DETERMINATION OF GENETIC DIVERSITY AND POPULATION STRUCTURE IN EGGPLANT

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by
Tesfa ALTAYE

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IZMIR

We approve the thesis of **Tesfa ALTAYE**

Examining Committee Members:

Prof. Dr. Anne FRARY
Department of Molecular Biology and Genetics, Izmir Institute of Technology

Prof. Dr. Sami DOGANLAR
Department of Molecular Biology and Genetics, Izmir Institute of Technology

Prof. Dr. Talat YALÇIN
Department of Chemistry, Izmir Institute of Technology

Assoc. Prof. Dr. Çağlar H. KARAKAYA
Department of Molecular Biology and Genetics, Izmir Institute of Technology

Assoc. Prof. Dr. Gülistan MEŞE ÖZÇIVICI
Department of Molecular Biology and Genetics, Izmir Institute of Technology

16 January 2015

Prof. Dr. Anne FRARY
Supervisor, Department of Molecular Biology and Genetics
Izmir Institute of Technology

Prof. Dr. Ahmet KOÇ
Head of the Department of
Molecular Biology and Genetics

Prof. Dr. Bilge KARAÇALI
Dean of the Graduate School of
Engineering and Sciences

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ABSTRACT

DETERMINATION OF GENETIC DIVERSITY AND POPULATION STRUCTURE IN EGGPLANT

Eggplant (Solanum melongena), an economically important crop in the genus Solanum, is known by different names in different countries. The diversity of some eggplant germplasm has studied using morphological, biochemical and molecular techniques by different scientists. Here, we studied the genetic diversity of 79 eggplant accessions collected from 28 countries in a molecular level and the efficiency of Sequence related amplified polymorphism also evaluated in comparison with other studies. The genetic diversity of 73 S. melongena accessions and six outgroups were assessed using ten sequence related amplified polymorphisms (SRAP) marker combinations. All the primer tested showed polymorphism and the average alleles registered per locus was 4.7. The primers included in the study showed moderate polymorphic information content. Genetic similarity was analyzed using Dice coefficient and the relation tree constructed using unweighted neighbor joining. The dendrogram revealed five groups and the similarity of the dendrogram ranged from 0.18 to 1 with mean value of 0.59. All the outgroups were distantly related to S. melongena accessions except the wild S. linnaeanum and S. incanum group C ,which showed strong similarity with the cultivated s. Melongena.The result reported in Dendrogram, PCoA and STRUCTURE analysis showed consistency. The genetic similarities registered in our accessions were not correlated with geographical diversity. Lack of exact provenance and accessions naming is one of the challenge beside uncoordinated work done to characterize eggplant accessions. Therefore, collecting worldwide accessions from different eggplant germplasm center and screening by power core may clarify the ongoing confusions of eggplant classification.

ÖZET

PATLICAN GENETİK ÇEŞİTLİLİK VE POPULASYON YAPISININ BELİRLENMESİ

Patlıcan (Solanum melongena), Solanum cinsi içinde ekonomik açıdan önemli bir bitkidir ve farklı ülkelerde farklı isimlerle bilinir. Simdiye kadar bilim insanları tarafindan patlıcan germplazmasının çeşitliliği; morfolojik, biyokimyasal ve moleküler teknikler kullanarak çalışilmiştir. Bu çalışmada, 28 ülkeden toplanmış 79 patlıcan çeşidinin moleküler seviyede genetik çeşitliliği incelenmiş ve sekans bağlantılı çoğaltılmış polimorfizm'in etkinliği diğer çalışmalarla karşılaştırılarak değerlendirilmiştir. On adet bağlantılı çoğaltılmış polimorfizm (SRAP) işaretleyici kombinasyonu kullanılarak 73 S. melongena çeşidinin ve 6 grup dışı örnegin genetik çeşitliliği belirlenmiştir. Denenen bütün primerler polimorfizm göstermiş ve allel ortalaması 4.7 olarak girilmiştir. Çalışmada kulanılan primer kombinasyonlari yeterli polimorfik bilgi sağlamıştır. Dice katsayısı kullanılarak genetik benzerlik analiz edilmis ve akrabalık ağacı unweighted neighbor joining kullanılarak çizilmistir. Dendogram 5 grup göstermiş ve 0,18-1 aralığında 0,59 ortalamada bulunmuştur. Grup dışı örnekler, kültür örneği S.melongena ile güçlü benzerlikleri olan S. linnaeanum and S. incanum ve grup C dışında, S. melongena türleriyle yakın ilişki göstermemiştir. Sonuçlar Dendogram'a girilmis ve PCoA ve STRUCUTRE analizleri tutarlılık göstermiştir. Çalismamizda genetik olarak birbirine benzeyen çesitlerin coğrafik bolgelerdeki dagilimlari arasinda iliski gozlenmemiştir. Türlerin kesin kaynağı ve çesitlerinin adlandırılmasındaki eksikliklikler bağlantı kurmaya engel teşkil etmiştir. Dolayısıyla, dünya çapında patlıcan germplazm merkezlerinden patlıcan türlerini toplayıp power core yazılımıyla görüntülemek devam eden sınıflandırma karışıklıklarını gidermemize yardımcı olacaktır.

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CHAPTER 1

INTRODUCTION

1.1. Definition and Origin of Eggplant

Eggplant (*Solanum melongena L.*) is a member of the Solanaceae family and is also commonly known as brinjal, an Arabic word popular in the Indian subcontinent, and aubergine, a French word popular in Europe. Eggplant is known by different names in other languages, some of which are vaatingan in Sanskrit; badanjan in Hindustani; baadangan and badenjan in Persian; bedengiam, baadanjaan and melongena in Arabic; patlican in Turkish; badnjan in Georgian; tabendjalts in Berber; berenjena in Spanish; and beringela in Portuguese (wikipedia.org/wiki/Eggplant). The genus Solanum contains economically important plants such as tomato, potato and eggplant and comprises more than 3500 species in seven subgenera such as *Archaesolanum, Bassovia, Leptostemonum, Lyciosolanum, Minon, Potatoe* and *Solanum* (Hawkes 1999). Eggplants are included in four of these subgenra: Subgenera *Leptostemonum, Solanum, Potatoe and Archaesolanum*. Subgenus *Leptostemonum* encompasses many species of eggplant including the three main cultivated species: *S. melongena, S. macrocarpon* and *S. aethiopicum*. Eggplant has 12 chromosomes like tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) and is an autogamous diploid with 2n=24.

Eggplant is an economically and nutritionally important crop like other solanceous vegetables such as tomato, potato and pepper. The three cultivated species are annuals and perennials in temperate and humid tropic conditions, respectively, and have wide environmental adaptation. Nevertheless, different cultivars of these species have different morphological and physiological responses such as yield and vigorousness when transferred to different environments. Eggplant is mostly produced and widely used in Asia and Africa; in addition, it is also produced and used in Europe and America. *S. melongena* is characterized by large phenotypic variability which includes diverse fruit weight, fruit shape, and colour. The related species *S. aethiopicum* is also morphologically highly variable, and accessions are assigned to four groups (Aculeatum, Gilo, Kumba, and Shum) based on their leaf and fruit morphology and

their uses. *S. aethiopicum* is native to Africa, and was later introduced to Brazil, West Indies and South America (Lester R.N., 1986; Daunay et al. 2001).

In comparison with other eggplant species, *S. melongena* has the widest climatic adaptation and can grow from low humidity to mountainous areas. It is generally grown in the tropical, hot, humid, equatorial climate of Africa, Asia and southern USA. *S. melongena* also grows in a vast area of the Mediterranean basin, which has hot, dry conditions. A wide range of adapted varieties is available in each production region, with seasonal production in temperate regions. Dry and humid tropical regions are favorable for the genetically variable *S. aethiopicum* which is mainly grown in Africa, West India, South America and Brazil. *S. macrocarpon* has a restricted growth zone in humid tropical forest regions and is less morphologically variable than other cultivated species (Lester et al., 1990, Daunay et al., 2001).

