

## Article

# Multi-Level Characterization of Eggplant Accessions from Greek Islands and the Mainland Contributes to the Enhancement and Conservation of this Germplasm and Reveals a Large Diversity and Signatures of Differentiation between both Origins

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**Abstract:** Crop landraces are found in many inhabited islands of Greece. Due to the particularity of environment and isolation from the mainland, Greek islands represent a natural laboratory for comparing the diversity of landraces from the islands with those of the Greek mainland. A collection of 36 Greek eggplant landraces and traditional cultivars from the mainland and the islands has been phenotypically and genetically characterized using 22 morphological descriptors and 5 SSR markers. The mineral composition (K, Mg, Cu, Fe, Mn, Zn) of fruits was also determined. The objectives of this study include the multi-level characterization of eggplant local landraces and the comparison of diversity among accessions from the Greek mainland and the islands. Characterization of eggplant landraces will contribute to the enhancement and prevention of genetic erosion in this local group and will provide a resource for future investigation and breeding. PCA analysis of morphological traits explained 45.4% of the total variance revealing the formation of two clusters, one with most of the island accessions, and another with most of the mainland ones. The SSR markers used exhibited high average values for the number of alleles/locus (4.6), expected heterozygosity (0.60) and PIC (0.55), while the observed heterozygosity was low (0.13). Both STRUCTURE and PCoA analyses based on SSR data revealed two genetic clusters, one made up mainly by the mainland accessions, while the other one was mainly made up by the island accessions. Although there was considerable variation among the landraces for the concentration of minerals studied, only average Mg concentration was significantly different between mainland and island accessions. Based on our data, the Greek eggplant landraces present considerable morphological and genetic diversity with some differentiation signatures between the island and the mainland accessions. Our results have

implications for conservation of Greek landraces and suggest that Greece might be considered as part of a secondary center of diversity for eggplant in the Mediterranean basin.

**Keywords:** *Solanum melongena*; germplasm; Greece; islands; mainland; phenotyping; genotyping; mineral composition; landraces

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## 1. Introduction

Greece lies at the southernmost part of the Balkan peninsula, at a crossroads between Europe, Asia, and Africa. The country is noted for its high species diversity, the extensive coverage of natural and semi-natural habitats, and many island complexes in the Archipelagos of Aegean Sea and the Ionian Sea [1]. Islands are natural laboratories for the study of the evolution and ecology of wild plants and endemisms [2–4]. Therefore, due to their isolation and special environmental features, islands are also considered as sites of special interest to prospect and conserve plant genetic resources [5,6]. The Greek archipelago includes ca. 7600 islands and islets in the Aegean Sea (including Crete) and ca. 300 islands and islets in the Ionian Sea [7]. Among them, 116 are inhabited, whereas only 79 have over 100 permanent residents [8]. The Aegean Archipelago is one of the largest archipelagos in the world, exhibiting high environmental and topographical heterogeneity, complex geological and palaeogeographical history, as well as high diversity and endemisms, rendering it an ideal stage for biodiversity and biogeographical studies [1,9]. Among the 13 phytogeographical regions proposed by Strid [10] to occur in Greece, five were identified in the Aegean [1]. The Ionian archipelago forms a distinct phytogeographical region, hosting 2027 plant taxa, 1827 of which are native and 89 are Greek endemics [5,11–13].

Landraces represent a significant part of agricultural biodiversity [14]. They are characterized by high genetic diversity, local adaptation, resilience to pathogens, and good organoleptic characteristics, although they often have low yield and display a lack of uniformity [15–17]. Over time, landraces have been gradually displaced from market-driven production, due to the consumers' demand for local high-quality plant products, landraces are currently recalling an increased interest [18,19]. With the shift to modern agriculture, Greece suffered a dramatic loss of its traditional agricultural germplasm, which was displaced by modern varieties produced by local breeders or imported [5,20]. Nowadays, landraces have regained an increased interest [18,19]. Due to the geographical morphology of Greece (almost 70% is hilly and/or mountainous) and the presence of small-size farms, landraces are still cultivated, especially by farmers in villages in remote regions and isolated islands, who maintain their seeds and use them mainly for their own consumption, and, to a lesser extent, for market purposes [5,13,19]. Therefore, these areas act as local in situ conservation spots and islands represent one of the most important sources of landraces and hotspots of agricultural biodiversity in Greece [5,20,21].

Eggplant (*Solanum melongena* L.) is the fifth most produced vegetable worldwide and has experienced a dramatic increase in yield and production in the last decade [22]. It was introduced in Greece around the 13th century and although initially used as an ornamental plant, it is now playing an important role in the Greek gastronomy, as an integral part of the Mediterranean diet. Besides commercial F1 hybrids and imported varieties, there are several traditional cultivars, e.g., Tsakoniki, and Santorini, which are adapted to the local environment, having excellent texture and cooking quality [23]. Apart from commercial varieties, several local landraces have been found during expeditions in the islands [5,24] or maintained in Greek Gene Bank (Hellenic Agricultural Organization Demeter, Thessaloniki, Greece). However, the diversity of Greek accessions has been barely explored, and so far, no studies have focused on the comparison of accessions from the mainland and the islands of Greece have been reported. Significant differences in genetic diversity of several plant species have been revealed when island populations are compared with mainland ones [25–28]. Factors such as geographic isolation, island size, plant breeding system, or life form, and in the case of crop species,

human factors, are shaping the difference levels [26,29–31]. Characterization of the genetic diversity and population structure of landraces and local germplasm is very important to implement the most appropriate strategies for their collection, management, efficient conservation, and utilization as a source of variation in breeding schemes [32]. Several studies have focused on the characterization of eggplant genetic resources, including the cultivated species, commercial cultivars, and their most recognized wild relatives, from different regions of origin in order to identify desired genotypes for use in eggplant breeding programs [33–36]. For instance, Liu et al. [36] examined 287 worldwide accessions for genetic diversity and population structure analysis, whereas Acquadro et al. [33] used high-throughput genotyping to assess the genetic relationships of brinjal, gboma, and scarlet eggplant complexes, which represent taxa belonging to the eggplant's primary secondary and tertiary gene pools. Moreover, Cericola et al. [34] used a combined marker-based and morphological approach to assess genetic diversity and illuminate the genetic relationship between "Occidental" and "Oriental" eggplant germplasm groups, while Hurtado et al. [35] evaluated the phenotypic and DNA-based diversity present in a collection of accessions sampled from three geographically separated centers of diversity.

Eggplant is an important source of plant-derived nutrients, valued for its composition in phytochemicals and especially minerals such as P, K, Ca, and Mg [37,38]. Raigón et al. [39] and Arivalagan et al. [38–40] studied the mineral composition and the nutritional value of eggplant landraces and germplasm accessions from different regions in order to identify mineral-rich germplasm for breeding purposes. Considerable differences were found in the mineral composition among varieties and germplasm accessions, revealing the existence of ample variation, which can be exploited for the selection of germplasm for nutritionally improved characteristics.

In this study, we report the assessment of the morphological and genetic diversity of eggplant germplasm from the Greek mainland and islands. In addition, the mineral content has been determined to obtain a more comprehensive view regarding the nutritional value of those accessions. The overall aim of this study is to acquire information for the efficient management and conservation of this valuable genetic material, for the identification of traits present in landraces that can be exploited for breeding purposes, and to compare the levels of diversity among the Greek mainland and island eggplant germplasm.

