groups of S. aethiopicum were intermingled in the cluster analysis. The four cultivar groups (Aculeatum, Gilo, Kumba, and Shum) are distinguished by simple morphological traits, like fruit size and shape, fruit bitterness, and the presence or absence of prickles and star leaf hairs [26,34], which allow the differentiation among cultivars based on morphological characterizations [21]. However several of these traits, like prickliness and presence/absence of star leaf hair, seem to have a simple genetic basis in scarlet eggplant [51] while, as occurs in common eggplant [75,76], other traits (fruit size and shape) are under control of a few major genes. The genetic flow occurring between different groups, as a result of spontaneous or artificial hybridization, may thus result in a lack of (or reduced) genetic differentiation. Indeed, in a previous study [26], it was reported that the Aculeatum group seems to have been derived from hybridization between S. aethiopicum group Kumba and S. anguivi. On the whole, the varietal groups that showed the highest genetic differentiation were Aculeatum, (characterized by the highest anthocyanin content and prickliness in respect to all the others) and Shum, which differed for the mean average values of 8 of 18 morphological traits analysed in a previous study [21].

PCoA including accessions of the sub-group 2 (Fig 4B), as expected grouped separately the entries of eggplant and its wild relative S. insanum from the close relatives S. incanum, S. lichtensteinii and S. linnaceanum, the latter being the most genetically differentiated from all the others. The S. melongena accessions analysed included types, hailing from Sri Lanka, India and China as well as from Ivory Coast and Spain and producing fruits of different shape and
Fig 4. Within-groups PCoA analyses in subgroups of germplasm panel of Solanum accessions: visualization of the genetic relationships within sub-group 1 (A; scarlet eggplant complex), sub-group 2 (B; brinjal eggplant complex) and sub-group 4 (C; New World species).

https://doi.org/10.1371/journal.pone.0180774.g004
colour. In a previous work [16] 191 eggplant accessions were scored for a set of 19 fruit and plant traits and the analysis of phenotypic data made it possible to classify the genotypes in three main fruit morphological groups producing: (i) elongated fruits, (mean ratio $f_s =$ fruit length/fruit maximum diameter around 5.05); (ii) semi-long fruits ($f_s$ from 1.2 to 2) and (iii) round fruits ($f_s$ around 1), which cut across the Oriental and Occidental divide. On the other hand STRUCTURE [77] analysis based on 24 microsatellite markers (22 genomic ones and two from EST), identified two major sub-groups, which to a large extent mirrored the provenance of the entries. In the present study, in spite of the wide set of polymorphisms detected, the accessions from different origin did not highlight a grouping together trend. This apparent discrepancy can be explained by either the difference in size of the two germplasm sets, but also by the number of markers applied, as the use of a limited number of selected markers might provide unrealistic estimates of genetic variability in the set of accessions in study.

PCoA including accessions of the sub-group 4 (Fig 4C) highlights that $S$. *sisymbriifolium*, $S$. *torvum* and *S. elaeagnifolium* are genetically far away from each other and that their grouping in the sub-group 4 is due to their common high genetic divergence from all the other entries. This is confirmed by previous molecular results that includes $S$. *torvum* and $S$. *sisymbriifolium* in different clades within subgenus Leptostemonum from the cultivated eggplants [23,30,54,55]. Furthermore, on the basis of PCoA analysis, the two accessions of $S$. *torvum* form a group ‘per se’ in respect to all the others.

Previous phylogenetic studies placed $S$. *elaeagnifolium* and the rest of species of the Elaeagnifolium clade closer to Old World species than either $S$. *sisymbriifolium* or *S. torvum* [19,23,54,55,73]. Recently, crossing data confirm that $S$. *elaeagnifolium* is closer to eggplant than either $S$. *sisymbriifolium* and *S. torvum*, as interspecific hybrids have been obtained which present intermediate fertility [58], and with which it is possible to obtain backcrosses with *S. melongena* (unpublished results).

**Conclusions**

One of the most exciting developments in the past decade has been the application of powerful and ultra-rapid nucleic acid sequencing techniques to the study of genetic relationships and phylogeny of crop species [78]. As previously reported by Bajaj et al. [79] in chickpea, our results demonstrate that the high-throughput genotyping of numerous genome-wide SNP markers represents a highly and more effective approach, in respect to the ones based on limited sets of genome-wide markers or a small set of gene sequences, for understanding the extent of natural allelic diversity and genetic relationships among and within wild and cultivated species belonging to eggplant complexes. The high number of detected polymorphisms were analysed by *FastSTRUCTURE* [48, 49], UPGMA and PCoA analyses and the three approaches showed to be complementary in the interpretation of data. On the whole, we confirm a wide genetic base and broad molecular diversity among wild and cultivated species within and among the three cultivated eggplant complexes and the New World eggplant CWRs. Thanks to a reduced complexity genome sequencing approach, we were able to fingerprint all accessions in the study and gathered information which may efficiently guide further exploration of the diversity and relationships in the large *Solanum* subgenus *Leptostemonum* group. The approach used and data obtained lay the foundation also to address the evaluation of gene flow among inter-fertile sympatric taxa [71], recent speciation and domestication processes of cultivated eggplants. In addition, the large number of markers distributed across the genome may also contribute to facilitate the transfer of target genomic regions controlling useful agronomic traits, such as biotic and abiotic stress tolerance or fruit quality traits, from related species into the genetic background of cultivated eggplants.
Supporting information

S1 Table. SNPs detected in the genome and in CDSs. In both cases, number and percentage of: (i) missing sites; (ii) detected SNPs, the percentage is evaluated as the ratio between detected SNPs/Genomic or CDS total SNPs-missing sites; (iii) heterozygous SNPs, the percentage is evaluated as the ratio between heterozygous SNPs/Genomic or CDS total SNPs-missing sites; (iv) homozygous SNPs, the percentage is evaluated as the ratio between homozygous SNPs/Genomic or CDS total SNPs-missing sites.

S1 Fig. Distribution of sequenced reads, after quality cleaning and trimming procedures, across a germplasm panel of 76 Solanum accessions (in million reads).

S2 Fig. FastSTRUCTURE output at K = 4 from full germplasm panel together with outputs of separate analyses performed with subsets of taxa. Asterisks indicate the best K choice based on the ΔK method.

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