Scientists do not agree on the origin of eggplant and different researchers have described the possible origin of the crop. A large distribution of eggplants found throughout south East Asia support the Indo-Chinese center of origin. According to Sampson (1936), the origin of eggplant is Africa, but there is no evidence of cultivation of *S. melongena* on the continent. Lester and Hasan (1991) supported Sampson's findings of eggplant's origin by using isozymes, seed proteins and morphometrics and they said that the Equatorial region (East Africa savanna) is the origin and center of diversity of eggplant. According to several Sanskrit (the primary liturgical language of Hinduism) documents, eggplant was widely used for food and medicine as early as 300 BCE and the plant was identified with various descriptive words. Those documents show that the possible and primary center of diversification of *S. melongena* was in Southeast Asia, specifically the Indo-Burmese zone (Khan 1979, Daunay et al., 2001). From a diversity and population structure study of 99 genotypes from 35 countries, genotypes from Asia were not significantly differentiated from Europe, Africa, and North America populations, while Europe, Africa, and North America were significantly differentiated from each other (Naegal et al. 2014). This result indicates Asia is the center of diversity, from which the other genotype pools were derived. A recent study of Chinese ancient literature revealed the domestication of eggplant in south west China and suggested that the domestication took place no later than 59 BC (Jin-Xiu Wang et al., 2008). In general, however, the Indo-Burmese origin of eggplant is supported by many researchers. De Candolle (1886), Vavilov (1928), Zeven and Zhukovsky (1975), and Isshiki et al (1994) are researchers whose work has supported

the Indo-Burmese zone as eggplant's origin of diversity. Daunay (2001) gave insight on the origin and diversification of the two cultivated species *S. macrocarpon* and *S. aethiopicum*, which are more diverse in West Africa. However, identification of the center(s) of origin and diversification of these species is difficult because they were domesticated over large areas.

1.2. Cultivated Eggplant Production

Eggplant has significantly increased in economic importance and production in the past ten years with total world production exceeding more than 48 million metric tonnes in 2012. All eggplant production data were taken from FAO (Food and Agriculture Organization of United Nations) 2012 database (ref). Eggplant is a very popular Solanum crop in Asia along with potato and tomato. In the past ten years, eggplant increased from 1.64 to 1.85 million ha in area coverage and from 29.5 to 48.4 million tonnes in production (Table 1.1), which shows the importance of the crop in terms of food and economical value. Around 94 % of eggplant production is located in Asia. According to FAO data, China is the leading producer, harvesting 28.8 million metric tonnes from 800,000 ha of land. India takes second place after China with 12.2 million metric tonnes from 700,000 ha of land, while the Netherlands takes first place in terms of eggplant productivity, which is around 447.6 metric tonnes per hectare. There is a wide difference in the yield of eggplant production which is due to the growth environment, technology and varieties. Iran with 1.3 million metric tonnes from 39,500 ha, Egypt with around 1.2 million metric tonnes from 45,251 ha and Turkey with around 0.8 million metric tonnes from 26,000 ha rank third, fourth and fifth, respectively (Table 1.2). In Africa, Egypt alone accounts for 73% of total eggplant production. Over ten years China's eggplant production increased by 13,392,008 tonnes (Table 1.1), which is the highest record registered in the FAO database. India also increased its production by 3,850,000 tonnes next to China. However, Turkey is the only country which decreased its production with 155,715 tonnes less production in 2012. Therefore, China is most responsible for the high percent increments of eggplant production in the past ten years. There are no statistics available for the two African species of eggplant; they are most often cultivated along with other species in gardens or in small fields near villages (Daunay et al., 2001) Because of this, obtaining the

correct eggplant production statistics in Africa is a problem even though it barely causes a significant increase in total yield.

Table 1. 1. Ten year world eggplant production
(Source: Food and Agricultural organization 2012)

Year	Area coverage ('000000 ha)	Production ('000000 tonnes)
2012	1.85	48.42
2011	1.82	46.84
2010	1.72	44.28
2009	1.69	43.17
2008	1.61	39.81
2007	1.66	37.62
2006	1.88	33.04
2005	1.83	32.08
2004	1.75	31.01
2003	1.68	29.71
2002	1.64	29.53

Table 1. 2. Top producers of eggplant
(Source: Food and Agricultural organization 2012)

Countries	Year	Area coverage ('000 Ha)	Production ('000000 Tonnes)
China	2012	800	28.80
India	2012	700	12.20
Iran	2012	39.5	1.30
Egypt	2012	45.25	1.19
Turkey	2012	26	0.80

Note: The aggregate data may include official, semiofficial or estimated data

1.3. Nutritional and Medicinal Value

S. aethiopicum and *S. macrocarpon* species bear round or oblong, fleshy fruit which are consumed at the immature stage like those of *S. melongena*. Their leaves are also eaten like spinach when they are glabrous. Immature and raw fruit of *S. melongena* are consumed and cooked with other vegetables and meat. In the agro-food industry the fruits are dehydrated, deep frozen or cooked in various food preparations and also used

to make jam. All three species can be bitter and bitterness depends on which part is consumed and the taste preference of people in the region. Its bitterness is minimized and released when cooked and mixed with other ingredients like salt, lemon, tahini, garlic and other spices. Eggplant is used in the cuisine of many countries and prepared in different ways: 'ratatouille' in France; 'parmigiana di melanzane' in Italy; 'patlıcan kızartması' 'karnıyarık,' 'İmam bayıldı' and 'patlıcan şakşuka' in Turkey; 'sambhar,' 'dalma,' 'chutney,' 'curry,' and 'achaar' in India; 'musakka' in Turkey, Greece, Middle East and Southern Asia; and 'kashk' in Iran.

Eggplant nutritional value is comparable to other common vegetables, but is lower than tomato (Grubben, 1977). Eggplant is a good source of minerals and vitamins and can be compared with tomato in terms of total nutritional value (Kalloo 1993, Singh and Kumar 2007). Eggplants have many ingredients such as fat, water, carbohydrate, water soluble sugar, amid protein and phenolic compounds that are beneficial for human health. Its fresh weight consists of 92.7% moisture, 4% carbohydrates and vitamins, 1.4% protein, 1.3% fiber, 0.3% fat, and 0.3% minerals (Khan, 1979). It provides relevant quantities of P, K, and Cu to the diet, with global mean values of 26.6 mg, 198.5 mg, and 0.062 mg per 100 g of fresh weight of these minerals, respectively (Marý´a D. Raigo´n et al., 2008). Different cultivars have various amount of antioxidant content which is important for medicinal purposes. According to Marý´a D. Raigo´n (2008), landraces of eggplant cultivars have higher amounts of phenolic compounds. Phenolics and other antioxidants are important substances that are found in plants and help to maintain human health. Research suggests that eating plenty of foods high in antioxidants helps slow the processes associated with aging and protects against many chronic diseases (Cao et al., 1996). Different findings support the benefits of eating vegetables and fruits as a reliable way to get needed amounts of antioxidant. Diets with a low proportion of vegetables are linked to a higher incidence of mortal diseases, like cancer or cardiovascular disease, which can be prevented by antioxidants (Doll, 1990; Bravo, 1998).