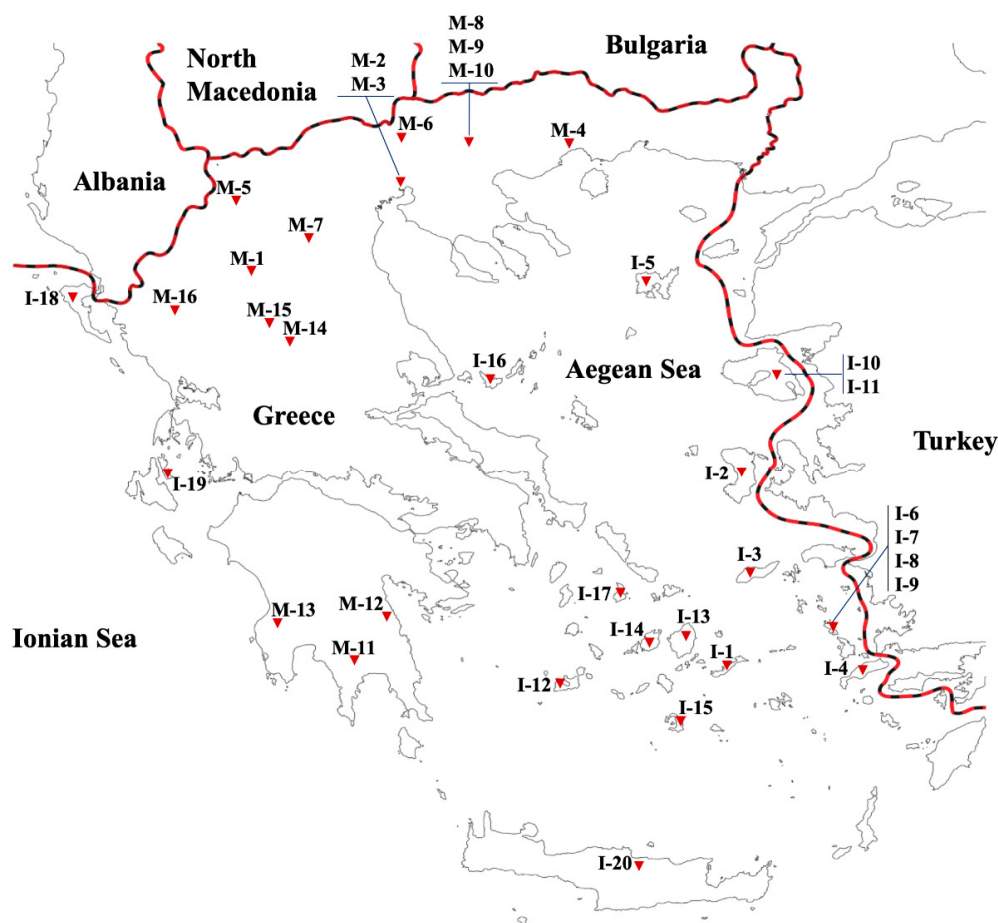
## 2. Materials and Methods

### 2.1. Plant Material and Cultivation Conditions

Thirty-six accessions, including landraces and cultivars from the mainland (16) and the islands (20) of Greece were selected to depict the Greek eggplant germplasm diversity (Table 1) and their geographical distribution (Figure 1). All materials are maintained at the National Greek Gene Bank (GGB; Institute of Genetic Resources and Plant Breeding Thermi, Greece), except I-6, I-7, and I-8, which are conserved at the Department of Agriculture of the University of Patras (Greece). Seeds were germinated in Petri dishes, following the Ranil et al. [41] protocol, and were subsequently transferred to seedling trays in a climatic chamber under a photoperiod and temperature regime of 16 h light (28 °C): 8 h dark (20 °C). One week later, the plantlets were transferred and kept in a heated greenhouse until transplantation. Nine plants per accession were grown in an open field plot at the University of Patras campus in Messolonghi, Greece (GPS coordinates: latitude, 38°36' N; longitude, 21°47'; 1.5 m a.s.l.) following a completely randomized block design experiment with 3 blocks and 3 plants per accession/block and spaced at 0.5 m within the row and 1 m between rows. Plants were pruned and trellised and drip-fertigated. Standard horticultural practices for eggplant production in this area for pest and weed control were performed. Accession ANP-025/07 (I-1) displayed an abnormal development at the seedling stage and was discarded for the morphological and mineral characterization, although it was kept for the genotyping.

**Table 1.** Names/collection number, geographic origin, collection site, geographical coordinates and status of the materials used for the study of morphological and molecular variation (SSRs) in a collection of Greek eggplants.

| Accession Code | Study Code | Territory | Geographic Region | Collection Site              | Longitude | Latitude | Altitude (m) | Status   |
|----------------|------------|-----------|-------------------|------------------------------|-----------|----------|--------------|----------|
| ANP-025/07     | I-1        | Island    | Aegean Sea        | Amorgos, Aegiali             | 36°00' N  | 25°00' E | 298          | Landrace |
| X-034/06       | I-2        | Island    | Aegean Sea        | Chios, Ag. Georgios Sikousis | 38°19' N  | 26°03' E | 369          | Landrace |
| IS-031/07      | I-3        | Island    | Aegean Sea        | Ikaria, Droutsoulas          | 37°36' N  | 26°11' E | 410          | Landrace |
| LKK-094/07     | I-4        | Island    | Aegean Sea        | Kos, Antimacheia             | 36°48' N  | 27°05' E | 133          | Landrace |
| X-116/06       | I-5        | Island    | Aegean Sea        | Lemnos, Kontopouli           | 39°55' N  | 25°20' E | 40           | Landrace |
| White Leros    | I-6        | Island    | Aegean Sea        | Leros Island                 | 37°09' N  | 26°49' E | 32           | Cultivar |
| Wide Purple    | I-7        | Island    | Aegean Sea        | Leros Island                 | 37°08' N  | 26°51' E | 6            | Landrace |
| Long Purple    | I-8        | Island    | Aegean Sea        | Leros Island                 | 37°08' N  | 26°51' E | 6            | Landrace |
| LKK-008/07     | I-9        | Island    | Aegean Sea        | Leros, Kamara                | 37°09' N  | 26°49' E | 29           | Landrace |
| M-039/06       | I-10       | Island    | Aegean Sea        | Lesvos, Paleokipos           | 39°05' N  | 26°45' E | 58           | Landrace |
| M-069/06       | I-11       | Island    | Aegean Sea        | Lesvos, Keramia              | 39°12' N  | 26°42' E | 14           | Landrace |
| MFS-030/07     | I-12       | Island    | Aegean Sea        | Milos, Mitakas               | 36°44' N  | 24°29' E | 50           | landrace |
| ANP-180/07     | I-13       | Island    | Aegean Sea        | Naxos, Agia Anna             | 37°04' N  | 25°21' E | 4            | Landrace |
| ANP-215/07     | I-14       | Island    | Aegean Sea        | Paros, Lefkes                | 37°07' N  | 25°12' E | 210          | Landrace |
| HL-027/07      | I-15       | Island    | Aegean Sea        | Santorini, Vourvoulos        | 36°26' N  | 25°26' E | 116          | Landrace |
| SAS-078/07     | I-16       | Island    | Aegean Sea        | Skopelos, Chora              | 39°07' N  | 23°43' E | 41           | Landrace |
| ATS-110/06     | I-17       | Island    | Aegean Sea        | Syros Island, Chrousa        | 37°24' N  | 24°55' E | 320          | Landrace |
| GRC-002/08     | I-18       | Island    | Ionian Sea        | Corfu, Skripero              | 39°42' N  | 19°46' E | 139          | Landrace |
| HL-237/07      | I-19       | Island    | Crete             | Iraklion, Moni Savvathianon  | 35°37' N  | 25°00' E | 467          | Landrace |
| IK-082/06      | I-20       | Island    | Ionian Sea        | Ithaki Island, Perachori     | 38°34' N  | 20°71' E | 343          | Landrace |
| K-153/06       | M-1        | Mainland  | Macedonia         | Grevena, Pontini             | 40°04' N  | 21°40' E | 819          | Landrace |
| EMI            | M-2        | Mainland  | Macedonia         | IPGRB/HAO DEMETER            | 40°32' N  | 22°59' E | 19           | Cultivar |
| Lagkada        | M-3        | Mainland  | Macedonia         | IPGRB/HAO DEMETER            | 40°32' N  | 22°59' E | 19           | Cultivar |
| KD-053/07      | M-4        | Mainland  | Macedonia         | Kavala, Platanotopos         | 40°50' N  | 24°03' E | 244          | Landrace |
| F-154/06       | M-5        | Mainland  | Macedonia         | Kastoria, Ampelokipoi        | 40°46' N  | 21°31' E | 638          | Landrace |
| SK-044/066     | M-6        | Mainland  | Macedonia         | Kilkis, Eptalofos            | 41°00' N  | 23°08' E | 451          | Landrace |
| K-054/06       | M-7        | Mainland  | Macedonia         | Kozani, Anarachi             | 40°29' N  | 21°34' E | 727          | Landrace |
| VG-011/083     | M-8        | Mainland  | Macedonia         | Serres                       | 41°05' N  | 23°35' E | 45           | Landrace |
| SK-056/06      | M-9        | Mainland  | Macedonia         | Serres, Platanakia           | 41°17' N  | 22°56' E | 313          | Landrace |
| Scoutari       | M-10       | Mainland  | Macedonia         | Serres/Skoutari              | 41°01' N  | 23°31' E | 14           | Cultivar |
| P-175/06       | M-11       | Mainland  | Peloponnese       | Lakonia, Lyra                | 36°38' N  | 22°57' E | 400          | Landrace |
| Tsakoniki      | M-12       | Mainland  | Peloponnese       | Leonidio                     | 37°10' N  | 22°51' E | 40           | Cultivar |
| P-084/06       | M-13       | Mainland  | Peloponnese       | Messinia, Kakana             | 37°18' N  | 21°44' E | 136          | Landrace |
| T-099/06       | M-14       | Mainland  | Thessaly          | Karditsa, Neo Ikonio         | 39°27' N  | 22°21' E | 107          | Landrace |
| T-527/06       | M-15       | Mainland  | Thessaly          | Trikala, Megarxis            | 39°36' N  | 21°45' E | 142          | Landrace |
| GRC 1430/04    | M-16       | Mainland  | Epirus            | Ioannina, Pogoni Vasiliko    | 40°00' N  | 20°35' E | 805          | Landrace |



