Genetic Diversity, Population Structure, and Resistance to *Phytophthora capsici* of a Worldwide Collection of Eggplant Germplasm

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Abstract

Eggplant (*Solanum melongena* L.) is an important solanaceous crop with high phenotypic diversity and moderate genotypic diversity. Ninety-nine genotypes of eggplant germplasm (species (*S. melongena, S. incanum, S. linnaeanum* and *S. gilo*), landraces and heirloom cultivars) from 32 countries and five continents were evaluated for genetic diversity, population structure, fruit shape, and disease resistance to *Phytophthora* fruit rot. Fruits from each line were measured for fruit shape and evaluated for resistance to two *Phytophthora* isolates seven days post inoculation. Only one accession (PI 413784) was completely resistant to both isolates evaluated. Partial resistance to *Phytophthora* fruit rot was found in 16 accessions from four *Solanum* species evaluated in this study. Genetic diversity and population structure were assessed using 22 polymorphic simple sequence repeats (SSRs). The polymorphism information content (PIC) for the population was moderate (0.49) in the population. Genetic analyses using the program STRUCTURE indicated the existence of four genetic clusters within the eggplant collection. Population structure was detected when eggplant lines were grouped by species, continent of origin, country of origin, fruit shape and disease resistance.

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Introduction

Cultivated eggplant, *Solanum melongena* L., is a high-value vegetable commodity in Europe and Asia. China and India are the major producers with 27.7 and 11.9 million tons per year, respectively [2011 FAO]. Eggplant is the third most important solanaceous crop worldwide after potato and tomato, and fourth most important in the U.S. [2011 FAO]. In the U.S., eggplants are a minor crop grown for specialty markets with an approximate production of 62 thousand tons annually [2011 FAO].

Unlike most other cultivated solanaceous crops (tomatoes, peppers, and potatoes), eggplants are an Old World species. Domestication of the cultivated eggplant is thought to have occurred in Asia as early as 59 B.C. [1–3]. Since that time, it has been transported and cultivated around the world [4,5]. Primary and secondary centers of diversity for eggplant are Asia and the Mediterranean basin in Europe, respectively. Studies have indicated that the progenitors of domesticated eggplant (*S. melongena*) originated in Africa and were derived from the closely related *Solanum incanum* (part of the eggplant complex) [6] and *Solanum linnaeanum* [5]. Both *S. incanum* and *S. linnaeanum* can form partially fertile hybrids with *S. melongena* making them potential sources for desirable traits such as abiotic and biotic disease resistance [7–9]. Wild relatives have traditionally been a good source of disease resistance for cultivated species that exhibit lower genetic diversity [10–15]. Domesticated heirloom varieties and landrace accessions may also harbor resistance, and are often more similar to modern cultivated varieties than wild species, making them a good source for desirable traits [16–17].

Multiple *Phytophthora* species are capable of causing disease symptoms on eggplants. Infected eggplants can display root and/or fruit symptoms. One causal agent of *Phytophthora* fruit rot is *Phytophthora capsici* L., an oomycete that affects multiple solanaceous species including eggplant, pepper, and tomato [18–22]. In the field, chemical management is expensive and provides limited protection against *Phytophthora capsici*-induced fruit rot in eggplant, which is the most common *Phytophthora*-induced symptom in eggplants [23]. Cultivated eggplants have some level of root rot resistance to moderately virulent isolates of *P. capsici* [20]. No cultivars, to date, have displayed any type of fruit resistance. Host resistance, an important part of a successful, sustainable management program, is not available for management of *Phytophthora* fruit rot in eggplants and currently, no known lines or cultivars are resistant to *P. capsici*. Partial fruit rot resistance to *P. capsici* has been identified in other solanaceous species such as peppers and tomatoes ([24], Granke et al. unpublished), but to our knowledge this has not been evaluated in eggplant.

In addition to disease resistance, fruit shape is an important attribute for each cultivar and many studies have been performed to identify the genetic basis of fruit shape in the Solanaceae [25–30]. Size, shape and color vary greatly between eggplant market classes, and it will be important to maintain this phenotypic diversity when incorporating disease resistance [4,31–32]. This
Table 1. Eggplant germplasm used for the study of morphological and molecular variation.