In the Ayurvedic, a Hindi system of medicine, white types of fruits were recommended for diabetic patients, and roots for the treatment of asthma (Daunay et al., 2007). Eggplant's effectiveness against otitis, cholera, dysuria, and toothache are explained in Sanskrit (Khan 1979). This is an indication of the use of eggplant in traditional medicine. In modern medicine, alkaloids play important roles in pharmaceuticals indicating the medicinal importance of eggplants, which contain high

amounts of alkaloids. Even though alkaloids have pharmaceutical importance, they have negative effects at excessive levels and can result in extreme bitterness. Fortunately, the fruit of cultivated species normally do not contain such high levels of alkaloids unless the plants have been subjected to extreme stress (Aubert et al., 1989a, b.). Eggplants also have negative effects on some people and cause an allergic reaction (Siddanakoppalu N. and Yeldur P., 2004).

1.4. Varietal Improvement

Different organizations and national institutes have made important efforts to collect eggplant germplasm across their countries and the world. Formerly, farmers in different countries preserved eggplant landraces. Today, the National Institutes of China, India, Japan, Taiwan, Thailand, Bangladesh, Russia, Germany, France, Netherland, Greece and the University of Birmingham in UK are important contributors to preserve eggplant germplasm. IPGRI (International Plant Genetic Resources Institute, formerly IBPGR), INRA (Institute de la Researche Agronomique), USDA (United States Department of Agriculture) and EGGNET (European Union project to collect and preserve eggplant) have also made significant efforts to collect eggplants in collaboration with the national institutes of different countries. Some of the institutes such as INRA, University of Birmingham, and Nijmegen Botanical Garden have collected vast amounts of *S. melongena* as well as accessions of the African cultivated eggplants, *S. aethiopicum and S. macrocarpon*.

All the international and national institutes use the collected germplasm to improve important traits of wild and cultivated species. In previous times, financial problems and shortages of resources have been barriers for eggplant research, as a result, work on varietal improvement and genetic diversity is insufficient (Daunay et al., 2001). However, more recently, the development of high productivity and disease resistant hybrids have increased the profit of farmers and drawn the awareness of seed companies.

Variety improvement includes different traits such as:

- Yield and its components such as early maturity, uniform harvest, rich color, taste and aroma, and storage quality;
- Resistance to diseases, insects and nematodes; resistance or tolerance to abiotic stress (drought, low or high temperatures, salinity);

- Nutritive value such as high dry matter, sugars, anthocyanin and total phenol contents, low level of polyphenol oxidase activity and orthodihydroxy phenolic compounds to avoid browning of cut fruits;
- Parthenocarpy, Market needs and consumer preference.

Different researchers have improved targeted traits in eggplant. The first hybrid *S. melongena* was developed in Japan in the 1930s and followed by hybrids developed in Europe in the 1970s (Daunay et al., 2001). This work has increased the homogeneity of production across Asia and Europe. All of Japan's and 80% of Europe's commercial cultivars are F1 hybrids, which encompassed only a small number of cultivars. India and China are also accelerating in use of F1 hybrids. Following the success of hybrids, breeders in different geographical region have attempted to improve productivity, fruit quality, resistance to disease and insects, and adaptation to agro climatic conditions. In general, farmers in Western Europe do not grow local varieties of *S. melongena* because of the availability of high yielding F1 hybrids. This may be a bottle neck for breeder because it causes significant loss of diversity. In Africa, Senegal has been working on breeding of *S. aethiopicum* since the 1980s (Seck 1986) while landraces of the less variable cultivated species *S. macrocarpon* are still used.

Conventional breeding techniques such as pedigree selection and backcross selection have been used in different areas and are supplemented by new biotechnological tools to improve targeted characters of a species. These techniques are mostly applicable in *S. melongena* cultivars in America, Europe and Asia. Crossability studies showed that the cross compatibility between any two Solanum species is not a simple absolute characteristic. According to Daunay (1998), who checked 41 wild species for crossability and the fertility of interspecific hybrids, 22 of the combinations gave rise to viable hybrids. Five of them (*S. dasyphyllum, S. liddii, S. pyracanthos, S. rubetorum and S. tomentosum*) were already reported in the literature and they tested 17 new species, never used before in crossing experiments. In addition to these 22 species, 14 other Solanum species including *S. aethiopicum* and *S. macrocarpon* were also reported for their crossability with *S. melongena*. Fertility of F1 hybrids was not similar across all crosses. In brief, the potential of brinjal eggplant germplasm as a source of valuable breeding characters is still insufficiently known, and those of the germplasm of other related cultivated and wild species are even less known and deserve intensive effort, since the genetic basis of *S. melongena* is narrow (Daunay et al., 2001). Identification of wild species that are compatible to produce hybrids with cultivated

eggplant would enable systematic evaluation of their genetic variability, particularly in the field of disease resistance (Daunay et al., 1995a). Studying diversity and crossability should continue until important wild species candidates are included in breeding programs.

Because of interspecific fertility problems, conventional breeding techniques are incapable of expanding the eggplant gene pool. As already mentioned, crossability between *S. melongena* and other distance subgenera or genera is low(Daunay et al., 1991) and transfer of important agronomic traits between eggplant species can be limited by sexual barriers (C.Collonnier et al., 2001). Bacteria wilt, fusarium wilt, verticillium wilt, root knot nematodes, fruit borers, fruit anthracnosis and mite resistance have been identified in some solanum species but have not yet been used to their full potential because of transfer difficulties (Daunay et al., 2001). Tissue culture techniques like somatic hybridization and anther culture (Ano, 1991; Blestsos et al., 1998; Daunay et al., 2001; Salas et al., 2011) have been used to solve some crossing problems, however hybrids have reduced fertility. In addition, techniques like pedigree selection which is applicable for mass selection and backcross selection are useful only for monogenic characteristics. In eggplant, variation is mostly quantitative, particularly for important agronomic characteristics such as parthenocarpic tendency, fruit number, fruit size, fruit weight, fruit shape, fruit firmness, flowering time, flower and fruit number per inflorescence, fruit set, calyx size and fruit glossiness, plant prickliness and hairiness (Frary et al., 2003, and Daunay et al, 2001). Although mutation of a single gene prevents the development of hairs, prickles and other characters, normal development is controlled by many genes (Daunay et al., 2001). Therefore, biotechnological techniques have been implemented for various applications, including protoplast fusion, genetic transformation, micropropagation and somatic embryogenesis (Sihachakr et al., 1994; Guri and sink, 1988; Leone et al., 1993; Iannacone et al., 1995; Ali et al., 1991, Mariana, 1992, Feri et al., 1995)

Marý´a D. Raigo´n (2008), work on nutritional quality of eggplant cultivars using commercial, landrace and hybrid eggplant varieties from different geographical areas, to evaluate improved content of phenolics and nutritionally relevant minerals (P, K, Ca, Mg, Na, Fe, Cu, and Zn) in fruit. Significant differences of content composition were detected between these varieties, which helps the breeder to select varieties with improved nutritional characteristics. The development of F1 hybrids increases the adoptability of crops throughout the world by improving qualitative and quantitative

yield. Heterosis was detected in selected breeding lines. Positive mean heterosis was noticed for dry matter content and Na concentration and negative mean heterosis was noticed for P, K and Ca concentrations.

Following the economic importance and demand of eggplant, dramatic progress has been made by increasing earliness, reducing prickliness and decreasing fruit bitterness. Regardless of these achievements, the range of pathogens controlled by genetic resistance is limited. Nevertheless, some promising work has been done regarding disease resistance. For example, *Ralstonia solanacearum* (bacterial wilt) resistance from *S. aethiopicum,* verticillium wilt resistance from *S. torvum,* fusarium wilt (*Fusarium oxyporum f. sp. melongena*), and fruit anthracnosis (*Colletotrichum gloeosporiodes*) resistances were transferred to cultivated eggplant from its wild relatives (Ano et al., 1991; Jarl et al., 1999; Rizza et al., 2002). These wild species have enormous potential to improve cultivated eggplant.