**Figure 1.** Distribution map of the 36 eggplant Greek accessions from the mainland and islands used in this study. Study codes as in Table 1.

## 2.2. Morphological and Mineral Composition Characterization and Data Analysis

Plants were characterized using 22 morphological descriptors for plant (4), leaf (6), flower (1), and fruit (11) traits obtained from the EGGNET descriptor list, of the EU-RESGEN PL 98-113 program [42,43], Biodiversity International (formerly International Board for Plant Genetic Resources) [44], and Kumar et al. [45] descriptors lists (Table 2). For all the traits, four measures were made per plant, except for plant traits, in which one measurement was made. For mineral composition analysis, one plant per block and accession and between one and three fruits per plant (depending on fruit size) were collected and analyzed at commercial maturity. Fruits were washed, peeled, cut into pieces, and bulked. Subsequently, the bulks were weighed, dried at 80 °C until constant weight, and powdered with a mechanical grinder (T 25 digital ULTRA-TURRAX®, IKA®-Werke GmbH & Co. Staufen, Germany). A total of 0.5 g of the dried samples were calcined in a muffle furnace (Thermconcept) at 550 °C for 5 h. The ashes were then dissolved in 10 mL of HCl 1M, filtered and the extract was brought to 50 mL with distilled water [46]. K was analyzed by flame photometry (Sherwood Model 410, Cambridge, UK), while the rest of the minerals (Mg, Fe, Cu, Mn and Zn) were analyzed by atomic absorption spectrophotometry (AAS) using a Thermo Elemental (SOLAAR AA Spectrometers, Cambridge, UK) spectrometer.

Averages for accession were used to calculate means, ranges, and coefficient of variation for mainland and island accessions using IBM® SPSS® Statistics 25 (IBM, Armonk, NY, USA). Significance of differences between means of mainland and islands was performed using the appropriate test at the significance level of  $p < 0.05$ . The results were analyzed using both parametric ( $t$ -test) and nonparametric (Wilcoxon-Mann-Whitney) methods, the latter in the cases of non-normality. A principal

component analysis (PCA) was performed using the function `prcomp` of the package `stats` (v3.6.1) in R [47] and represented graphically using the package `ggplot2` [48].

**Table 2.** Descriptors used for phenotyping of Greek eggplant accessions and their range/scale. All descriptors based on IBPGR descriptors [44], except NOLFF [42,43] and DSFS [45].

| Descriptor Code           | Descriptor Name                     | Descriptor Scale/Unit                                                                                                                                          |
|---------------------------|-------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Plant descriptors</i>  |                                     |                                                                                                                                                                |
| PGH                       | Plant Growth Habit                  | 3–7 (3 = upright; 7 = prostrate)                                                                                                                               |
| PH                        | Plant Height                        | cm                                                                                                                                                             |
| NOLFF                     | Number of Leaves to First Flower    | number                                                                                                                                                         |
| DSFS                      | Days Since Fruit Set                | number                                                                                                                                                         |
| <i>Leaf descriptors</i>   |                                     |                                                                                                                                                                |
| LPL                       | Leaf Petiole Length                 | cm                                                                                                                                                             |
| LBLe                      | Leaf Blade Length                   | cm                                                                                                                                                             |
| LBW                       | Leaf Blade Width                    | cm                                                                                                                                                             |
| LBLo                      | Leaf Blade Lobing                   | 1–9 (1 = very weak; 9 = very strong)                                                                                                                           |
| LSS                       | Leaf Surface Shape                  | 1–9 (1 = flat; 9 = very convex or bullate)                                                                                                                     |
| LP                        | Leaf Prickles                       | 0–9 (0 = none; 9 = more than 20)                                                                                                                               |
| <i>Flower descriptors</i> |                                     |                                                                                                                                                                |
| NOFPI                     | Number of Flowers Per Inflorescence | number                                                                                                                                                         |
| <i>Fruit descriptors</i>  |                                     |                                                                                                                                                                |
| FCP                       | Fruit Calyx Prickles                | 0–9 (0 = none; 9 = more than 30)                                                                                                                               |
| FPL                       | Fruit Pedicel Length                | cm                                                                                                                                                             |
| FL                        | Fruit Length                        | cm                                                                                                                                                             |
| FB                        | Fruit Breadth                       | mm                                                                                                                                                             |
| FLBR                      | Fruit Length to Breadth Ratio       | 1–9 (1 = broader than long; 9 = several times longer than broad)                                                                                               |
| FS                        | Fruit Shape                         | 3–7 (Position of widest part of fruit: 3 = $\frac{1}{4}$ way from base to tip; 5 = $\frac{1}{2}$ way from base to tip; 7 = $\frac{3}{4}$ way from base to tip) |
| FC                        | Fruit Curvature                     | 1–9 (1 = none, fruit straight; 9 = U shaped)                                                                                                                   |
| FW                        | Fruit Weight                        | g                                                                                                                                                              |
| FPC                       | Fruit Predominant Color             | 1–9 (1 = milk white; 9 = black)                                                                                                                                |
| FAC                       | Fruit Additional Color              | 1–9 (1 = milk white; 9 = black)                                                                                                                                |
| FFC                       | Fruit Flesh Color                   | 3–7 (3 = white; 7 = green)                                                                                                                                     |