| Species   | ID    | Accession | Plant ID | Country        | Source        |
|-----------|-------|-----------|----------|----------------|---------------|
| *S. melongena* | 101   | C-S-16    | -        | Spain          | UT Valencia   |
| *S. melongena* | 102   | Griff 1276| 468      | Thailand       | USDA-GRIN     |
| *S. melongena* | 103   | Griff 14182| New Orleans Market | U.S.         | USDA-GRIN     |
| *S. melongena* | 104   | Griff 14186| Hastings purple thornless | U.S.         | USDA-GRIN     |
| *S. melongena* | 105   | H15       | -        | Spain          | UT Valencia   |
| *S. melongena* | 106   | IIVA-371  | -        | Spain          | UT Valencia   |
| *S. melongena* | 107   | MM 108 bis| -        | France         | AVRDC         |
| *S. melongena* | 108   | MM 114    | Berengena larga negra | Spain         | AVRDC         |
| *S. melongena* | 109   | MM 1171   | Large Santa Olalla | Costa Rica   | AVRDC         |
| *S. melongena* | 110   | MM 1363   | -        | Costa Rica     | AVRDC         |
| *S. melongena* | 111   | MM 1364   | -        | Costa Rica     | AVRDC         |
| *S. melongena* | 112   | MM 1365   | -        | Guatemala      | AVRDC         |
| *S. melongena* | 113   | MM 141    | Violette d’Avignon | France       | AVRDC         |
| *S. melongena* | 114   | MM 1750   | Listada di Gandia | Spain        | AVRDC         |
| *S. melongena* | 115   | MM 346    | Berengena redonda | Spain        | AVRDC         |
| *S. melongena* | 116   | MM 39     | Noire de Chateaurenard | France     | AVRDC         |
| *S. melongena* | 117   | MM 522    | Waimanolo long B1 | U.S.        | AVRDC         |
| *S. melongena* | 118   | MM 56     | Violette de Toulouse | France     | AVRDC         |
| *S. melongena* | 119   | MM 61     | Zebrina   | Spain          | AVRDC         |
| *S. melongena* | 120   | MM 64     | Ronde de Valence | France      | AVRDC         |
| *S. melongena* | 121   | MM 69     | Monstrueuse de New York | U.S.       | AVRDC         |
| *S. melongena* | 122   | MM 91     | Black Beauty | U.S.         | AVRDC         |
| *S. melongena* | 123   | PI 102727 | No. 202  | Uzbekistan     | USDA-GRIN     |
| *S. melongena* | 124   | PI 105346 | Lao Lai Hei Chieh | China       | USDA-GRIN     |
| *S. melongena* | 125   | PI 115505 | Giant of Benares | India       | USDA-GRIN     |
| *S. melongena* | 126   | PI 140446 | 5917     | Iran           | USDA-GRIN     |
| *S. melongena* | 127   | PI 140456 | 7015     | Iran           | USDA-GRIN     |
| *S. melongena* | 128   | PI 141968 | No. 1    | China          | USDA-GRIN     |
| *S. melongena* | 129   | PI 143410 | Badenjan  | Iran           | USDA-GRIN     |
| *S. melongena* | 130   | PI 169641 | 1448     | Turkey         | USDA-GRIN     |
| *S. melongena* | 131   | PI 169650 | 2259     | Turkey         | USDA-GRIN     |
| *S. melongena* | 132   | PI 171851 | 6753     | Turkey         | USDA-GRIN     |
| *S. melongena* | 133   | PI 175914 | 9043     | Turkey         | USDA-GRIN     |
| *S. melongena* | 134   | PI 179500 | 9877     | Iraq           | USDA-GRIN     |
| *S. melongena* | 135   | PI 179997 | 10598    | India          | USDA-GRIN     |
| *S. melongena* | 136   | PI 181896 | Aleppo 3 | Syria          | USDA-GRIN     |
| *S. melongena* | 137   | PI 181963 | Homs 21  | Syria          | USDA-GRIN     |
| *S. melongena* | 138   | PI 193599 | Long Violet | Ethiopia     | USDA-GRIN     |
| *S. melongena* | 140   | PI 199516 | M 19     | Greece         | USDA-GRIN     |
| *S. melongena* | 141   | PI 200881 | -        | Afghanistan    | USDA-GRIN     |
| *S. melongena* | 142   | PI 204731 | -        | Turkey         | USDA-GRIN     |
| *S. melongena* | 143   | PI 213193 | M-57/29  | Greece         | USDA-GRIN     |
| *S. melongena* | 144   | PI 217962 | Banjal Bemba | Pakistan     | USDA-GRIN     |
| *S. melongena* | 145   | PI 223844 | -        | Philippines    | USDA-GRIN     |
| *S. melongena* | 146   | PI 230333 | Kairyo-onaga | Japan        | USDA-GRIN     |
| *S. melongena* | 147   | PI 230334 | Kitta Horyo | Japan        | USDA-GRIN     |
| *S. melongena* | 148   | PI 230335 | Taiwan-naga | Japan        | USDA-GRIN     |
| *S. melongena* | 149   | PI 232078 | Kopek    | South Africa  | USDA-GRIN     |
| *S. melongena* | 150   | PI 232079 | Mofale   | South Africa  | USDA-GRIN     |
| *S. melongena* | 151   | PI 233916 | -        | El Salvador    | USDA-GRIN     |
| Species  | ID   | Accession | Plant ID      | Country   | Source      |
|----------|------|-----------|---------------|-----------|-------------|
| *S. melongena* | 152  | PI 234632 | Early Round Purple | South Africa | USDA-GRIN   |
| *S. melongena* | 153  | PI 241506 | Badanjan | Iran | USDA-GRIN   |
| *S. melongena* | 154  | PI 249570 | Makhua Proh | Thailand | USDA-GRIN   |
| *S. melongena* | 155  | PI 250677 | No. 1 | Afghanistan | USDA-GRIN   |
| *S. melongena* | 156  | PI 263727 | Rosita | Puerto Rico | USDA-GRIN   |
| *S. melongena* | 157  | PI 267104 | Cylinder A-132 | Soviet | USDA-GRIN   |
| *S. melongena* | 158  | PI 269600 | 423 | Pakistan | USDA-GRIN   |
| *S. melongena* | 159  | PI 276104 | Motale | South Africa | USDA-GRIN   |
| *S. melongena* | 160  | PI 286099 | No. 62-46-2 | U.S. | USDA-GRIN   |
| *S. melongena* | 161  | PI 286100 | No. 62-48-2 | U.S. | USDA-GRIN   |
| *S. melongena* | 162  | PI 290467 | Lungi de Impant | Hungary | USDA-GRIN   |
| *S. melongena* | 163  | PI 290469 | Cu-e-da-juan | Hungary | USDA-GRIN   |
| *S. melongena* | 164  | PI 304839 | G2562 | Brazil | USDA-GRIN   |
| *S. melongena* | 165  | PI 320501 | 24 | Canada | USDA-GRIN   |
| *S. melongena* | 166  | PI 320504 | 28 | Canada | USDA-GRIN   |
| *S. melongena* | 167  | PI 320509 | 35 | Canada | USDA-GRIN   |
| *S. melongena* | 168  | PI 349612 | Terongglatik | Indonesia | USDA-GRIN   |
| *S. melongena* | 169  | PI 351129 | Kurume Long | Japan | USDA-GRIN   |
| *S. melongena* | 170  | PI 358232 | Dolg | Macedonia | USDA-GRIN   |
| *S. melongena* | 171  | PI 358242 | Monska Pata | Macedonia | USDA-GRIN   |
| *S. melongena* | 172  | PI 358244 | Renski dolg | Macedonia | USDA-GRIN   |
| *S. melongena* | 173  | PI 358822 | Sredno Dolg | Macedonia | USDA-GRIN   |
| *S. linnaeanum* | 174  | PI 388846 | WL-74 | Italy | USDA-GRIN   |
| *S. linnaeanum* | 175  | PI 388847 | WL-85 | Italy | USDA-GRIN   |
| *S. melongena* | 176  | PI 391646 | Liu-ye-ch'ieh | China | USDA-GRIN   |
| *S. melongena* | 177  | PI 413782 | 22–73 | Cote D'Ivoire | USDA-GRIN   |
| *S. melongena* | 178  | PI 413783 | 3–73 | Burkina Faso | USDA-GRIN   |
| *S. melongena* | 179  | PI 413784 | 13–73 | Burkina Faso | USDA-GRIN   |
| *S. melongena* | 180  | PI 419198 | Tsu Yang | China | USDA-GRIN   |
| *S. linnaeanum* | 181  | PI 420415 | 52 | Colombia | USDA-GRIN   |
| *s. gilo* | 182  | PI 43119 | BGH 5008 | Brazil | USDA-GRIN   |
| *S. melongena* | 183  | PI 452122 | Lunga Violetta di Romagna | Italy | USDA-GRIN   |
| *S. melongena* | 184  | PI 452123 | Tonda di Manfredonia | Italy | USDA-GRIN   |
| *S. melongena* | 185  | PI 462370 | Neznyj 36 | Soviet | USDA-GRIN   |
| *S. melongena* | 186  | PI 470273 | - | Indonesia | USDA-GRIN   |
| *S. melongena* | 187  | PI 478390 | O 81 | China | USDA-GRIN   |
| *S. melongena* | 188  | PI 491192 | Kemer | Turkey | USDA-GRIN   |
| *S. incanum* | 189  | PI 500922 | Chipusni | Zambia | USDA-GRIN   |
| *S. melongena* | 190  | PI 560903 | Six Leaves | China | USDA-GRIN   |
| *S. melongena* | 191  | PI 561139 | 37 | Kazakhstan | USDA-GRIN   |
| *S. melongena* | 192  | PI 561140 | 36 | Kazakhstan | USDA-GRIN   |
| *S. melongena* | 193  | PI 593748 | 37 | Thailand | USDA-GRIN   |
| *S. melongena* | 194  | PI 593806 | 171 | Thailand | USDA-GRIN   |
| *S. melongena* | 195  | PI 593885 | 314 | Thailand | USDA-GRIN   |
| *S. melongena* | 196  | PI 595220 | Gator | United States | USDA-GRIN   |
| *S. melongena* | 197  | PI 600912 | Little fingers | U.S. | USDA-GRIN   |
| *S. melongena* | 198  | PI 606714 | Pompano market | U.S. | USDA-GRIN   |
| *S. melongena* | 199  | PI 639121 | Puerto Rican beauty | Puerto Rico | USDA-GRIN   |
| *S. melongena* | 200  | PI 639122 | Blackee | U.S. | USDA-GRIN   |

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Eggplant Diversity, Structure and Resistance

Phenotypic diversity does not always translate to high levels of genetic diversity [4,33]. Modern varieties of eggplant often have lower genetic diversity, and new traits are often bred into commercial varieties from landraces or wild relatives with higher genetic diversity [34]. The characterization of genetic diversity is important for maintenance and utilization of germplasm resources (wild, landrace, heirloom, breeding lines and cultivars), and the development of core collections [3,35]. Genetic bottlenecks (domestication, selection of lines by market class, etc.) have limited the variability existing within cultivated lines [3].