1.5. Genetic Diversity and Its Importance

Genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or genomes of organisms. Eggplant is characterized by great morphological diversity both at intra- and interspecific levels (Furini and Wunder 2004), which makes taxonomic classification difficult (Knapp et al., 2013). Understanding the importance of studying genetic diversity, scientists devoted their time to study diverse genomic collections of Solanum species. Before the start of molecular markers, they used morphological and biochemical studies. However, these approaches could not solve the taxonomical confusion because of a high level of morphological variability and a limited amount of work done (T.K. Behera et al., 2006). Morphological data can lead to ambiguous interpretations because of vast variability. To overcome these problems, isozyme variation was analysed (Lester and Hasan, 1991; Isshiki et al., 1994; Karihaloo and Gottlieb, 1955), chloroplast DNA used (Sakat et al., 1991; Sakata and Lester, 1997; Isshiki et al., 1998), and genomic DNA also used (Kaihaloo et al., 1995; Mace et al., 1999 and etc..) to assess genetic diversity. Allozyme variation was used in order to elucidate the phylogenetic relationships of Solanum species (Isshiki et al., 1994), which showed similar results as chloroplast DNA analysis (Sakata and Lester, 1997). A study on *S. melongena* and its wild relatives using AFLP markers also showed consistent result with isozyme and chloroplast DNA analysis (Mace et al., 1999). All of these

results on eggplant diversity conflicted with conventional classification based on morphology. More recently other molecular markers are available for polymorphism studies of eggplant and related species. Using molecular data is advantageous compared to morphological data because genotypic data are highly conserved and not influenced by environment, which can help clarify the study of morphologically complex organisms.

Despite ongoing work, some eggplant species have not yet been properly classified. The evolution and classification of eggplant research has been inferring Lester and Hassan (1991) work done with limited data sets. The material even did not include prickly Chinese cultivars (Jin-Xiu-Wang et al., 2008). In a recent study of wild relatives of eggplant, the names of species were reviewed and keys and characters were provided to resolve ambiguous classification (Knapp et al., 2013). The greatest diversity of landraces and cultivars of *S. melongena* is found in Asia (India, China and Southeast Asia), with secondary centers in the Middle East and around the Mediterranean. *S. melongena* is characterized by large phenotypic variability which includes diverse plant form (from small fruited spiny plants to large fruited non-spiny plants), fruit shape (ovoid, globular, oblong, semi long, long, very long and serpentine), and colour (green, mottled green, white, pink, mauve, violet, striped purple or black). The related species *S. aethiopicum* is also morphologically highly variable, and accessions are assigned to four groups based on their leaf and fruit morphology and their uses. Aculeatum, Gilo, Kumba, and Shum are the four groups under the name *S. aethiopicum*. Aculeatum is better known by the name *S. integrifolium poir*; Gilo as *S.gilo radddi* or *S. olivare paill* and *Poir*; kumba as *S. aethiopicum*; and Shum as *S. zuccagnianum Dunal. S. integrifolium* (Aculeatum) is characterized by small fruit, glabrous leaves, prickliness, sub-spherical fruits, juiciness and bitterness. *S. gilo* or *S. olivare* (Gilo) is characterized by large fruit of diverse shape and number, hairy leaves, fruits tasting like carrots or green beans. *S. zuccagnianum* (Shum) is characterized by short, branched stems with small leaves, hairless, prickle-less and has small fruits. S. *aethiopicum* (Kumba) is characterized by glabrous leaves and large, sweet and fleshy fruits. *S. macrocarpon* expresses less diversity in its phenotype than *S. aethiopicum*. The fruit is recognized by its leafy calyx and this species has less variability in fruit shape and color than the other two cultivated species.

Knowledge of the diversity of wild and semi-cultivated eggplants provides an opportunity for breeders to easily compile a list of wild species that could be useful for

eggplant breeding. Studies of plant evolution unravel questions about crop migration and domestication and provide information about similarities in agronomic traits and disease resistance of species. Studying diversity of species will help to select appropriate breeding materials to solve the interspecific cross incompatibility problem because of distant relationships (Isshiki et al., 1994). Assessment of genetic variability within the germplasm is also important to explore new agronomic characters or genes of interest for breeding purposes. In addition, it gives information for conserving genetic resources. Hence, such work is important for genetic improvement and new trait exploitation. Collecting diverse germplasm will help us to challenge future food shortages and to adopt newly developed varieties in different agro-ecological zones. This also allows future eggplant breeders to evaluate wild germplasm for any character of agronomic interest. Characterization of the genetic diversity of this germplasm is important to maintain and utilize these resources. Limited information on characterization of genetic diversity is a bottleneck for the breeder. Exploiting diversity supports the breeder to develop varities resistant to biotic and abiotic stress, adapted to different agro-ecologies and with increased quantitative and qualitative productivity.

A low frequency of polymorphism has been reported in solanaceous plants as a result of their autogamous nature (Nunome et al., 2003 and Stàgel et al., 2008). In addition, breeders have also been responsible for reduced eggplant genetic diversity by concentrating on a few core inbred lines in many breeding programs. Therefore, the released cultivars display high similarity (Ali Z., et al., 2011). As a result, the information we get from germplasm collections will be useful for the management of eggplant germplasm and improving the breeding programs of eggplant.

In contrast to other solanaceous species, eggplant has been less often used in molecular genetics research, probably because it is produced and consumed less widely than tomato and potato. Eggplant has many unique traits compared with the two Solanum model species, including larger fruit size, high temperature and water-stress tolerance, parthenocarpy without negative pleiotropic effects, and stable verticillium and bacterial wilt resistance (Fukuoka et al., 2012). In addition, information about eggplant evolution will shed light on the domestication process involved in other solanaceous crops such as tomato, potato and chili pepper (Weese and Bohs, 2010). Therefore, the accumulation of genomic information about eggplant will not only facilitate genetics research and molecular breeding of eggplant itself, but will also make

this species a valuable and unique member of the Solanaceae for comparative biological studies of the genetics, physiology, development, and evolution of this taxon.

a

Shume Kumpa

Aculeatum Gilo

(Source: Plant names from University of Melbourne 2013)

Figure 1. 1. Pictorial representations of the three cultivated species. a) *S. aethiopicum* b) *S. macrocarpon* c) *S. melongena*

(Cont. on next page)

b

(Source: Plant names from University of Melbourne 2013)

c

(Source: Knapp S. et al., 2012)

Figure 1.1. (Cont.)

1.6. Molecular Markers

Molecular markers disclose neutral site of variation at the DNA sequence level. Unlike morphological marker they are numerous in number (Jones R.N. et al., 1997), and efficient to study diversity and population structure of organisms. Molecular markers and marker mapping are applicable in many area of modern biology. Some of the marker has enormous potential to explore genetic diversity by detecting nucleotide differences between cultivars and species. Despite work done on diversity, breeding and genotype identification using molecular markers in the Solanaceae, eggplant has not benefited like other Solanum species such as tomato, potato and pepper. Researchers used different molecular markers to study the diversity and structure of eggplant and allied species. Studies have been performed to determine the genetic diversity of eggplant using markers such as random amplified polymorphic DNA (RAPD) (Kaihaloo et al., 1995; Singh et al., 2006 and Nunome et al., 2001), amplified fragment length polymorphism (AFLP) (Prohens et al.,2005; Furini and Wunder, 2004; Mace et al., 1999), RAPD and AFLP (Nunome et al., 2001), simple sequence repeats (SSR) (Ge H. et al., 2013; Mu˜noz-Falcón et al., 2011; Demir et al., 2010; Tumbilen et al., 2009, Stàgel et al., 2008; Behera et al., 2006; Nunome et al., 2003; Nunome et al., 2009), sequence related amplified polymorphism (SRAP) (Li et al., 2010), sequence tagged microsatellite site (STMS) (T.K. Behera et al., 2006) and inter simple sequence repeat (ISSR) (Isshiki et al., 2008). All of the aforementioned studies on Solanum species mainly focused on exploring the genetic diversity and relationships among species/accessions.