### 2.3. SSR Characterization and Data Analysis

Total genomic DNA was extracted for each accession from nearly 100 mg of young leaf tissue [49]. DNA quality (230/260 and 260/280 nm ratios) and concentration were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit® 2.0 Fluorometer (Applied Biosystems, Waltham, MA, USA), respectively, while DNA integrity was evaluated by agarose gel electrophoresis (0.8%). Three genomic SSRs developed by Vilanova et al. [50] and two by Nunome et al. [51] were used due to their high polymorphism for the genetic characterization of the samples, which were organized in one multiplex reaction according to the expected allele size range (Table 3). The PCR amplification was performed in a total volume of 12 µL including 7.21 µL water, 1.2 µL 1× PCR buffer, 0.6 µL MgCl<sub>2</sub> (50 mM), 0.24 µL dNTPs (10 mM), 0.06 µL forward primer with M13 tail (10 µM), 0.24 µL fluorochrome (FAM, VIC, NED, and PET, 10 µM), 0.3 µL reverse primer (10 µM), 0.15 µL Taq DNA Polymerase (5 U/µL), and 2 µL DNA template (20 ng/µL). The PCR was performed following the program: 95 °C for 3 min for a denaturation step, 30 cycles of 30 s at 95 °C followed by 30 s at 65 °C and 30 s at 72 °C and finally 72 °C for 5 min for the last extension step. The PCR products were subsequently diluted in formamide and sequenced by capillary electrophoresis through

an ABI PRISM 3100-Avant sequencer (Applied Biosystems, Waltham, MA, USA) using a 500 LIZ GeneScan size standard (Applied Biosystems, Waltham, MA, USA). The fragments were analyzed using the GeneScan software (Applied Biosystems, Waltham, MA, USA) to obtain the electropherograms and the alleles were identified with the Genotyper DNA Fragment Analysis software (Applied Biosystems, Waltham, MA, USA).

**Table 3.** Characteristics of the SSR markers used for the molecular characterization of Greek eggplant accessions.

| SSR Locus | Motif                                 | Forward Primer and Reverse Primer (5'→3')            | Size Range (bp) | T° Annealing | Dye | Source               |
|-----------|---------------------------------------|------------------------------------------------------|-----------------|--------------|-----|----------------------|
| csm4      | (GA) <sup>15</sup>                    | GCGTACCAATTCTAACCACAAG<br>GTAATCCGCTTCCCATTCTC       | 238–254         | 60           | PET | Vilanova et al. [50] |
| csm27     | (GA) <sup>23</sup>                    | TGTTTGGAGGTGAGGGAAAG<br>TCCAACTCACCGGAAAAATC         | 193–210         | 60           | VIC | Vilanova et al. [50] |
| csm32     | (AG) <sup>23</sup>                    | TCGAAAGTACAGCGGAGAAAG<br>GGGGTTTGATTTTCATTTTC        | 248–254         | 60           | NED | Vilanova et al. [50] |
| emi02c21  | (AC) <sup>13</sup> A(TA) <sup>4</sup> | TGTGAGGAGAAGAATCAGAGGATCA<br>CGCGACTAAGTTTGTTCCTGAAA | 126–136         | 60           | VIC | Nunome et al. [51]   |
| eme11f04  | (TC) <sup>16</sup>                    | ACCCCCAAATCAAATCATTTACCC<br>GGCATGGTTAGGGTTTTAGCGTT  | 88–100          | 60           | FAM | Nunome et al. [51]   |

The SSRs analysis was performed with the software PowerMarker [52] and GenAlEx 6.503 [53]. For each marker, the following parameters were calculated: number of alleles ( $N_a$ ), major allele frequency ( $f$ ), number of effective alleles ( $N_e$ ), number of genotypes ( $N_g$ ), polymorphic information content (PIC) that was calculated using the following formula  $PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i p_j$  (where  $n$  is the total number of alleles detected,  $p_i$  is the frequency of the  $i$ th allele, and  $p_j$  is the frequency of the  $j$ th allele) [54], observed heterozygosity ( $H_o$ ) calculated as the number of heterozygous alleles/number of alleles, expected heterozygosity ( $H_e$ ) calculated as  $H_e = 1 - \sum_{i=1}^n p_i^2$  (where  $p_i$  is the frequency of the  $i$ th allele) [55] and the inbreeding coefficient ( $F$ ) calculated as  $F = 1 - (H_o/H_e)$  [56]. Molecular analysis of variance (AMOVA) among groups (mainland vs. islands), among accessions within a group, and within individuals was performed with 999 permutations ( $\alpha = 0.05$ ) using GenAlEx 6.503 software [53]. Correlations between morphological and SSR distance matrices were investigated using a Mantel test ( $\alpha = 0.05$ ) with 999 permutations using GenAlEx 6.503 software [53].

Principal coordinates analysis (PCoA) was performed using the function `dudi.pco` of the package `ade4` (v1.7-13) in R [47] and represented graphically using the package `ggplot2` [48]. A model-based Bayesian structure implemented in the software STRUCTURE (version 2.3.4) [57] was used to estimate the population structure. Twenty runs of STRUCTURE were performed by setting the number of clusters ( $K$ ) from 1 to 10 with a length of the burn-in period of 500,000 steps followed by 150,000 Monte Carlo Markov chain (MCMC) replicates, assuming an admixture model and uncorrelated allele frequencies. No prior knowledge of the population of origin was introduced. The  $\Delta K$  method [58] was used to identify the most likely number of clusters ( $K$ ) using STRUCTURE HARVESTER 0.6.94 software [59]. Each accession was assigned to its corresponding group based on maximum membership probability, as indicated by Remington et al. [60].

### 3. Results

#### 3.1. Morphological Characterization

A wide diversity was observed for the morphological traits in the germplasm collection of Greek eggplant (Table 4, Table S1). For traits measured in a quantitative scale, accession values encompassing a broad range of the scale were observed for most of the traits, except for LBL<sub>o</sub> (3.00 to 5.00), LP (0.00 to 0.92), FS (4.00 to 5.33), and FC (1.00 to 5.00). A wide variation was also observed for the quantitative traits, especially for PH, LPL, NOFPI, FPL, FL, FB and FW. Although the ranges of variation between

accessions from the mainland and islands overlapped, island accessions showed a broader coefficient of variation (CV) for all the quantitative traits, except for NOLFF and LBW. However, significant ( $p < 0.05$ ) differences between the two groups were found only for LBW, LBL<sub>o</sub>, FCP, FB, FLBR, FC, and FW.

The orthogonal transformation by PCA of the morphological traits revealed that the total variation was explained by 22 principal components (PCs), although the first five PCs explained altogether 71.5% (Supplementary data S2). The bi-dimensional representation of the first two PCs revealed that the PC1, which accounted for 32.1% of the variation, was able to separate the island from the mainland accessions (Figure 2A). In fact, most of the island accessions (13 out of 19) exhibited positive PC1 values, while most of the mainland accessions showed negative PC1 values (14 out of 16). Similarly, most of the island accessions (15 out of 19) displayed positive values for the PC2, which accounted for 13.3% of the variation, while mainland accessions (11 out of 16) displayed negative PC2 values. Interestingly, although the accessions from the same geographical area fell relatively close in the PCA, like the island accessions I-6, I-7, I-8, and I-9 from Leros or I-10 and I-11 from Lesvos and the mainland accessions M-8, M-9, and M-10 from Serres, they showed a certain degree of morphological diversity. No high correlation absolute values were found between the morphological traits and the first two PCs (Table S2). However, moderate to weak PC1 positive correlations were found for FB and FW and negative correlation for FLBR and FC, while weak PC2 positive correlations were found for LBW, LPL, LBL<sub>e</sub>, and NOFPI, and no negative PC2 correlations were found ( $< -0.3$ ).