Population structure analysis has recently been gaining popularity as a method to understand and visualize spatial and temporal differences between subpopulations [19,36-40]. Information on population structure can provide insight about connections between phenotypic variation and the distribution of genetic diversity. Population structure should also be taken into account when testing and incorporating desirable traits. If population structure is present in materials being evaluated for association mapping, spurious associations may be found between a particular genotype and the trait of interest [31,41]. Many studies have looked at the genetic diversity of eggplants within specific countries or regions, and a recent study compared genetic differentiation and structure in three centers of diversity; however, few studies have looked at diversity and population structure in a global collection of eggplants [2,4,31,34,42-46].

We evaluated the fruit shape index, Phytophthora fruit rot resistance, genetic diversity and population structure of a diverse collection of eggplant germplasm using 22 simple sequence repeats (SSRs). Our objectives were to evaluate a worldwide collection of eggplant germplasm and cultivated species.

Plant Material

Ninety-four accessions of eggplants (S. melongena), three accesses of S. linnaeanum, one accession of S. gilo, and one accession of S. incanum were obtained from the United States Department of Agriculture Germplasm Resource Information Network (ars-grin.usda.gov), Universidad de Technologia de Valencia, and the INRA (French National Institute for Agricultural Research) (Table 1). Accessions represented 32 countries on five different continents, and included primary and secondary centers of diversity. Seeds were sown into 72-cell trays containing a soilless peat mixture (Suremix Michigan Grower Products, Inc. Galesburg, MI) in a polyethylene greenhouse (MSU Horticulture Teaching and Research Center, Holt, MI). Eight weeks after sowing, seedlings were transplanted into a field at the MSU Plant Pathology Research Center (East Lansing, MI). Individual accessions were planted into single plots. Each individual line was established in 3 m long plot and 12 lines were planted per row. Within rows, plants were spaced 0.45 m apart. Rows were spaced 2.4 m apart, covered with black plastic mulch, and grown according to local practices. Immature eggplant fruits of marketable size were hand harvested throughout the growing season and brought to the lab for inoculation and evaluation.

Isolates

Two virulent P. capsici isolates, previously evaluated on pepper, tomato and eggplant, were selected from the long-term collection of Dr. Mary K. Hausbeck at MSU [18-20]. Isolates were characterized by host of origin, mefenoxam sensitivity [insensitive (I) or sensitive (S)] and mating type (A1 or A2). Isolate 12889 (pepper, I, A1) and isolate OP97 (cucumber, S, A1) were maintained on unclarified V8 agar at 25°C under constant light. Prior to inoculations, isolates were activated by inoculating and recovering each isolate from an individual pepper fruit to ensure virulence.

Inoculation and Evaluation

For inoculation, a single 6 mm-diameter plug from an actively growing P. capsici isolate on V8 agar was placed, mycelium side down onto a non-wounded eggplant fruit surface-disinfested in 10% bleach for 5 min and rinsed with distilled water. Control eggplants were inoculated with a single 6 mm-diameter sterile plug of agar. Plugs were covered with a sterile microcentrifuge tube and affixed into place with petroleum jelly. Eggplants were placed into a humidity chamber consisting of an aluminum pan with a ring of moistened paper towel around the edge, covered with plastic wrap, sealed with tape and kept under constant light at room temperature. Inoculation was scored after 4-6 weeks as follows: rating of 0 = no visible symptoms, a rating of 1 = <25% symptomatic area, 25%>2<50%, 50%>3<75%, and a rating of 4=75% symptomatic area of the fruit.
Table 2. Fruit shape parameters and mean disease ratings for each isolate.

| Species          | Accession | Meana | OP97 | Fruitb |         |         |
|-------------------|-----------|-------|------|--------|---------|---------|
|                   |           | Ratio | Length | Width  | Cluster |         |
| S. melongena      | C-S-16    | 5.59  | 4.12  | 22.98  | 1       |         |
| S. melongena      | Griff 1276| 1.14  | 4.30  | 4.92   | 2       |         |
| S. melongena      | Griff 14182| 2.59  | 6.11  | 14.97  | 3       |         |
| S. melongena      | Griff 14186| 1.75  | 7.83  | 13.48  | 1       |         |
| S. melongena      | H15       | 1.93  | 6.17  | 11.81  | 2       |         |
| S. melongena      | IVIA-371  | 2.08  | 7.26  | 14.98  | 4       |         |
| S. melongena      | MM 108 bis| 5.23  | 4.21  | 21.52  | 1       |         |
| S. melongena      | MM 114    | 6.57  | 3.44  | 22.23  | 2       |         |
| S. melongena      | MM 1171   | 2.81  | 5.43  | 15.14  | 1       |         |
| S. melongena      | MM 1363   | 5.05  | 5.12  | 25.77  | 2       |         |
| S. melongena      | MM 1364   | 3.02  | 6.02  | 17.78  | 5       |         |
| S. melongena      | MM 1365   | 1.87  | 7.88  | 14.55  | 2       |         |
| S. melongena      | MM 141    | 4.67  | 5.14  | 23.84  | 1       |         |
| S. melongena      | MM 1750   | 2.49  | 7.11  | 17.52  | 5       |         |
| S. melongena      | MM 346    | 1.31  | 8.73  | 11.35  | 2       |         |
| S. melongena      | MM 39     | 5.37  | 4.29  | 22.89  | 1       |         |
| S. melongena      | MM 522    | 8.03  | 3.27  | 26.14  | 1       |         |
| S. melongena      | MM 56     | 2.42  | 6.83  | 16.33  | 2       |         |
| S. melongena      | MM 61     | 1.97  | 5.88  | 11.53  | 2       |         |
| S. melongena      | MM 64     | 1.16  | 8.65  | 9.99   | 2       |         |
| S. melongena      | MM 69     | 1.34  | 8.78  | 11.43  | 4       |         |
| S. melongena      | MM 91     | 1.92  | 7.49  | 14.19  | 4       |         |
| S. melongena      | PI 102727 | 2.44  | 6.10  | 14.76  | 4       |         |
| S. melongena      | PI 105346 | 1.17  | 8.94  | 10.33  | 4       |         |
| S. melongena      | PI 115505 | 1.72  | 6.57  | 11.27  | 2       |         |
| S. melongena      | PI 140446 | 1.77  | 7.14  | 12.55  | 2       |         |
| S. melongena      | PI 140456 | 3.48  | 6.31  | 21.85  | 5       |         |
| S. melongena      | PI 141968 | 4.46  | 4.52  | 19.91  | 2       |         |
| S. melongena      | PI 143410 | 1.35  | 7.94  | 10.68  | 5       |         |
| S. melongena      | PI 169641 | 3.78  | 5.19  | 19.34  | 4       |         |
| S. melongena      | PI 169650 | 4.66  | 4.22  | 19.20  | 4       |         |
| S. melongena      | PI 171851 | 4.31  | 4.14  | 17.78  | 4       |         |
| S. melongena      | PI 175914 | 2.92  | 4.83  | 14.08  | 5       |         |
| S. melongena      | PI 179500 | 3.64  | 4.41  | 16.01  | 2       |         |
| S. melongena      | PI 179997 | 3.34  | 4.75  | 15.88  | 5       |         |
| S. melongena      | PI 181896 | 1.91  | 6.60  | 12.54  | 5       |         |
| S. melongena      | PI 181963 | 3.99  | 4.04  | 15.95  | 5       |         |
| S. melongena      | PI 193599 | 1.84  | 6.45  | 11.64  | 5       |         |
| S. melongena      | PI 199516 | 1.74  | 8.76  | 14.23  | 2       |         |
| S. melongena      | PI 200881 | 3.82  | 5.85  | 22.10  | 1       |         |
| S. melongena      | PI 204731 | 2.73  | 7.55  | 18.06  | 5       |         |
| S. melongena      | PI 213193 | 1.07  | 9.21  | 9.75   | 5       |         |
| S. melongena      | PI 217962 | 3.26  | 4.75  | 15.36  | 4       |         |
| S. melongena      | PI 223844 | 2.89  | 5.27  | 14.82  | 2       |         |
| S. melongena      | PI 230333 | 7.15  | 3.62  | 25.64  | 4       |         |
| S. melongena      | PI 230334 | 6.84  | 3.10  | 21.22  | 5       |         |
| S. melongena      | PI 230335 | 7.43  | 3.52  | 26.16  | 1       |         |
| S. melongena      | PI 232078 | 4.01  | 4.57  | 18.15  | 5       |         |
| S. melongena      | PI 232079 | 2.36  | 5.61  | 13.14  | 1       |         |
Table 2. Cont.