1.6.1. Sequence Related Amplified Polymorphisms (SRAPs)

Several polymerase chain reaction (PCR) markers are available and differ in complexity, reliability and information generation capacity. SRAP markers are one type of PCR marker (Li and Quiros 2001). Li and Quiros' original thinking was to simplify the AFLP detection procedure (Vos et al., 1995), increase throughput and improve reproducibility compared to RAPD markers. They developed the markers by skipping restriction enzyme digestion and ligation of target DNA fragments and adapters, minimizing the rounds of PCR reactions in the AFLP protocol, and by designing a

special PCR running program. Most SRAP and AFLP markers are dominant and are evenly distribution across chromosomes (Li and Quiros 2001). Youssef et al., (2011) study on genetic diversity of Musa accessions supported the effectiveness of SRAP. They found that SRAP produced threefold more loci than AFLP.

SRAP markers target amplification to open reading frames (ORFs), which is relatively conserved among species (Ferroil et al., 2003). The SRAPs have forward and reverse primers with lengths of 17 and 18 bases, respectively. These primers consist of 14 to 15 bases of core sequence starting at the 5' end. For example, the forward primer sequence consists of 14 bases, where the first 10 bases starting at the 5' end have no specific constitution called filler sequence, followed by CCGG sequence. Similarly, the reverse primer consists of 15 bases, where the first 11 bases starting at 5' are filler sequence and are followed by AATT sequence. Three selective bases are added to the 3' end of both forward and reverse primers. These primers should not form hairpins or other secondary structure, have a GC content of 40 to 50%, and contain different filler sequence from 10 or 11 bases long. The aim of using CCGG sequence in the core sequence of the forward primer is to target exons in the open reading frame (ORF). In general, there is a higher amount of GC content in gene coding sequences of plant genomes (Lin et al., 1999) and they used the difference of GC content between gene coding sequences and other sequences as an advantage.

SRAP markers combine simplicity, reliability, flexibility, detection of multiple loci, cost effectiveness and facile sequencing of selected bands, which have many advantages compared with some other markers and allow researchers to perform SRAP routinely with limited facilities or in well-established genomics labs. The forward SRAP primer can be combined with many reverse primers, which can enhance the effectiveness and efficiency of SRAP marker detection (Li and Quiros 2001). The markers are applicable for various purposes including map construction, genomic fingerprinting, gene cloning and gene tagging. These markers have been used to detect genetic diversity for various organisms since genomic sequence information is not necessary for SRAP markers. After SRAP markers were used in a diversity study of *Brassica napus* (Riaz et al., 2001), they were also used to detect the diversity of different organisms such as vegetables (Y.Jiang and J.P.Liu, 2011) grasses (Xie et al., 2009), cereals (Yang et al., 2010, Dai et al., 2012), legume crops (Vandemark et al., 2006, Salem S. Alghamdi et al., 2011), oil crops (Zhang et al., 2010b), fruits (Guo et al., 2012), medicinal plants (Ortega et al., 2007), ornamental plants (Feng et al., 2009b), and

woody plants (Li et al., 2010). H. Li and his colleagues (2010) used SRAP markers to study genetic diversity of Chines eggplant collection for the first time and gave an efficient result. Thus, SRAP technology has been commonly used in analysis of genetic diversity of many plant species.

1.7. Goals of Research

The objective of this study was to determine the genetic variation at the molecular level among collection of eggplants from four continents and representing 28 countries. Understanding the actual genetic diversity of these eggplants at the molecular level can give more precise characterization than morphological diversity. Accordingly, the genetic distances and population structure of 73 S. melongena accessions and six outgroups were determined. This study will add more information on genetic diversity of *S. melongena*, with a final goal to improve molecular breeding and conservation of genetic resources to challenge food shortage, disease and adaptation problems. Moreover, the work also evaluates the performance of SRAP markers in a world eggplant collection for the first time and gives insight on the consistency of result with other diversity studies. It also gave us insight on the correlation of actual genetic diversity and geographical diversity.

CHAPTER 2

MATERIAL AND METHODS

2.1. Plant Material

We used eggplant germplasm collected from different geographical regions by Institut National de la Recherche Agronomique (INRA) in the past years. The eggplant collection represents six species and a total of 79 accessions (Table 2.1), with their geographical origin. Most of the accessions are from commonly cultivated *S. melongena* that comprise 73 accessions. The other five species are *S. aethiopicum, S. linnaeanum, S. incanum, S. viarum, and S. violaceum* and were used as outgroups. The 79 accessions are from four continents. The highest number of genotypes is from Asia followed by Europe, with 38 and 32 accessions, respectively. Africa is represented by three accessions and North America is represented by six accessions.

Table 2. 1. Accessions and species used for this study

No.	Species Name	Accession number	Geographical Origin	Continent
1	*S. melongena*	MM 0006	India	Asia
2	*S. melongena*	MM 0014	Greece	Europe
3	*S. melongena*	MM 0021	Italy	Europe
4	*S. melongena*	MM 0023	Italy	Europe
5	*S. melongena*	MM 0039	France (SUD-EST)	Europe
6	*S. melongena*	MM 0054	France (SUD-EST)	Europe
7	*S. melongena*	MM 0055 (A0012)	France (SUD-EST)	Europe
8	*S. melongena*	MM 0058	France (SUD-EST)	Europe
9	*S. melongena*	MM 0061	Spain (Gandia)	Europe
10	*S. melongena*	MM 0064	Spain (Gandia)	Europe
11	*S. melongena*	MM 0069	USA	North America
12	*S. melongena*	MM 0091	USA	North America
13	*S. melongena*	MM 0101	Japan	Asia
14	*S. melongena*	MM 0102	Japan	Asia
15	*S. melongena*	MM 0106	Spain (Valencia)	Europe
16	*S. melongena*	MM 0113	Spain	Europe
17	*S. melongena*	MM 0124	China	Asia
18	*S. melongena*	MM 0125	USSR	Europe
19	*S. melongena*	MM 0128	Romania	Europe
20	*S. melongena*	MM 0152	Sri Lanka	Asia
21	*S. melongena*	MM 0154	Yugoslavia	Europe
22	*S. melongena*	MM 0164	Bulgaria	Europe
23	*S. melongena*	MM 0165 (A0067)	Philippines	Asia
24	*S. melongena*	MM 0166 (A0119)	Philippines	Asia
25	*S. melongena*	MM 0178	USA	North America
26	*S. melongena*	MM 0194 (A0003)	France(SUD-EST)	Europe

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Table 2.1. (cont.)