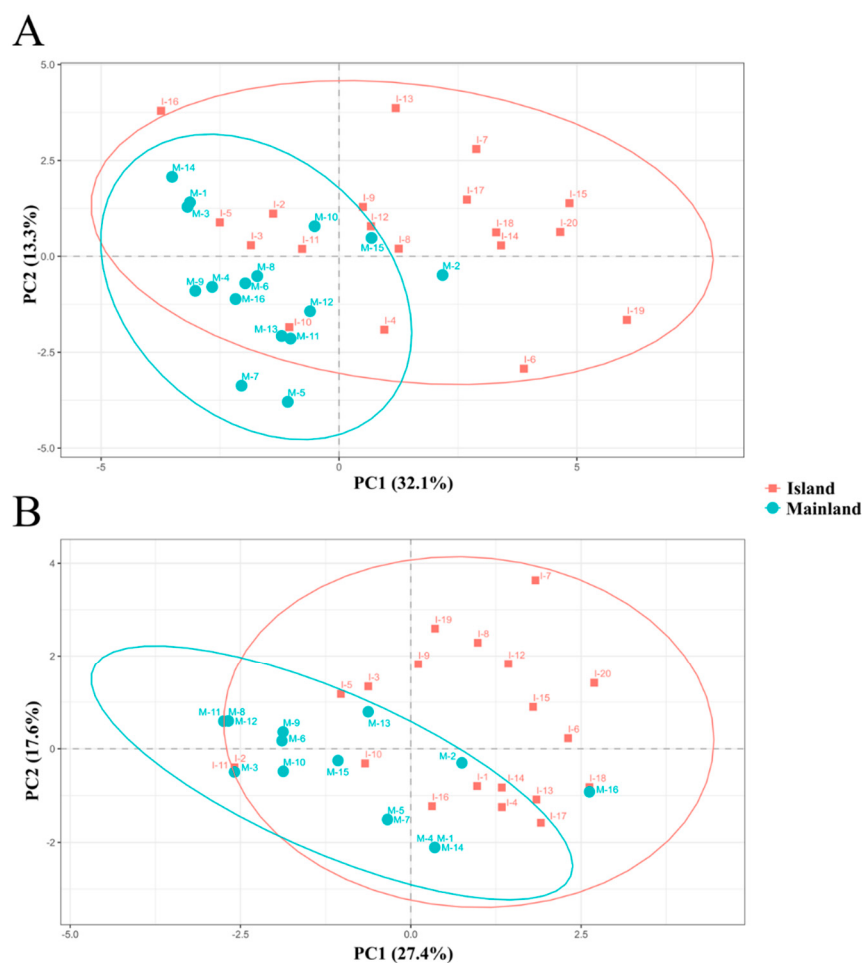
**Table 4.** Mean, range (between brackets), and coefficient of variation (CV %) for the morphological and mineral descriptors studied in eggplant accessions of the Greek islands ( $n = 19$ ) and the mainland ( $n = 16$ ) and significance of mean differences among these two groups ( $p < 0.05$ ).

|                          | Mainland                |        | Islands                 |        | <i>p</i> -Value |
|--------------------------|-------------------------|--------|-------------------------|--------|-----------------|
|                          | Mean<br>(Range)         | CV (%) | Mean<br>(Range)         | CV (%) |                 |
| <i>Plant descriptors</i> |                         |        |                         |        |                 |
| PGH                      | 4.36<br>(3.00–6.50)     | 23.70  | 5.09<br>(3.00–7.00)     | 21.50  | 0.052           |
| PH                       | 80.59<br>(56.83–106.00) | 16.30  | 71.72<br>(46.50–106.17) | 21.90  | 0.083           |
| NOLFF                    | 4.97<br>(4.00–6.67)     | 13.30  | 5.41<br>(4.20–6.83)     | 12.50  | 0.060           |
| DSFS                     | 50.51<br>(42.83–58.33)  | 8.20   | 48.26<br>(41.00–55.67)  | 9.10   | 0.129           |
| <i>Leaf descriptors</i>  |                         |        |                         |        |                 |
| LPL                      | 9.83<br>(6.79–12.29)    | 17.60  | 10.22<br>(6.53–14.63)   | 19.40  | 0.541           |
| LBL <sub>e</sub>         | 31.51<br>(27.50–36.97)  | 8.40   | 31.4<br>(25.57–37.48)   | 10.50  | 0.918           |
| LBW                      | 20.43<br>(16.35–25.62)  | 11.50  | 22.14<br>(18.16–25.63)  | 9.80   | <b>0.032 *</b>  |
| LBL <sub>o</sub>         | 3.38<br>(3.00–5.00)     | 23.80  | 4.37<br>(3.00–5.00)     | 21.90  | <b>0.004 *</b>  |
| LSS                      | 4.88<br>(3.00–7.00)     | 27.90  | 5.42<br>(3.00–7.00)     | 19.80  | 0.203           |
| LP                       | 0.18<br>(0.00–0.92)     | 126.40 | 0.21<br>(0.00–0.92)     | 125.70 | 0.892           |

Table 4. Cont.

|                           | Mainland                  |        | Islands                   |        | <i>p</i> -Value |
|---------------------------|---------------------------|--------|---------------------------|--------|-----------------|
|                           | Mean<br>(Range)           | CV (%) | Mean<br>(Range)           | CV (%) |                 |
| <i>Flower descriptors</i> |                           |        |                           |        |                 |
| NOFPI                     | 1.15<br>(1.00–1.40)       | 11.10  | 1.22<br>(1.00–1.60)       | 16.90  | 0.388           |
| <i>Fruit descriptors</i>  |                           |        |                           |        |                 |
| FCP                       | 1.51<br>(0.00–4.00)       | 83.90  | 3.32<br>(0.33–7.00)       | 57.90  | <b>0.003 *</b>  |
| FPL                       | 6.33<br>(4.91–8.22)       | 16.70  | 6.24<br>(3.58–9.72)       | 32.10  | 0.840           |
| FL                        | 18.46<br>(14.50–22.32)    | 13.00  | 18.4<br>(11.2–43.85)      | 42.80  | 0.246           |
| FB                        | 61.93<br>(50.91–87.78)    | 16.20  | 85.1<br>(52.75–110.76)    | 20.30  | <b>0.000 *</b>  |
| FLBR                      | 7.49<br>(5.00–8.50)       | 11.50  | 5.2<br>(1.33–8.33)        | 44.40  | <b>0.002 *</b>  |
| FS                        | 4.69<br>(4.00–5.33)       | 7.00   | 4.36<br>(3.33–5.00)       | 12.20  | 0.106           |
| FC                        | 3.3<br>(1.00–5.00)        | 32.30  | 1.81<br>(1.00–4.00)       | 53.70  | <b>0.000 *</b>  |
| FW                        | 246.33<br>(177.32–368.40) | 19.70  | 329.64<br>(215.66–500.67) | 20.80  | <b>0.000 *</b>  |
| FPC                       | 6.52<br>(2.00–8.00)       | 22.10  | 5.83<br>(1.00–8.17)       | 40.50  | 0.550           |
| FAC                       | 4.31<br>(1.00–7.50)       | 48.00  | 4.78<br>(1.00–8.17)       | 48.20  | 0.527           |
| FFC                       | 4.73<br>(3.00–5.33)       | 15.10  | 4.45<br>(3.00–6.00)       | 20.60  | 0.218           |
| <i>Minerals</i>           |                           |        |                           |        |                 |
| K (mg/g DW)               | 25.05<br>(20.07–31.50)    | 13.21  | 23.14<br>(19.80–29.00)    | 11.58  | 0.073           |
| Mg (mg/g DW)              | 2.22<br>(1.96–2.46)       | 6.75   | 2.05<br>(1.63–2.43)       | 12.19  | <b>0.015 *</b>  |
| Cu (mg/Kg DW)             | 9.56<br>(2.91–18.14)      | 47.91  | 7.65<br>(2.35–26.93)      | 69.67  | 0.278           |
| Fe (mg/Kg DW)             | 31.8<br>(25.14–43.94)     | 14.68  | 31.48<br>(22.02–41.84)    | 15.78  | 0.850           |
| Mn (mg/Kg DW)             | 12.25<br>(9.61–13.67)     | 8.82   | 11.74<br>(9.79–13.84)     | 9.71   | 0.198           |
| Zn (mg/Kg DW)             | 49.75<br>(30.30–77.26)    | 26.85  | 49.39<br>(29.53–76.90)    | 31.14  | 0.942           |

\* indicates a significant difference among islands and the mainland accessions at *p*-value < 0.05.