| Species       | Accession  | Mean<sup>a</sup>       | Fruit<sup>b</sup> | Cluster<sup>c</sup> |
|---------------|------------|------------------------|-------------------|---------------------|
|               |            | 12889 | OP97 | Ratio | Length | Width |       |
| S. melongena  | PI 233916  | MS   | S    | 2.37  | 5.6    | 13.16 | 5     |
| S. melongena  | PI 234632  | S    | S    | 0.93  | 8.72   | 7.90  | 5     |
| S. melongena  | PI 241506  | MS   | S    | 2.73  | 6.08   | 16.37 | 2     |
| S. melongena  | PI 249570  | S    | S    | 1.53  | 5.79   | 9.96  | 5     |
| S. melongena  | PI 256077  | S    | MS   | 3.07  | 5.27   | 16.05 | 4     |
| S. melongena  | PI 263727  | S    | S    | 1.95  | 8.01   | 12.82 | 4     |
| S. melongena  | PI 267104  | S    | S    | 3.93  | 4.86   | 18.811| 5     |
| S. melongena  | PI 269600  | S    | S    | 1.71  | 7.13   | 11.56 | 4     |
| S. melongena  | PI 276104  | S    | S    | 2.22  | 7.03   | 15.56 | 2     |
| S. melongena  | PI 286099  | S    | S    | 5.81  | 4.52   | 25.27 | 5     |
| S. melongena  | PI 286100  | S    | S    | 6.45  | 4.73   | 28.12 | 5     |
| S. melongena  | PI 290467  | S    | S    | 3.34  | 6.28   | 20.96 | 3     |
| S. melongena  | PI 290469  | S    | S    | 2.16  | 6.99   | 14.89 | 4     |
| S. melongena  | PI 304839  | S    | S    | 2.85  | 6.17   | 17.51 | 4     |
| S. melongena  | PI 320501  | S    | S    | 2.18  | 7.23   | 15.48 | 1     |
| S. melongena  | PI 320504  | S    | S    | 4.58  | 6.58   | 29.07 | 1     |
| S. melongena  | PI 320509  | S    | S    | 2.46  | 6.93   | 17.07 | 4     |
| S. melongena  | PI 349612  | S    | S    | 1.44  | 5.09   | 7.38  | 4     |
| S. melongena  | PI 351129  | S    | S    | 5.61  | 4.70   | 26.48 | 4     |
| S. melongena  | PI 358232  | S    | S    | 4.59  | 4.90   | 22.35 | 4     |
| S. melongena  | PI 358242  | S    | S    | 2.16  | 7.18   | 14.53 | 4     |
| S. melongena  | PI 358244  | S    | S    | 5.36  | 4.70   | 24.73 | 4     |
| S. melongena  | PI 368822  | S    | S    | 3.18  | 5.81   | 18.22 | 1     |
| S. linnaeanum | PI 388846  | MR   | S    | 1.03  | 2.38   | 2.46  | 3     |
| S. linnaeanum | PI 388847  | MR   | S    | 1.07  | 1.89   | 2.01  | 3     |
| S. melongena  | PI 391646  | S    | S    | 5.33  | 8.20   | 25.77 | 1     |
| S. melongena  | PI 413782  | S    | R    | 0.79  | 1.48   | 1.16  | 3     |
| S. melongena  | PI 413783  | MR   | R    | 0.46  | 4.37   | 2.02  | 3     |
| S. melongena  | PI 413784  | R    | R    | 0.69  | 5.85   | 4.05  | 3     |
| S. melongena  | PI 419198  | S    | MS   | 5.63  | 4.38   | 24.25 | 1     |
| S. linnaeanum | PI 420415  | S    | S    | 1.06  | 2.07   | 2.14  | 3     |
| S. gilo       | PI 441908  | R    | MR   | 0.83  | 5.15   | 4.27  | 3     |
| S. melongena  | PI 452122  | S    | S    | 5.79  | 4.08   | 23.75 | 1     |
| S. melongena  | PI 452123  | S    | S    | 1.29  | 9.60   | 12.16 | 1     |
| S. melongena  | PI 462370  | S    | S    | 1.15  | 10.94  | 12.02 | 5     |
| S. melongena  | PI 470273  | S    | S    | 3.30  | 4.88   | 15.86 | 3     |
| S. melongena  | PI 478390  | S    | S    | 0.75  | 9.54   | 7.08  | 4     |
| S. melongena  | PI 491192  | S    | S    | 4.95  | 4.46   | 22.06 | 1     |
| S. incanum    | PI 500922  | MS   | R    | 1.03  | 2.56   | 2.63  | 3     |
| S. melongena  | PI 560903  | S    | S    | 0.95  | 8.70   | 8.15  | 1     |
| S. melongena  | PI 561139  | S    | S    | 2.94  | 5.58   | 16.15 | 1     |
| S. melongena  | PI 561140  | S    | S    | 3.48  | 4.65   | 16.2063| 4    |
| S. melongena  | PI 593748  | S    | S    | 2.65  | 5.77   | 15.18 | 1     |
| S. melongena  | PI 593806  | S    | S    | 3.79  | 4.20   | 15.86 | 1     |
| S. melongena  | PI 593885  | S    | S    | 1.12  | 6.00   | 6.57  | 2     |
| S. melongena  | PI 595220  | S    | S    | 2.70  | 4.56   | 12.54 | 5     |
| S. melongena  | PI 600912  | S    | S    | 4.58  | 3.35   | 15.3  | 2     |
| S. melongena  | PI 606714  | S    | S    | 2.40  | 5.60   | 13.26 | 3     |
temperature (25°C). Three fruits (replicates) from each eggplant line were evaluated per isolate. The experiment was performed three times (runs). An experiment replicate included three fruits for each isolate of every eggplant accession evaluated in a completely randomized design (CRD) blocked by isolate. One line (PI 500922) was repeated only one time for a total of two experimental replicates of three fruit per isolate due to poor fruit set. Two control fruits were inoculated with a sterile plug of V8 agar for each line.

Eggplant fruits were evaluated for disease severity seven days after inoculation. Fruits were evaluated on the following progressive scale based on the percentage of symptomatic fruit surface to account for differences in fruit size: 0 = no visible symptoms (resistant (R)), 1 = <25% of the fruit was symptomatic (moderately resistant (MR)), 2 = 25% to <50% (moderately susceptible (MS)), 3 = 50% to <75% (susceptible (S)), 4 = ≥75% symptomatic area (susceptible (S)) (Fig. 1). Visible mycelia growth was assessed as 0 = absent, 1 = present. Phytophthora isolations were performed on 10% of symptomatic fruits by peeling back the external layer of the fruit and plating three small portions of fruit tissue at the disease margin onto V8 agar plates amended with benomyl, ampicillin, PCNB, and mefenoxam to confirm the causal agent of the symptoms [47]. Phytophthora capsici was identified using morphological characteristics according to Waterhouse [48] and isolate mefenoxam sensitivity was confirmed by transferring the recovered isolates to V8 plates amended with 100 ppm mefenoxam according to Lamour and Hausbeck [47].