27	*S. melongena*	MM 0198 (A0321)	Netherland	Europe
28	*S. melongena*	MM 0203	USA	North America
29	*S. melongena*	MM 0304	Netherland	Europe
30	*S. melongena*	MM 0357	China	Asia
31	*S. melongena*	MM 0360	China	Asia
32	*S. melongena*	MM 0364	USSR (Moldovia)	Europe
33	*S. melongena*	MM 0367	USSR (Krasnodar)	Europe
34	*S. melongena*	MM 0377	India (Kerala)	Asia
35	*S. melongena*	MM 0380 E	India (Varanasi)	Asia
36	*S. melongena*	MM 0396	India	Asia
37	*S. melongena*	MM 0400	Thailand	Asia
38	*S. melongena*	MM 0401	France (Bretagne, Brest)	Europe
39	*S. melongena*	MM 0409	Italy	Europe
40	*S. melongena*	MM 0412 (A0209)	Japan	Asia
41	*S. melongena*	MM 0459 (A0060)	India	Asia
42	*S. melongena*	MM 0477 BIS	Nepal	Asia
43	*S. melongena*	MM 0480	USSR (Krasnodar)	Europe
44	*S. melongena*	MM 0500 (A0215)	France (Guadeloupe)	Europe
45	*S. melongena*	MM 0508	Bulgaria	Europe
46	*S. melongena*	MM 0563	Italy	Europe
47	*S. melongena*	MM 0653	India(Karataka)	Asia
48	*S. melongena*	MM 0656 (A0129)	Taiwan	Asia
49	*S. melongena group G*	MM 0687	Malaysia	Asia
50	*S. melongena*	MM 0725	Tahiti	Europe
51	*S. melongena*	MM 0915	Lebanon	Asia
52	*S. melongena*	MM 12389	Malaysia	Asia
53	*S. melongena*	MM 1281 C	Thailand	Asia
54	*S. melongena group H*	MM 1303	Thailand	Asia
55	*S. melongena*	MM 1392	Sudan	Africa
56	*S. melongena*	MM 1445	India	Asia
57	*S. melongena*	MM 1448	India	Asia
58	*S. melongena*	MM 1449 BIS	India	Asia
59	*S. melongena*	MM 1487	China (Hangzhou)	Asia
60	*S. melongena*	MM 1498 bis	India	Asia
61	*S. melongena*	MM 1536	Malaysia	Asia
62	*S. melongena*	MM 1542	Thailand	Asia
63	*S. melongena*	MM 1551	Malaysia	Asia
64	*S. melongena*	MM 1564	Indonesia	Asia
65	*S. melongena*	MM 1597	India	Asia
66	*S. melongena group G*	MM 1677	Thailand	Asia
67	*S. melongena*	MM 1713	USA	North America
68	*S. melongena*	MM 1724	Indonesia (Java)	Asia
69	*S. melongena*	MM 1725	Ukraine	Europe
70	*S. melongena*	MM 1750	Spain	Europe
71	*S. melongena*	MM 1751	Italy	Europe
72	*S. melongena*	MM 1788	Italy	Europe
73	*S. melongena*	MM 1789	Vietnam	Europe
74	*S. viarum*	MM0374	Nepal	Asia
75	*S. violaceum*	MM0497	Japan	Asia
76	*S. incanum group A*	MM0663	Uganda	Africa
77	*S. incanum group C*	MM0715	Israel (Telaviv)	Asia
78	*S. aethiopicum aculeatum group*	MM 0134	France(Martinique)	Europe
79	*S. linnaeanum*	MM 0195	Tunisia	Africa

The eggplant accessions were planted in seedling plates that contained peat and perlite in a greenhouse. Eight seeds from each accession were planted in a seedling plate row. Newly expanded leaves were collected in 2ml tubes by chopping into small pieces. Fresh leaves were extracted immediately or stored for several days at -80 ^{o}C temperature before extraction.

2.2. DNA Isolation

Genomic DNA was extracted from young leaf tissue following the procedure given by Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) with a little modification. Total genomic DNA of all accessions was extracted from fully expanded leaves mixed with cetyltrimethylammonium bromide (CTAB) and an iron ball in 2 ml tubes and put in a tissue lyser to grind the tissue. DNA was isolated from eight individual plants from each of 79 accessions and stored in a 4 ^{o}C refrigerator. After rehydration, 5 µL of DNA for each individual was combined with the DNAs of the other individuals of the same accession. These pooled DNA samples were the material used for further experiments. DNA quality and quantity were assessed on a 1% (w/v) agarose gel stained with ethidium bromide.

2.3. SRAP Marker Analysis

SRAP analysis was carried out according to previously established protocols described by Li and Quiros (2001). One hundred thirty two SRAP primer combinations were initially screened in four representative, randomly selected samples. Forward and reverse primers are shown in Table 2.2. Primer combinations were selected based on the strength of amplification and banding patterns. Ten primer combinations that produced consistent amplifications and clear polymorphic bands were selected from the 132 SRAP primer combinations.

The PCR reaction mixtures (25.25 µl total volume) contained 18 µl of distilled water, 2.5 µl of 10x PCR buffer, 1.5 µl of MgCl2, 0.5 µl of dNTPs, 0.5 µl of forward primer, 0.5 µl of reverse primer, 0.5 µl of Taq polymerase and 0.25 µl of Bovine Serum Albumin (BSA). The amplifications were performed in a Veriti® Thermal Cycler (Applied Biosystems® PCR instruments) programmed with the following PCR

protocol: 94 °C for 5 min, followed by 5 cycles of three steps; 1 min denaturing at 94 °C, 1 min annealing at 35 °C , and 1 min elongation at 72 °C. In the following 35 cycles the annealing temperature increased to 50 °C while the other steps remained similar, and with a final elongation step at 72 °C for 10 min and conservation at 4 °C. Amplified PCR products were electrophoresed for at least 2h at 120 mA and 95 volt in a 2% agarose gel. PCR products were diluted with loading solution (LS) at the ratio of 10 to 1.

Table 2. 2. Selected primer combinations (SPCs) of SRAP primers used in this study

Abbreviations	Primer combinations	Forward primer (5' to 3')	Reverse primer (5' to 3')
SPC1	me5-em6	me5: TGAGTCCAAACCGGAAG	em6: GACTGCGTACGAATTGCA
SPC2	me6-em1	me6: TGAGTCCAAACCGGACT	em1: GACTGCGTACGAATTAAT
SPC3	me6-em11	me6: TGAGTCCAAACCGGACT	em11: GACTGCGTACGAATTCCA
SPC4	me7-em11	me7: TGAGTCCAAACCGGACA	em11: GACTGCGTACGAATTCCA
SPC5	me8-em11	me8: TGAGTCCAAACCGGAAC	em11: GACTGCGTACGAATTCCA
SPC6	me10-em6	me10: TGAGTCCAAACCGGACG	em6: GACTGCGTACGAATTGCA
SPC7	me10-em10	me10: TGAGTCCAAACCGGACG	em10: GACTGCGTACGAATTCAG
SPC8	me11-em10	me11: TGAGTCCAAACCGGTCC	em10: GACTGCGTACGAATTCAG
SPC9	me11-em11	me11: TGAGTCCAAACCGGTCC	em11: GACTGCGTACGAATTCCA
SPC10	me12-em1	me12: TGAGTCCAAACCGGTAG	em1: GACTGCGTACGAATTAAT

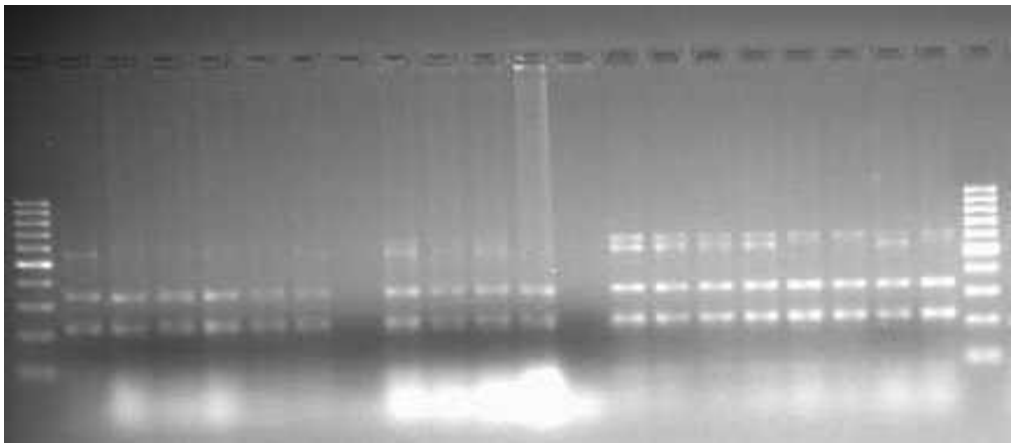

Figure 2.1. Gel electrophoresis result from SPC9 for the first twenty accessions in table 2.1

Columns represent the accessions we used and the rows indicate the number of alleles detected using SPC9 for the first 20 accessions. Clearly amplified bands were scored from these gel electrophoresis results and unclear bands were not included in our data to found reliable data. Similarly the gel results of 79 accessions were scored for 10

SRAP primer combinations. The first column and the last column in this picture represent the ladder (figure 2.1.)