**Figure 2.** Principal component analysis (PCA) similarities based on the morphological characterization of 35 eggplant accessions used in this study (A) and principal coordinates analysis (PCoA) based on the genotyping characterization of all the accessions used in this study from the mainland (16) and island (20) Greek areas (B). The first and second principal coordinates (PC) are displayed. Study codes as in Table 1.

### 3.2. Concentration of Minerals

The concentrations of the macrominerals K and Mg were much higher than those of the microminerals Cu, Fe, Mn and Zn (Table 4). As occurred for morphological traits, a wide variation was observed among the accessions for mineral composition, with ranges of variation in the collection of over 10-fold for Cu, and ranges of the coefficient of variation of up to 67.2% for Cu in the accessions from the islands. However, for all traits, an overlap of the values was observed for the six minerals analyzed among the two groups, and significant differences between averages of mainland and insular groups were observed only for Mg, with slightly larger values in the mainland group.

### 3.3. SSR Characterization

All five SSRs were polymorphic and amplified between three (em311f04) and seven (csm4) alleles totaling 23 alleles, resulting in an average of 4.6 alleles per SSR locus (Table 5, Table S3). The major allele frequency was, on average, slightly higher than 0.5, ranging between 0.31 (csm4) and 0.65 (eme11f04). The average number of effective alleles ( $N_e$ ; 2.74) was lower than the number of alleles ( $N_a$ ; 4.60), ranging from 1.91 (eme11f04) to 4.41 (csm4), while the number of genotypes ( $N_g$ ) was higher than  $N_a$ , ranging from 4 (emi02c21) to 10 (csm4), except emi02c21, which showed the same number of alleles and genotypes, indicating that all loci were in homozygosity. The PIC value had an average value of 0.55,

with a range between 0.39 (eme11f04) and 0.75 (csm4). The  $H_o$  showed low values with an average of 0.13, ranged from 0 (emi02c21) to 0.17 (csm27), much lower than those of  $H_e$  (0.60 on average), which ranged from 0.47 (eme11f04) to 0.77 (csm4). On the contrary,  $F$  values were high, ranging from 0.66 (eme11f04) to 1 (emi02c21). The comparison between mainland and island accessions revealed that the latter presented generally higher genetic diversity (Table 5). In this respect, mainland accessions showed a lower number of alleles compared to the island accessions (3.2 and 4.2, respectively), as well as a lower number of effective alleles (2.22 vs. 2.79), genotypes (3.8 vs. 5.6), and PIC (0.42 vs. 0.53) but a higher frequency of the major allele (0.60 vs. 0.48). Average values for  $H_o$  and  $H_e$  were also much lower in the mainland accessions (0.03 and 0.48) than in the islands' accessions (0.20 and 0.61), while  $F$  values were much higher in the mainland accessions (0.92) than in those from the islands (0.68).

**Table 5.** Diversity statistics of SSR markers for all the Greek eggplant accessions and for the island and the mainland groups. Diversity statistics evaluated include: number of alleles ( $N_a$ ), major allele frequency ( $f$ ), number of effective alleles ( $N_e$ ), number of genotypes ( $N_g$ ), polymorphic information content (PIC), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), and inbreeding coefficient ( $F$ ).

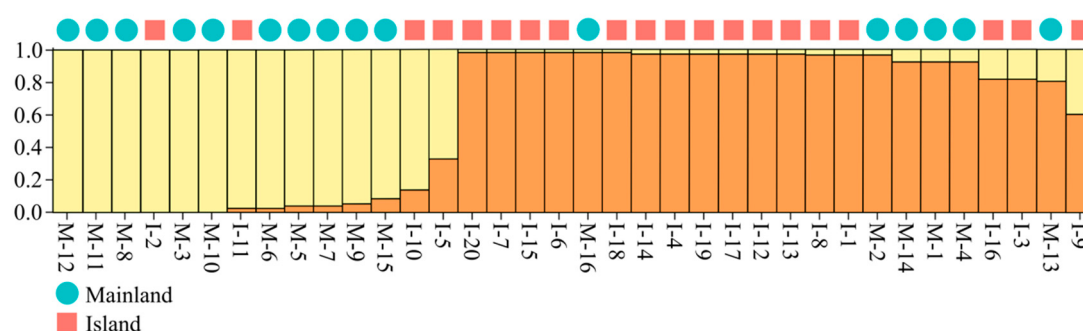
| SSR Locus                           | $N_a$         | $f$              | $N_e$            | $N_g$          | PIC              | $H_o$            | $H_e$            | $F$              |
|-------------------------------------|---------------|------------------|------------------|----------------|------------------|------------------|------------------|------------------|
| <i>All accessions (n = 36)</i>      |               |                  |                  |                |                  |                  |                  |                  |
| csm4                                | 7             | 0.32             | 4.41             | 10.00          | 0.76             | 0.14             | 0.77             | 0.83             |
| csm27                               | 4             | 0.50             | 2.63             | 7.00           | 0.58             | 0.17             | 0.62             | 0.75             |
| csm32                               | 5             | 0.53             | 2.57             | 7.00           | 0.55             | 0.17             | 0.61             | 0.73             |
| emi02c21                            | 4             | 0.61             | 2.19             | 4.00           | 0.48             | 0.00             | 0.54             | 1.00             |
| eme11f04                            | 3             | 0.65             | 1.92             | 5.00           | 0.40             | 0.17             | 0.48             | 0.66             |
| Mean $\pm$ SE                       | 4.6 $\pm$ 0.7 | 0.522 $\pm$ 0.05 | 2.746 $\pm$ 0.43 | 6.6 $\pm$ 1.00 | 0.552 $\pm$ 0.05 | 0.130 $\pm$ 0.03 | 0.605 $\pm$ 0.04 | 0.796 $\pm$ 0.05 |
| <i>Islands accessions (n = 20)</i>  |               |                  |                  |                |                  |                  |                  |                  |
| csm4                                | 6             | 0.33             | 4.65             | 8.00           | 0.75             | 0.20             | 0.79             | 0.76             |
| csm27                               | 4             | 0.53             | 2.28             | 5.00           | 0.47             | 0.25             | 0.56             | 0.57             |
| csm32                               | 5             | 0.58             | 2.53             | 7.00           | 0.56             | 0.25             | 0.61             | 0.60             |
| emi02c21                            | 3             | 0.50             | 2.20             | 3.00           | 0.44             | 0.00             | 0.55             | 1.00             |
| eme11f04                            | 3             | 0.50             | 2.29             | 5.00           | 0.47             | 0.30             | 0.56             | 0.49             |
| Mean $\pm$ SE                       | 4.2 $\pm$ 0.6 | 0.485 $\pm$ 0.04 | 2.79 $\pm$ 0.46  | 5.6 $\pm$ 0.90 | 0.538 $\pm$ 0.05 | 0.200 $\pm$ 0.05 | 0.612 $\pm$ 0.04 | 0.686 $\pm$ 0.09 |
| <i>Mainland accessions (n = 16)</i> |               |                  |                  |                |                  |                  |                  |                  |
| csm4                                | 4             | 0.37             | 3.44             | 5.00           | 0.66             | 0.07             | 0.71             | 0.91             |
| csm27                               | 3             | 0.50             | 2.57             | 4.00           | 0.54             | 0.07             | 0.61             | 0.90             |
| csm32                               | 3             | 0.47             | 2.26             | 4.00           | 0.46             | 0.06             | 0.56             | 0.89             |
| emi02c21                            | 4             | 0.75             | 1.71             | 4.00           | 0.39             | 0.00             | 0.41             | 1.00             |
| eme11f04                            | 2             | 0.94             | 1.13             | 2.00           | 0.11             | 0.00             | 0.12             | 1.00             |
| Mean $\pm$ SE                       | 3.2 $\pm$ 0.4 | 0.604 $\pm$ 0.10 | 2.22 $\pm$ 0.39  | 3.8 $\pm$ 0.50 | 0.429 $\pm$ 0.09 | 0.039 $\pm$ 0.01 | 0.482 $\pm$ 0.10 | 0.923 $\pm$ 0.02 |