Ten immature fruits of marketable size collected from each line were measured for maximum length (cm) and maximum width (cm) using a hand caliper. Fruit shape was calculated as the ratio of maximum length to midpoint width for each line. Fruit shape ratios were rounded to the nearest whole number. Values between 0 and 1 were considered round, 2–3 were considered oval, 4–5 were semi-elongate and >5 were considered elongate.

Phenotype Statistical Analyses
Mean values for disease ratings for each accession were estimated using the PROC MEANS function of SAS software v9.3 (SAS Institute, Cary, NC). Significant differences between disease values (ratings) for eggplant accessions and isolates were estimated using the PROC MIXED function of SAS software. Significant differences were detected between experiment runs and each run was analyzed separately using Fisher’s LSD test (P≤0.05). Accession by isolate interactions were calculated using the ANOVA slice option of PROC MIXED when P≤0.05. Lines with a consistent disease mean value of ≥2 in each run of the experiment were considered susceptible, with a consistent mean value <2 were termed moderately susceptible, lines with a

Table 2. Cont.

| Species      | Accession | Mean* | Fruitb | Clusterc |
|--------------|-----------|-------|-------|----------|
| S. melongena | PI 639121 | S     | S     | 1        |
| S. melongena | PI 639122 | S     | S     | 5        |

*aMean disease rating across all experimental replicates for each isolate, 12889 and OP97.

*bMean fruit parameters for ratio (fruit length:fruit width), length (cm), and width (cm).

*cSTRUCTURE genetic cluster assignment based on 22 SSR markers.

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consistent mean value \(<1\) were moderately resistant, and lines with a mean value \(=0\) were resistant. Significant differences for pathogen growth were estimated using the PROC GLIMMIX function of SAS at \(P = 0.05\).

Fruit shape significant differences between lines and countries were calculated using the PROC mixed function of SAS software v9.3. Countries represented by less than four accessions were excluded from analyses. Unequal sample sizes among countries

Table 3. Solanum spp. fruit shape, width and length variation between countries of origin.

| Country   | Shape | Width (cm) | Length (cm) |
|-----------|-------|------------|-------------|
| China     | 3.0   | cd         | A           |
| France    | 3.8   | b          | Cd          |
| Iran      | 2.3   | de         | Ab          |
| Italy     | 3.5   | bc         | Abc         |
| Japan     | 6.8   | a          | E           |
| Macedonia | 3.8   | b          | Cd          |
| S. Africa | 4.2   | d          | D           |
| Spain     | 3.1   | c          | Bc          |
| Thailand  | 2.2   | d          | Cd          |
| Turkey    | 3.9   | b          | D           |
| USA       | 3.6   | bc         | Cd          |

*Categories with less than five individuals representing a country were not included in analyses.

Mean fruit shape calculated as the ratio of fruit length to fruit width.

Mean fruit length from peduncle to blossom end measured in cm.

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Table 4. Polymorphic primers evaluated against 99 eggplant lines.

| SSR       | Forward sequence | Reverse sequence | Allele* | Genetic Diversity | PICb  | Source          |
|-----------|------------------|------------------|---------|------------------|-------|-----------------|
| BM61461   | CTCATTACACTTCATACAACAG | TGCAGTAGGTGTTGCTACGG | 6      | 0.18             | 0.18  | SolCAP          |
| GPM5203   | CACCAACACATCCTTTCAACC | ATATAATGGTGTTGCGGCAC | 4      | 0.24             | 0.23  | SolCAP          |
| CB164833  | CCGGCCAGGTGCTATTATACACAAC | CGGCCGAGGTACAACGCC | 3      | 0.57             | 0.49  | SolCAP          |
| T0633     | GATGGGCTATGGCTGCTGT | ACATCCCIAATGGTTGTTG | 2      | 0.03             | 0.03  | SolCAP          |
| CA516334  | ACCACCCCTCATCAACACC | ATTTTGGCGGTGGTTGCAAC | 6      | 0.61             | 0.55  | SolCAP          |
| GPM5178   | GATTTTGCATGGTCAACTCATG | ACAGTGGAAATAAGTTGAAGCA | 5      | 0.73             | 0.69  | SolCAP          |
| GP1102    | GAACCTTCTCATCTGTATGT | TTTGCCCCCATATTGTAATC | 2      | 0.45             | 0.35  | SolCAP          |
| C2_A153480 | AGTGAAGTCGACTCATCAACAATCCTC | GAAACCAATGGGCTTACTGAAAGA | 7      | 0.60             | 0.51  | SolCAP          |
| C2_A1596210 | AGCTCTATTACATTAAAAACTAGTCCTCAT | TCTTTCTTTGTTGGGGAGCTAATTC | 2      | 0.50             | 0.37  | SolCAP          |
| AF348141  | CCAATCCGAACTTTACGGT | CCATACCGAGGTTGTCCTCTCT | 5      | 0.68             | 0.62  | NCBI            |
| CAMS5362  | CCCCTTGACACTTGATTA | TATGCCCCCTCTGGATAGAC | 4      | 0.48             | 0.42  | Minamiyama 2007 |
| GO0496268.1 | GTTGGCTGTGTTACCAACCT | CTTCTCTGCACCTCCACAAC | 2      | 0.48             | 0.37  | NCBI            |
| C2_A1513200 | TATGGGCTGGGTGCTCCTCCAC | AGATTTTCCCTCTGGCGGCTTTGCTG | 3      | 0.11             | 0.10  | SolCAP          |
| C2_A15132410 | TGGTATGTTGCTGGGATTGTGATTG | AGATCGTGGAGAGACTGGAAGATAC | 4      | 0.64             | 0.57  | SolCAP          |
| CSM7F     | CGACGATACACTTTGAACAG | CCAAATAGCGAGTTTTTCAAAGA | 2      | 0.50             | 0.37  | Hurtado 2012    |
| CSM27     | TGGTGGAGGGTGGGAAGAGG | TCAACCTCAGGGAAAAATC | 3      | 0.57             | 0.50  | Hurtado 2012    |
| CSM30     | CATCTGTCTGGTGCTTGTG | TTTAGCTGTCGCCATACCC | 3      | 0.50             | 0.40  | Hurtado 2012    |
| CSM31     | CAAAGGATAGTCTCGATGC | CGGGTAGCTGTCGTTGTCG | 6      | 0.76             | 0.71  | Hurtado 2012    |
| CSM43     | ATTTTACCCCCGAAAAATG | ACCGGCTCTAGGTTTTCGAC | 4      | 0.62             | 0.55  | Hurtado 2012    |
| CSM44     | CGCTGTTGATACCCATCATC | TTGCACAAATCCTGTTTC | 3      | 0.46             | 0.36  | Hurtado 2012    |
| CSM54     | ATGGCTCTCCTTTGGCGAAG | TTGGTGGGGATCTGAGAAG | 3      | 0.44             | 0.37  | Hurtado 2012    |
| CSM73     | TTCAACATAGCCTTGACCTTCA | ATAGCAGGGTTTGGAACCTCA | 4      | 0.63             | 0.56  | Hurtado 2012    |

*Number of unique alleles detected in the population.

Polymorphism information content for each marker.