2.4 Genetic Analysis

The data collected from gel electrophoresis of PCR products were analyzed by DARwin software. Presence/Absence dissimilarity index, and Dice and Jaccard coefficients were used for analysis. Each accession was genotyped for each SRAP locus with band presence coded as (1) and absence as (0), only clear bands were scored. The dissimilarities of accessions were estimated using two different measures. Dice coefficient, d*ij* = b+c/2a+ (b+c); and Jaccard, d*ij* = b+c/ a + (b+c), where d_{ij} is the dissimilarity between two individuals, i and *j*, a is the number of bands present in i and j, b is the number of bands present in i and absent in j, c is the number of bands absent in i and present in j. After the data were analyzed using Dice and Jaccard coefficients and unweighted neighbor joining clustering method; the Dice coefficient was selected for further analysis because of its high cophenetic correlation value. Then, we constructed the relationship tree of our accessions using Neighbor-Joining method.

The polymorphic information content (PIC) of all primer combinations was calculated using the following equation, PIC = 2*(frequency of presence) * (frequency of absence) (Roldan-Ruiz et al. 2000). Moreover, a principal coordinate analysis was carried out to show the multi-dimensional relationships between entries. The widely used program, STRUCTURE SOFTWARE 2.3 (Pritchard et al., 2000) was used to determine population structure using a Bayesian-based model. To determine number of clusters (K) values of 1-8 were tested using a burn-in period of 50000 and 100000 rounds from 10 independent simulations. To determine the delta K value, we used the web-based STRUCTURE HARVESTER program (Evanno et al., 2005).

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1. Results

3.1.1. SRAP Results

In the present study, ten selected primer combinations from the total 132 tested SRAP primer combinations were utilized for evaluating genetic diversity within *S. melongena* and five outgroup species (Table 2.2). These ten primer combinations consisting of seven forward and four reverse primers were selected based on their ability to prime PCR amplification of four randomly selected genotypes.

SRAP analysis was carried out on all 79 accessions and a total of 47 bands were amplified by ten different SRAP combinations. All the primers showed polymorphism (Table 3.1). The numbers of bands and polymorphic bands produced by the SRAP primer combinations varied from three to eight. The highest number of bands was observed for primer combination SPC1 and the lowest was for SPC4. The average number of bands amplified per locus was 4.7. Total number of band and number of polymorphic bands changed when outgroups were removed. The total number of bands decreased from 47 to 32 and the number of polymorphic bands also decreased from 47 to 27. The average number of alleles or band per primer combination decreased to 3.2 when the outgroups were removed and the average percentage of polymorphic band decreased to 84.4%.

Table 3. 1. Results for the 10 SRAP primer combinations used for analyzing 79 eggplant accessions

Primer combinations used	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands	Total number of bands within *S. melongena* accessions	Number of polymorphic bands within *S. melongena* accessions	Percentage of polymorphic bands among *S. melongena* accessions
SPC1	8	8	100	5	5	100
SPC2	5	5	100	2	2	100
SPC3	6	6	100	3	1	33.33

(Cont. on next page)

Table 3.1. (Cont.)

SPC4	3	3	100	3	3	100
SPC5	4	4	100	2	2	100
SPC6	4	4	100	3	3	100
SPC7	4	4	100	2	1	50
SPC8	4	4	100	4	4	100
SPC9	4	4	100	4	3	75
SPC10	5	5	100	4	3	75
Total	47	47	100	32	27	84.4
Average	4.7	4.7		3.2	2.7	

According to Table 3.2, the average polymorphism information content (PIC) was 0.14 with 0.02 as the lowest and 0.26 as the largest values in 0 to 0.5 scales. The results varied for *S. melongena* accessions, which had PIC values ranging from 0.03 to 0.22 with an average PIC of 0.14. The highest PIC was registered for SPC4 and SPC9 in all tested accessions and within *S. melongena* accessions, respectively. The lowest PIC was registered by SPC8 and SPC3 in all tested accessions and within *S. melongena* accessions, respectively. The PIC values of six primer combinations increased, three decreased and SPC10 gave the same value when the outgroups were removed from the data.

Table 3. 2. Polymorphic information content (PIC) of used primer combinations

SRAP primer combinations	PIC with outgroups	PIC without outgroups
SPC1	0.13	0.17
SPC2	0.11	0.14
SPC3	0.04	0.03
SPC4	0.26	0.19
SPC5	0.06	0.07
SPC6	0.17	0.16
SPC7	0.15	0.16
SPC8	0.02	0.1
SPC9	0.21	0.22
SPC10	0.13	0.13
Average	0.141	0.140

Note: Different way of calculating PIC. So, to change the values in 0 to 1 scale multiply the result by two.

3.1.2. Genetic Similarity Analysis

The coefficient and clustering method that yields a high cophenetic correlation (r) is considered as a suitable method for analyzing genetic diversity (Romesburg 1984). Cophentic correlation shows the level of agreement between the dendrogram and genetic data. According to the scale given by Rohlf (1992), a very good fit result is above 0.9 and a poor fit is below 0.8. In this study the cophenetic correlation for Dice and Jaccard's coefficients were 0.991 and 0.988, which indicated very good fits between the data matrix and dendrogram. Dice coefficient had a higher cophentic correlation than Jaccard's coefficient. Accordingly, Dice coefficient and unweighted neighbor joining clustering were selected for analyzing the genetic diversity.

The dendrogram (Fig 3.1) was comprised of five main groups. The dendrogram had similarity values ranging from 0.18 to 1 with a mean value of 0.59. According to the dissimilarity matrix, the minimum similarity of 0.18 was registered between *S. viarum* and *S. violaceum* assigned in group three and five; and the maximum similarities of 1 were registered between 23 accessions and 22 of them were from S. melongena accessions. The other one species, *S. linnaeanum* accession *(*MM 0195) and *S. melongena* accession (MM 0198 (A0321)) were assigned in accessions with maximum similarity under different species name and collected from different continent. The outgroups, except *S. linnaeanum and S. incanum* were comparatively found to be distantly related to the other accessions. Hence, *S. linnaeanum* was similar to the cultivated *S. melongena* accessions.