### 3.4. SSR Molecular Analysis of Variance, Genetic Structure, and Multivariate Analysis

The AMOVA revealed that the variation among accessions accounts for the greatest proportion of molecular variance (69.62%), followed by the variation within individuals due to heterozygosity (19.25%), and finally by the variation among mainland and island groups, which accounts for just 11.12% of the total variation (Table 6). The  $\Delta K$  statistic in the genetic structure analysis presented a maximum peak at  $K = 2$ , suggesting that two genetic clusters exist in the Greek materials evaluated (Figure S1). With very few exceptions, the percentage of the ascription of accessions to their cluster was over 80%, revealing a low degree of genetic admixture (Figure 3). Cluster one included 14 accessions, of which 10 (71.4%) were from the mainland and 4 (28.6%) were from islands. Cluster two was made up by 22 accessions, of which 6 (27.3%) are from the mainland, and 16 (72.7%) are from the islands.

**Table 6.** Molecular analysis of variance in 36 Greek eggplant accessions.  $p$ -value estimates are based on 999 permutations ( $\alpha = 0.05$ ). The following sources of variation were considered: between groups (mainland vs. islands), among accessions within a group, and within individuals. Acronyms:  $df$  = degrees of freedom,  $SS$  = Sums of Squares,  $MS$  = mean squared deviations,  $F_{st}$  = genetic differentiation among populations within the total sample,  $F_{is}$  = genetic differentiation among individuals within populations,  $F_{it}$  = genetic differentiation among individuals within the total sample.

| Source            | df | S.S.   | M.S. | Variance Components | Percentage of Variation | F-Statistics   | p-Value |
|-------------------|----|--------|------|---------------------|-------------------------|----------------|---------|
| Between groups    | 1  | 9.19   | 9.19 | 0.19                | 11.12                   | $F_{st}$ 0.111 | 0.001   |
| Among accessions  | 34 | 89.42  | 2.63 | 1.16                | 69.62                   | $F_{is}$ 0.783 | 0.001   |
| Within accessions | 36 | 11.50  | 0.32 | 0.32                | 19.25                   | $F_{it}$ 0.807 | 0.001   |
| Total             | 71 | 110.11 |      | 1.66                |                         |                |         |



**Figure 3.** Genetic population structure of the 36 Greek eggplant accessions from the mainland and island areas based on SSRs data ( $K = 2$ ). Each individual is represented by a vertical bar that is partitioned into colored segments that represent the estimated ratio of membership of an individual to a cluster. Cluster one is in yellow while cluster 2 is in orange. Study codes as in Table 1.

The PCoA analysis based on SSRs data explained all the variation with 17 PCs, although the first five PCs accounted for 75.8% (Table S4). Like in the PCA analysis with morphological data, the PC1, which accounted for 27.4%, allowed to separate the island accessions, with positive values of PC1 (15 out of 20), from the accessions of the mainland, with negative values of the PC1 (11 out of 16) (Figure 2B). On the contrary, PC2, which accounted for 17.6%, cannot clearly separate the two groups that spread from positive to negative values. Like the PCA on morphological traits, the accessions from the same geographical area showed a certain degree of genetic diversity. Finally, the Mantel test showed a weak, although significant, correlation between genetic and morphological data ( $r = 0.244$ ,  $p = 0.002$ ) (Table S5).

#### 4. Discussion

In a climate change scenario coupled with an unprecedented increase in food demand, due to dietary changes and relentless growing population [61], local germplasm characterization and enhancement is imperative. The genetic variability and allelic diversity of local landraces could mitigate the effects of a changing environment and may guarantee food security of domestic markets [62]. In addition, heirlooms and ecotypes can harbor allelic combinations useful for the development of new more resilient and locally adapted varieties [14]. In this respect, this study aimed to assess the morphological and genetic diversity of the eggplant germplasm collection from Greece which is distributed and cultivated in a wide variety of niches throughout the distinctive country's topography. Moreover, the characterization of Greek eggplant landraces will prevent genetic erosion of this local group and provide a valuable resource for utilization in research and breeding.

#### 4.1. Morphological Diversity

The morphological characterization displayed considerable diversity for most of the traits studied. The most heterogeneous traits were those related to fruit appearance, such as shape and size, followed by others related to vegetative traits, such as plant and organ size and type of growth, alongside with skin and flesh color. The solanaceous fruits exhibit considerable morphological diversity, including size, shape, and color, both within and between different species [63]. Morphological variations of fruits in terms of shape, size, and color are a result of adaptive evolution; in addition, variation observed in the vegetative traits could be due to the accumulation of mutations on neutral traits, to artificial or natural selection or to both [63,64]. These same types of morphological traits were also the most variable ones when eggplant accessions collected from Greece, Spain, Turkey, and remote centers of diversity were studied [23,34,35,65,66]. Consistent with previous works, the amount of diversity is comparable to that observed in the characterization of germplasm from other geographical areas of the Mediterranean region [34,35,64–66], providing further evidence that several areas of the Mediterranean region can be considered as secondary centers of diversity for eggplant [35,64].

PCA analysis revealed the existence of two discrete groups, one formed almost exclusively by the island accessions and the other mostly by those from the mainland ones. On average, the accessions from the islands have higher values for fruit weight, fruit calyx prickles, leaf blade width, leaf blade lobing, fruit breadth, but lower ones for fruit curvature and fruit length to breadth ratio than those from the mainland. The assignment of the eggplant accessions studied into groups according to their agromorphological characteristics correlated with their geographic origin has also been reported in other eggplant studies [35,67–69].

#### 4.2. Mineral Composition

As occurred for morphological traits, the wide variation observed for mineral composition agrees with other works in which a significant number of eggplant accessions, commercial varieties, and hybrids were evaluated [65]. In the present study, significant differences were found only for Mg among island and mainland accessions, while non-significant differences were observed for Fe, Mn, Zn, Cu, and K. Raigón et al. [39] and Arivalagan et al. [38–40] studied the mineral composition and the nutritional value of eggplant landraces and germplasm accessions from different regions in order to identify mineral-rich germplasm for breeding purposes. For comparison, we transformed the values detected in the aforementioned studies, to the same units as in our study. The eggplant accessions studied had lower K and Cu concentration than those of Raigón et al. [39] and Arivalagan et al. [38,40]. Mg content in our study was similar to that of Raigón et al. [39] and higher than in Arivalagan et al. [38,40], while Zn concentration was significantly higher than in these studies. The environment, cultivation methods, as well as the cultivar used can influence the mineral composition of eggplants [70,71], and explain the differences observed in minerals' concentrations among our study and the others. To our knowledge, this is the first time that an inclusive uniform mineral content characterization of eggplant fruits from a field-grown germplasm collection was examined.