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were accounted using the Kenward-Rogers degrees of freedom option implemented in SAS software. Line mean values for fruit shape, length and width were calculated using the lsmeans statement of SAS software. Correlations between fruit shape parameters and disease susceptibility were estimated using the PROC CORR function of SAS. Disease susceptibility correlations were evaluated for each isolate and experimental replicate separately.

**Genetic Analyses**

Genomic DNA was extracted from young green leaves of eggplants using the Nucleo Spin II DNA extraction kit (Macherey-Nagel Germany, CAT# 740770) according to the manufacturer’s instructions. DNA was normalized to 5 ng/μl using the NanoDrop ND 1000 spectrophotometer and NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE).

One hundred ninety-two primers from previously published SSR markers ([3,49], solgenomics.org) or designed (Primer 3 http://primer3.sourceforge.net/) from putative Solanaceae defense-related genes (NCBI http://ncbi.nlm.nih.gov) were tested against a subset of the eggplant collection to identify polymorphic markers. Reactions were performed in 15 μl total volume and contained 1 μl DNA, and 0.13 μl GoTaq (Promega Corporation Madison, WI), 0.9 μl 25 mM MgCl₂, 0.3 μl dNTPs, and 0.6 μl each of forward and reverse primers (Integrated DNA Technologies, Inc., Coralville, IA), with 8.45 μl ddH₂O. PCR reactions were performed in a programmable thermal cycler (Eppendorf, Westbury, NY) using the program: initial denaturation, 94°C (3 min) followed by 35 cycles at 94°C (30 s), 60°C (30 s) and 72°C (1 min), with a final extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis in 4% (wt/vol) agarose gel in 1× Tris-borate-EDTA buffer, stained with ethidium bromide (5 μg/ml) for visualization and compared to a 100-bp ladder (Invitrogen Life Technologies, Burlington, ON, Canada) to determine amplicon sizes. SSR markers identified as polymorphic in the population were used for genetic diversity, population structure and trait associations.

### Table 5. Genetic differentiation (pairwise Fst) estimates of SSRs for *S. melongena* grouped by continent.

| Category | Africa | Asia | Europe | N. America |
|----------|--------|------|--------|------------|
| Asia     | 0.00   | -    |        |            |
| Europe   | 0.04*  | 0.02 | -      |            |
| N. America | 0.04* | 0.00 | 0.03* | -          |
| S. America | 0.03  | 0.11* | 0.05  | 0.06*      |

*Categories with less than four lines were excluded from analyses and are not shown.

Average values for SSRs are presented. * indicates value was outside the 2.5% and 97.5% confidence intervals at 1000 bootstraps.

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### Table 6. Genetic diversity estimates for SSRs for *S. melongena* grouped by continent and country of origin.

| Category | AlleleNo | G_D | PIC |
|----------|----------|-----|-----|
| Africa   | 2.71     | 0.51 | 0.43 |
| Asia     | 3.10     | 0.48 | 0.41 |
| Europe   | 2.81     | 0.48 | 0.41 |
| N. America | 3.10  | 0.50 | 0.44 |
| S. America | 2.67  | 0.46 | 0.39 |
| China    | 2.18     | 0.39 | 0.32 |
| France   | 2.14     | 0.38 | 0.31 |
| Iran     | 2.23     | 0.42 | 0.35 |
| Japan    | 1.91     | 0.35 | 0.28 |
| Macedonia | 2.18  | 0.40 | 0.33 |
| South Africa | 2.14 | 0.41 | 0.34 |
| Spain    | 2.50     | 0.42 | 0.36 |
| Thailand | 2.36     | 0.42 | 0.36 |
| Turkey   | 2.36     | 0.40 | 0.34 |
| USA      | 2.77     | 0.48 | 0.42 |

*Categories with less than four lines were excluded from analyses and are not shown.

Mean values are presented for the average number of alleles (AlleleNo), genetic diversity (G_D) and the polymorphism information content (PIC).

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Genetic Diversity and Population Structure

Genetic diversity was estimated using Powermarker v3.25 [50] and significance at each locus was determined with 1000 permutations using the Exact test; overall genetic diversity was estimated using the Mantel test as implemented in Powermarker. Genetic distance matrix values were calculated using Euclidean distance with the unweighted pair group method with arithmetic mean (UPGMA) and visualized in MEGA5 [50,51].

Population structure of the germplasm was analyzed using STRUCTURE v2.3.4 [52]. Following preliminary analyses, burnin length, MCMC chain replication and lambda were selected to be 200,000, 500,000 and 1.52, respectively. Population number (K) was determined empirically by comparing posterior distribution likelihoods independently among 3 independent runs of K = 1 to 20 as described by Evanno et al. [53]. Data included 22 polymorphic SSRs and were analyzed using the admixture model and correlated allele frequencies without previous population information [52,54]. Wright’s subpopulation fixation index (Fst), the proportion of the total genetic variance within a subpopulation, significance between populations was determined using 1000 bootstrap replicates as implemented in Powermarker [55].

Visualization of the resulting Q (proportion of membership based on a 0 to 1 scale) of each accession into predefined categories (country, continent, species, disease susceptibility and fruit shape) was generated using the Population Sorting Tool (PST) in R [19,56] (J.J. Morrice, unpublished). Individuals with Q >0.6 membership in a single subpopulation were labeled as such. Individuals with Q<0.6 membership in a single subpopulation were considered admixed. Significance of population structure predefined categories was estimated using the population differentiation test implemented in Powermarker. Significance at each locus and overall was determined using 1000 permutations. Countries represented by less than four individuals were excluded from analyses. Significance of pairwise Fst differentiation was based on 2.5% and 97.5% confidence intervals (P= 0.05) based off of 1000 bootstrap replications.

Results

Phytophthora capsici Disease Resistance and Fruit Shape

Significant differences between experimental runs indicated the effect of environmental variability during fruit growth and development on fruit disease resistance was high. In each repetition of the experiment, there were significant differences among plant accessions (P<0.0001). No significant differences in disease severity were found between isolates in any replicate of the experiment (P=0.32, P=0.43, and P=0.43). The interaction between accessions and isolate was significant for each run (Run 1: P=0.0008; Run 2: P<0.0001; and Run 3 P<0.0001) of the experiment. Differences in pathogen growth (absence/presence) and the interaction between pathogen growth and accession were not significant in any replicate (approximately P=1.0 for each). The majority of the accessions were susceptible at 7 days post inoculation to isolates OP97 (89%) and 12889 (87%), respectively (Table 2). Symptoms included brown discoloration of the fruit and water soaking, with occasional external mycelial growth (Fig 1). Eggplant accession PI 413784 was the only line completely R to both isolates tested. Susceptibility to one isolate did not always result in susceptibility to the other isolate. Lines PI 413782 and Gril 1276 were R (rating = 0) to isolate OP97 and S or MS to isolate 12889. S. melongena lines, MM1365, PI 193599, PI 263727 and PI 419198 were MS (rating <2) to isolate OP97. Eggplant lines H15 and PI 441908 were R to isolate 12889 and S and MS to isolate OP97, respectively. Two of the S. linnaeanum lines, PI

Table 7. Genetic differentiation (pairwise Fst) estimates of SSRs for S. melongena grouped by country.