#### 4.3. Molecular Diversity

Many studies assessed the genetic diversity and population structure of local varieties collections from different origins using SSRs [34–36,45,70,71]. The SSR markers used, were highly informative and effective for genetic diversity analysis. For example, the PIC value in the whole collection ranged from 0.39 to 0.75 with a mean value of 0.55. This value is higher than those observed in previous studies [36,45,72,73], while it was lower than those calculated by Hurtado et al. [35] and Cericola et al. [34]. A PIC value greater than 0.5 indicates loci of high polymorphism, PIC values between 0.25 and 0.5 indicate loci of intermediate polymorphism, and PIC values less than 0.25 indicate loci of low polymorphism [72]. In addition, the number of alleles/locus in the whole collection was high (4.6), higher than that (3.67) revealed by Augustinos et al. [23], when they studied a limited number

of Greek traditional eggplant cultivars. Taking into consideration other studies on eggplants from different geographic origin or different cultivar types based on SSR data, the number of alleles/locus calculated in our eggplant collection is lower than that detected by Hurtado et al. [35] and Liu et al. [36], but almost similar to those by Demir et al. [74], Muñoz-Falcón et al. [73], Tümbilen et al. [66], and Vilanova et al. [50,75]. Two of the SSR markers used in our study (csm27, csm32), have also been used by Hurtado et al. [35] and Vilanova et al. [50]. In their studies, they observed higher average number of alleles/locus as compared to those detected by us, an observation that might be explained by the fact that their collections originate from a broader geographic area, or belong to different cultivar types, in contrast to our collection which originates from a single and more geographically restricted area. However, when Hurtado et al. [35] calculated the average number of alleles/locus only for the Spanish eggplants included in their collection, it was almost similar to ours (5.0). Expected heterozygosity ( $H_e$ ) values were high, with an average value of 0.6, quite similar or higher to the values observed in other similar studies, providing further evidence of the utility and highly informative nature of the SSRs used. The estimated values of observed heterozygosity ( $H_o$ ) (average 0.13) were expected, since eggplant is basically an autogamous plant [76] and thus most heritage and commercial varieties are expected to be homozygous [34]. Muñoz-Falcón et al. [77] detected significantly lower values for Spanish landraces (0.02) and for non-Spanish landraces (0.02), whereas Cericola et al. [34] also found that most eggplant landraces used in their study had low heterozygosity values, and only 38 out of 238 materials had  $H_o > 0.10$ . Low  $H_o$  was also detected by Augustinos et al. [23] and Liu et al. [36] (0.04 and 0.03, respectively) and Vilanova et al. [50,75]. The high level of homozygosity in eggplant landraces shows that pure lines can easily be derived by individual selection from these materials [75]. Although genetic diversity based on molecular data is dependent on the type and number of markers used and the accessions tested [66], the relatively high PIC values and average number of alleles/locus detected and also the high levels of heterozygosity observed compared to other similar studies, indicate that considerable diversity exists in our eggplant collection. Although, the number of SSR markers we used has been limited, their high PIC values indicate that they provide meaningful information on the diversity of Greek eggplants. Consequently, Greece, together with other countries such as Spain and Turkey, might be considered as part of a secondary center of diversity for the eggplant in the Mediterranean basin. Further analysis with high-throughput methods may provide a confirmation for our hypothesis.

Remarkably, the island accessions seem to be more variable than those from the mainland, as indicated by the average number of alleles/locus (4.2 vs. 3.2) and PIC values (0.53 vs. 0.42). Higher values of  $H_o$  were observed in the island accessions when compared to the mainland ones (0.20 vs. 0.03), suggesting that a higher degree of outcrossing occurs in the island accessions than in those from the mainland. Although endemic island populations tend to have less diverse variation in comparison to continental populations [25,31,78], due to founder effect events [78,79], of limited population size and genetic drift [80], the heterozygote excess in our case could be possibly due to selective advantages enjoyed by heterozygote individuals, due to the agroecological conditions of the island environment, as in the case of dill [12]. Another possible explanation is that Greek islands in the Aegean Sea are in proximity with Asia minor and the Turkish coastline and thus genetic exchange could be favored through pollen dispersal, human influence and transportation. Environmental conditions also have a critical impact on the relationship between plants and pollinators. Rain can disrupt this pollen transfer and hinder sexual reproduction in plants [81]. The dry climate and the lack of rainfall in the islands may promote cross-pollination, therefore, increasing the proportion of heterozygotes in the island accessions. Consistent with the PCA analysis of the morphological traits, the genetic STRUCTURE analysis differentiates two genetic clusters, one made up mostly by mainland and another by the island accessions. These results are also confirmed in the PCoA analysis. The fact that some island accessions fell in the mainland genetic cluster and vice versa probably has contributed to the limited differentiation observed in the AMOVA analysis between the mainland and the island groups (11.12%).

Discrimination among accessions originating from the islands and the mainland has also been reported for Greek dill by Ninou et al. [12] and for okra landraces by Kyriakopoulou et al. [82].

#### 4.4. Comparison of Morphological and Molecular Diversity

Undoubtedly, even though genetic characterization has become predominant in the last decade due to the efficiency and the plummeting cost of sequencing and genotyping, the morphological phenotyping is still essential and complementary to the genetic assessment [83]. The correlations between morphological and molecular data usually vary depending on the eggplant genotype, the morphological descriptors, and the molecular markers used [35]. In this study, the Mantel test detected a weak, although significant, correlation between genetic and morphological data ( $r = 0.244$ ,  $p = 0.002$ ), in agreement with previous eggplant studies where weak or no correlation between genotypic and phenotypic data have been found [23,34,35,77]. However, these results need to be confirmed by additional analyses with larger molecular markers datasets.

### 5. Conclusions

In conclusion, our results show a remarkable phenotypic and genetic variation existing in the Greek eggplant landraces, suggesting that Greece, among other Mediterranean countries, such as Italy and Spain, might be considered as a secondary center of diversity for eggplant. Both morphological and genetic data revealed clustering of eggplant accessions into two major groups based on the geographic origin (mainland vs. island accessions). Some differentiation signatures in morphological, molecular, and fruit composition were detected between both groups underlying the significant impact of the environment, geographic isolation, and the agroecological conditions in the differentiation and characteristics of the eggplant landraces from the Greek islands and mainland. There was a weak but significant correlation between the morphological and genetic data, indicating that both types of data provide complementary information and therefore they should be taken into consideration for the characterization of plant material. Our results show that local Greek populations of eggplant are a promising material which can be of interest not only for conservation purposes but also for the manipulation of value-added traits through breeding programs and for commercial exploitation.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/9/12/887/s1>, Table S1: Average values of the morphological and mineral characterization of the eggplant accessions of Greek islands ( $n = 19$ ) and the mainland ( $n = 16$ ). Table S2: Percentage of the variance explained by each principal component (PC) of the PCA analysis, correlations between the PCs and the morphological traits evaluated in Greek eggplant accessions and the eigenvalues of the accessions for each PCs. Table S3: SSR alleles identified in the eggplant accessions of Greek islands and the mainland. Table S4: Percentage of the variance explained by each principal component (PC) of the PCoA analysis and eigenvectors in Greek eggplant accessions for each PC. Table S5: Mantel test between morphological and genetic data. Figure S1: Delta K values for 2 to 10 genetic clusters for thirty-six accessions (cultivars and landraces) in Greek eggplant accessions. Delta K was calculated according to Evanno et al. (2005).

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