| Category | France | Iran | Japan | Macedonia | S. Africa | Spain | Thailand | Turkey | USA |
|----------|--------|------|-------|-----------|-----------|-------|----------|--------|-----|
| France   | 0.00   | 0.04 | 0.04  | 0.04      | 0.04      | 0.04  | 0.04     | 0.04   | 0.05|
| Iran     | 0.01   | 0.13 | 0.08  | 0.13      | 0.15      | 0.15  | 0.15     | 0.15   | 0.06|
| Japan    | 0.06   | 0.08 | 0.04  | 0.04      | 0.04      | 0.04  | 0.04     | 0.04   | 0.04|
| Macedonia| 0.04   | 0.04 | 0.04  | 0.04      | 0.04      | 0.04  | 0.04     | 0.04   | 0.04|
| S. Africa| 0.05   | 0.05 | 0.05  | 0.05      | 0.05      | 0.05  | 0.05     | 0.05   | 0.05|
| Spain    | 0.05   | 0.05 | 0.05  | 0.05      | 0.05      | 0.05  | 0.05     | 0.05   | 0.05|
| Thailand | 0.05   | 0.05 | 0.05  | 0.05      | 0.05      | 0.05  | 0.05     | 0.05   | 0.05|
| Turkey   | 0.05   | 0.05 | 0.05  | 0.05      | 0.05      | 0.05  | 0.05     | 0.05   | 0.05|
| USA      | 0.05   | 0.05 | 0.05  | 0.05      | 0.05      | 0.05  | 0.05     | 0.05   | 0.05|

- Categories with less than four lines were excluded from analysis and are not shown.
- Average values for SSRs are presented; * indicates value was outside the 2.5% and 97.5% confidence intervals at 1000 bootstraps.

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Table 8. Genetic differentiation (pairwise Fst) estimates of SSRs for eggplant germplasm grouped by disease resistance.

| Category¹  | R/MR | MS | S  |
|------------|------|----|----|
| R/MR       | -    | 0.19 | 0.52* |
| MS         | 0.01 | -  | 0.15 |
| S          | 0.00 | 0.03* | -  |

¹12889 appears below the diagonal and OP97 values are above the diagonal; MS = moderately susceptible, R/MR = resistant/moderately resistant, S = susceptible.
²Average values for SSRs are presented; * indicates value was outside the 95% confidence interval at 1000 bootstraps.

Table 9. Genetic differentiation (pairwise Fst) estimates of SSRs for S. melongena germplasm grouped by fruit shape.

| Category²  | Elongate | Oval | Round |
|------------|----------|------|-------|
| Oval       | 0.10*    |      |       |
| Round      | 0.06*    | 0.00 |       |
| Semi-Elongate | 0.04 | 0.02 | 0.03* |

²Fruit shape category based on the ratio of mean length:mean width for each line.
³Average values for SSRs are presented; * indicates value was outside the 2.5% and 97.5% confidence interval at 1000 bootstraps.

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388847 and PI 338846, and the S. incanum accession PI 500922 were R or MR to isolate 12889. Lines PI 181896, PI 233916, MM 56, and PI 413783 were MS to isolate 12889. Phytophthora capsici isolates were successfully recovered from diseased fruits and mefenoxam sensitivity was confirmed for each isolate (data not shown).

Fruit shape and size varied considerably in the population (Fig 2, Table 2). S. melongena accessions had fruit shape ratios ranging from 1 (round) to 8 (elongate). The wild species evaluated (S. linnaeanum, S. gilo, and S. incanum) had a fruit shape ratio of approximately 1 (round) with fruit ≤3 cm (Table 2). Solanum melongena line PI 413783 had the lowest fruit shape ratio (0.46) and line MM 522 had the highest fruit shape ratio (8).

When evaluated by country, S. melongena fruits from Japan had the highest length:width ratio indicating fruits were slender and elongated. Fruits from Thailand had the lowest fruit shape ratio, indicating fruits were more round. Fruit length and width also varied greatly between countries. Fruits from China were the widest and Japan the narrowest. Fruits from Japan were also the longest and fruits from Thailand were the shortest (Table 3).

Diversity of SSR Loci in the Eggplant Germplasm Collection

The 192 primers evaluated yielded 22 polymorphic markers that were used for characterizing and evaluating genetic diversity of the eggplant collection (Table 4). A total of 83 alleles were detected among the 22 SSRs, ranging from 2 to 7 alleles per locus with an average allele diversity of 3.8 alleles per locus. The mean genetic diversity index of the collection was 0.49 ranging from 0.03 (T0633) to 0.76 (CSM31) (Table 4). The mean polymorphism information content (PIC) value was 0.42 and individual markers ranged from 0.03 to 0.71 for the population. The highest PIC value was 0.35 in PI 290467 and the lowest PIC value was 0.085 in Grif 1276. Genetic diversity was equally distributed within continents (0.46–0.51), and pairwise Fsts indicated low to moderate to high (0.43–0.52) (Table 5). Genetic diversity within countries was similar (0.35–0.48) (Table 6), and pairwise Fst values suggested low (0.00) to great (0.17) genetic differentiation among countries (Table 7).

Pairwise Fsts for disease resistance to isolates 12889 and OP97 showed little (0.00) to very great (0.52) genetic differentiation between categories (Table 8). MR/R phenotypes only had high and significant (P=0.05) genetic differentiation with S phenotypes for isolate OP97. Significant genetic differentiation was also detected between the MS and the S category for isolate 12889. No significant genetic differentiation was found between the R/MR category and the MS categories for either Phytophthora isolate (Table 8–9). Genetic diversity of fruit shape categories was moderate to high (0.43–0.52) (data not shown). Pairwise Fst differentiation between fruit shape categories was low (0.00) to moderate (0.1) (Table 9). Individuals with an elongate fruit shape were significantly differentiated from those with a round or oval fruit shape. Significant differentiation was also detected between round shaped individuals and semi-elongated individuals (Table 9).

Population Structure Analysis

Population structure of the 99 accessions was estimated using the STRUCTURE software and the 22 polymorphic SSRs. Accessions were grouped into four genetic clusters (Ln = - 3381.8). S. linnaeanum, S. gilo and S. incanum accessions were placed into genetic Cluster 3, while S. melongena individuals were distributed through each of the clusters (Table 2). Seventy-eight individuals could be assigned to a single cluster based on membership, while the remaining 21 individuals could not be assigned. When compared with the UPGMA tree, STRUCTURE-inferred clusters largely overlapped with the grouping of branches based on genetic distance (Fig 3). Relationships between the inferred clusters according to the UPGMA tree indicated that Cluster 3 was more differentiated from Clusters 1, 2 and 4, and that Clusters 1 and 4 and Clusters 2 and 4 were less differentiated from each other. Pairwise Fsts between clusters were significant and ranged from 0.08 to 0.17, indicating 8–17% of the
variation was explained by genetic differences between clusters. Cluster 1 had moderate differentiation from Clusters 2, 3, and 4. Cluster 2 had great differentiation from Cluster 3, and Cluster 4 had moderate differentiation from Clusters 2 and 3 following the guidelines suggested by Hartl and Clark [55]. Population structure was detected when individuals were grouped by continent of origin (Fig. 4A), country of origin (Fig. 4B), species, fruit shape (Fig. 5) and disease resistance to isolates 12889 and OP97 (Fig. 6), as some clusters were more frequent than others in each grouping (Fig. 4–6). Cluster 3 individuals were not represented in Asia, and Cluster 4 individuals were not represented in Africa (Fig. 4). For both isolates, individuals from Cluster 4 were not represented in the MR/R categories for either isolate, had low representation in the MS category, and were highly represented in the S category. Cluster 3 individuals were highly represented in both the R/MR and S categories, but not the MS for both isolates (Fig. 6). When grouped by fruit shape (round, oval, semi-elongate and elongate), Cluster 1 was under represented in the oval and elongate fruit.

**Figure 3. UPGMA genetic distance matrix differences between eggplant lines.** Lines are colored based on their STRUCTURE inferred subpopulations. Cluster 1 individuals are denoted by purple squares, Cluster 2 individuals are black-outlined light yellow circles, Cluster 3 individuals are sky blue counterclockwise triangles, Cluster 4 individuals are steel blue diamonds, and admixed individuals are open squares. 
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Figure 4. Population structure grouped by country (A) and continent (B) of origin for eggplant (*S. melongena*) germplasm. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (dark blue). A white space and black tick marks separate subgroups of individuals. (A) Population structure grouped by country of origin for the *S. melongena* germplasm. Only countries represented by four or more individuals were included. (A).

Figure 5. Population structure grouped by disease resistance to isolate 12889 (A) and OP97 (B). Individuals were grouped into a resistant and moderately resistant category (R/MR), a moderately susceptible category (MS), and a susceptible category (S) based on their mean disease ratings. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (dark blue). A white space and black tick marks separate subgroups of individuals.

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shape categories. Clusters 2 and 4 both had low representation in the round category. Cluster 3 was not represented in round or elongated individuals and had minor representation in the oval shape category.

Discussion

This study investigated Phytophthora fruit rot resistance, fruit shape, population structure and genetic diversity in a worldwide collection of eggplant. The overall estimate of genetic diversity of the collection was moderate (0.5) in our study, similar to a recent report on eggplant diversity, though this is likely to be an underestimation due to limited sampling [3]. Bayesian clustering, using SSR markers, identified four genetic clusters in the eggplant collection. Most individuals belonged predominantly to one of the four clusters, while 20% were admixed according to the inferred clustering. Admixture, an indicator of migration or interbreeding between genetic clusters, was moderate in our population. Inferred genetic clusters did not directly correspond with the predefined categories of continent, country, fruit shape or Phytophthora fruit rot resistance, though some clusters did appear more frequently in one category compared to another.

On eggplant, fruit rot is the most common symptom of P. capsici seen in the field [23]. Symptoms start as small water soaked lesions, turning brown and eventually covering the whole fruit. Advanced symptoms can include complete rotting of the fruit and visible mycelia on the external surface of the fruit [23]. Isolate-specific interactions and partial fruit rot resistance have been identified in other solanaceous species (tomatoes and peppers) suggesting a multigenic host response, but no studies have looked at Phytophthora fruit rot in eggplant [24] (Granke et al. unpublished). In our study, the eggplant accessions evaluated demonstrated partial and isolate-specific resistance to Phytophthora fruit rot. Most lines evaluated were completely susceptible to both isolates (~90%). Several eggplant accessions displayed isolate-specific resistance; these individuals were placed into genetic Clusters 2 and 3, and were from S. America, Asia, Africa and Europe. Two of these geographic regions are known centers of eggplant diversity, and likely harbor additional sources of resistance [2–5]. Only one of the 99 lines evaluated, a Cluster 3 landrace eggplant collected in Burkina Faso in the early 1900s, had complete resistance to both isolates evaluated. This accession also showed high levels of genetic similarity to the wild eggplant relatives evaluated, S. incanum, S. gilo, and S. linnaeanum. While further evaluation with more isolates is necessary, PI 413784 appears to be a promising source of host resistance to Phytophthora fruit rot in eggplant.

When categorized by disease resistance (S, MS, MR, R) for each isolate, there was significant genetic differentiation among eggplant genotypes infected with isolates OP97 or 12889. Individuals that were R and MR to isolate OP97 were significantly differentiated from individuals that were S. Only S individuals were significantly differentiated from the MS individuals when inoculated with isolate 12889. These results emphasize the importance of utilizing different P. capsici isolates when breeding for resistance. The three wild relatives, S. linnaeanum, S. gilo, and S. incanum, showed partial or isolate-specific resistance to the two isolates evaluated in this study.

When grouped by species, S. linnaeanum, S. gilo, and S. incanum individuals evaluated were predominantly in genetic Cluster 3. Solanum incanum has long been part of the eggplant complex and ancestral individuals are thought to be one of the progenitors of modern eggplant [43,44]. Solanum linnaeanum is a related species and has only recently been included as a possible progenitor of the modern eggplant with limited crossability [5,8]. Genetic Cluster 3 individuals were also detected in the S. melongena category, supporting gene movement between S. melongena and its wild relatives, S. incanum and S. linnaeanum. These S. melongena individuals may have been misclassified, but are more likely the result of introgression since the wild species were small fruited and prickly.

Cultivated eggplant, similar to pepper and tomato, is a phenotypically diverse species with varying levels of genotypic diversity [42,44,46,57]. Solanum melongena fruit shape, size and color is a byproduct of domestication, selection, and breeding for specific market classes. Phenotypic evaluation of eggplant fruit shape varied greatly among the S. melongena accessions evaluated, while the wild species, S. incanum, S. gilo, and S. linnaeanum, had no variation in fruit shape. Maintaining market class variation may be difficult when incorporating traits like fruit rot resistance, which was most often observed in accessions with a limited size range.

Significant differences in this population were observed in fruit shape, length and width among eggplant lines when grouped by country of origin, representing different market classes, in this study. Since eggplant has market classes particular to geographic areas, it was expected that population structure categorized by fruit shapes and country of origin would correspond with the inferred genetic clusters. Significant differentiation was seen between S. melongena individuals with elongated fruit shapes and those with round and oval fruit shapes. Individuals with a round fruit shape were also significantly differentiated from semi-elongate fruit shape individuals. These results are consistent with limited breeding among market classes. However, inferred population structure did not correspond with the fruit categories and this may be the result of limited sampling in each geographic location. While only genetic Cluster 3 was not represented in the round or elongate shape category, all other clusters were represented by at least one individual in each category.

When grouped by country and continent, significant population structure and moderate genetic diversity was evident among the
categorized evaluated. The highest levels of genetic diversity were seen within the continents of Africa and N. America. The highest level of genetic diversity for countries was in the USA. The increased genetic diversity in Africa is likely due to the prevalence and intercrossing of wild and related species, as Africa is the center of origin for cultivated eggplant species. The increased genetic diversity in N. America and the USA may be the result of breeding programs integrating wild relatives and varieties from around the world. The diversity could also be from the movement of Asian and European varieties into the U.S., which may be market under different names. Overall differentiation among countries was similar to the differentiation among continents, and future core collections should include individuals from areas with high genetic diversity and genetic differentiation. In particular, genotypes from China were not significantly differentiated from any other country, while genotypes from Thailand, Japan, Spain, and Macedonia and the U.S. were frequently significantly differentiated from other countries. Similarly, Asia was not significantly differentiated from populations from Europe, Africa and N. America, while N. America, Europe and Africa were all significantly differentiated from each other, Asia, as a center of diversity and domestication, and in particular genotypes from China, may be more akin to the ancestral population from which these other pools were derived, making them more similar and less differentiated from other eggplants.

Cultivated eggplant, compared to other solanaceous species, is an understudied crop with worldwide importance. This study provides an overview of the population structure, genetic diversity and Phytophthora fruit rot resistance of a geographically diverse set of eggplant. The estimates of genetic diversity and the four genetic clusters found in this study are likely to be lower than actual genetic diversity and structure of eggplant due to limited sampling and molecular markers. A previous study using a subset of SSRs in a smaller collection of eggplant was able to identify more allelic variation at each locus [3]. While population structure was significant for disease resistance, fruit shape, continent and country, the genetic clusters did not completely correspond with these predefined categories in our study, which may be due, in part, to unequal samples in each category. Future studies involving eggplant diversity, disease resistance and other agronomic traits should aim to include individuals from around the world for maximum diversity, and will need to consider the effect of population structure on marker-trait associations.

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Author Contributions

Conceived and designed the experiments: RN. Performed the experiments: RN. Analyzed the data: RN. Contributed reagents/materials/analysis tools: MK. Wrote the paper: RN LMQ-O MKH.

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