The largest group, group one contained 75 accessions of the cultivated *S. melongena* and other two species S. *incanum group C, and S. linnaeanum*. Thus, group one encompassed 94.9 % of the total accessions tested with a minimum similarity of 0.61 between *S. melongena* (MM1750) from Spain and *S. incanum group C (*MM0715) from Israel, a maximum similarity of 1.00 observed in between 23 accessions and a mean value of 0.81. The accessions registered under high similarity of all accessions found in group one. The first group divided to three subgroups (Fig. 3.1 and Table. 3.3). Subgroup A, B and C consisted of 66, 7 and 2 accessions, respectively. The highest percentage of group one accessions assigned in subgroup 1A, the remaining 9.3% and 2.7% assigned in subgroup 1B and 1C, respectively. Subgroup 1A comprised *S. melongena* accessions and one *S. linnaeanum* accession with a minimum similarity of 0.71 registered in between accession MM 0006 from India and MM 1542 from

Thailand. All the accessions assigned under group one with maximum similarity found in subgroup 1A. The highest similarity registered in between accessions MM 0014 (Greece), MM 021 (Italy) and MM 023 (Italy); MM 0113 (Spain), MM 0124(China) and MM0128 (Romania); MM 0058 (France), MM 0061 (Spain) and MM 0357 (China); MM 0396 (India), MM 0401(France) and MM 0409 (Italy); MM 0380 E (India), MM 0400 (Thailand), MM 0563 (Italy), MM 0656 (A0129) (Taiwan) and MM 1445 (India); MM 0091 (USA) and MM 0101 (Japan); MM 0166 (A0119) (Philippines) and MM 0377 (India); and MM 0198 (A0321) (Netherland) and MM 0195 (Tunisia). This group had the mean similarity of 0.86.

Subgroup 1B composed of *S. melongena* accessions from Europe and Asia with a minimum similarity of 0.85 between MM 0725 and MM 1750; and a maximum similarity of 0.97 between MM 0194 (A0003) and MM 0725. The last subgroup 1C composed of two accessions, one from *S. melongena* and the other was S. incanum group C. The accessions that showed the minimum similarity in group one isolated to subgroup 1B and subgroup 1C. We sub grouped group one to see the geographical distribution of the accessions (table 3.3). However, the sub groups contained mixture of accessions from different continents. The result showed that the geographical distribution doesn't directly correlate to the dissimilarities of accessions. Except group one the remaining group 2, 3, 4 and 5 consisted of one accession each. , *S. incanum group A* from Uganda, *S. viarum* from Nepal, *S. aethiopicum aculeatum group* from France (Martinique) and *S. violaceum* from Japan was grouped in to group 2, 3, 4 and 5, respectively. The result showed the distant relation of those out groups except *S. linnaeanum* and *S. incanum group C*, which grouped together with *S. melongena* accessions. One *S. melongena* accessions grouped together with *S. incanum* group C in subgroup 1C.

Pair-wise similarity index within *S. melongena* ranged from 0.697 to 1. The minimum similarity within S. melongena accessions was 0.69 registered in between MM1542 from Thailand and MM1750 from Spain. Accessions that showed higher similarities of 1 collected from different countries. The similarity of germplasm is not restricted to geographical origin, whereas accessions registered under highest similarity found in Asia and Europe. The pairwise similarity comparison of *S. melongena* accessions with outgroups showed the high similarity between *S. melongena* (MM0198 (A0321)) from Netherland and *S. linnaeanum* (MM 0195) from Tunisia; and the minimum similarity of 0.24 in between *S. violaceum* (MM0497) from Japan and *S.*

melongena (MM 1542) from Thailand. *S. linnaeanum, S. incanum group C, S. incanum group A, S. aethiopicum aculeatum group, S. viarum, and S. violaceum* had an average similarity of 0.91, 0.71, 0.54, 0.47, 0.44 and 0.43 with *S. melongena* accessions, respectively. The result showed the close relation of the wild species *S. linnaeanum* and *S. incanum* group c to the cultivated species *S. melongena* accessions. *S. linnaeanum* had a minimum similarity of 0.8 with MM 1750. The two wild species *S. linnaeanum* and *S. incanum* group c found in the same group with *S. melongena* accessions in the dendrogram (fig. 2). The outgroup *S. incanum group C, S. incanum group A, S. aethiopicum aculeatum group, S. viarum, and S. violaceum* showed maximum similarity with *S .melongena* accession MM0367 from USSR.

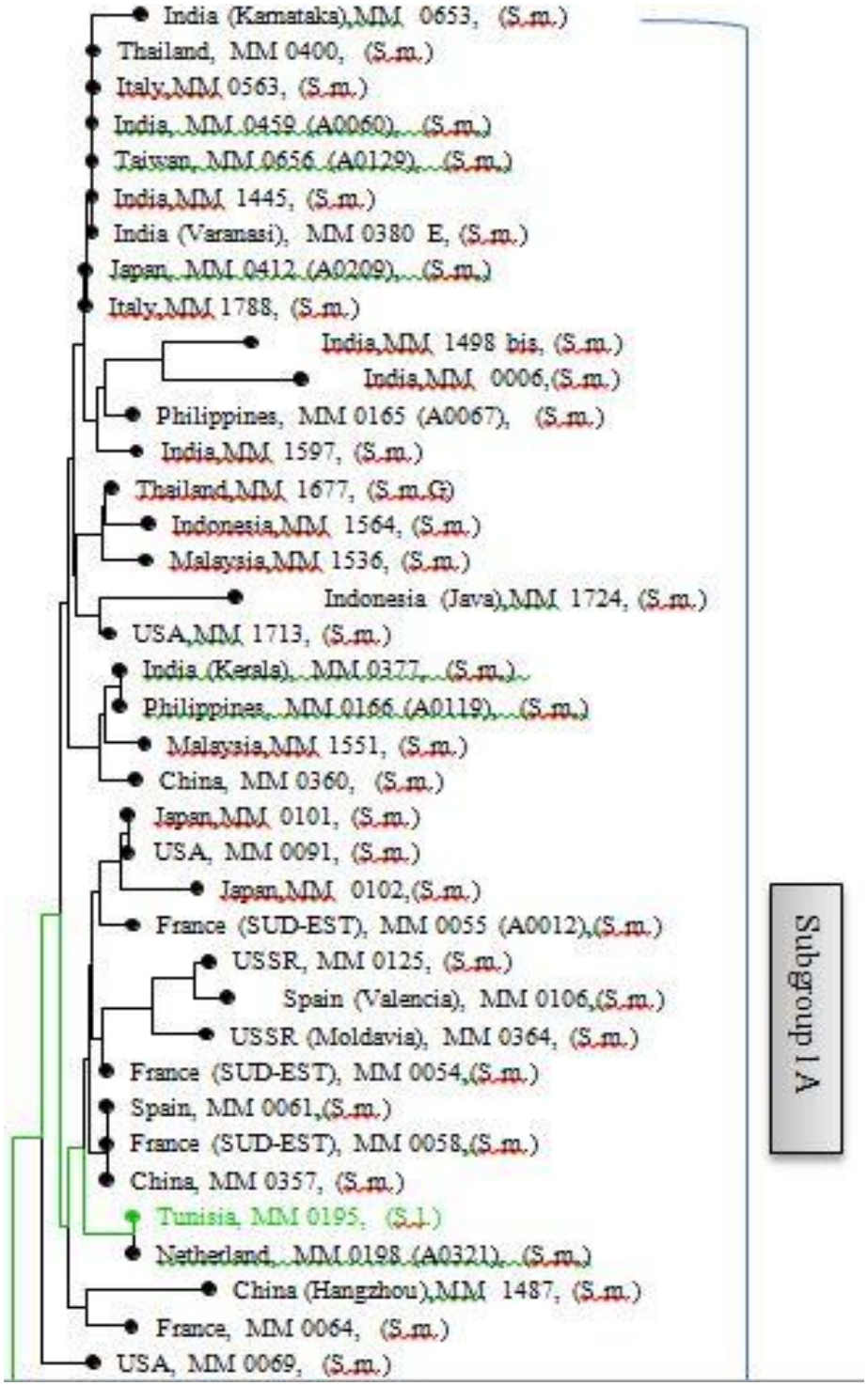

Figure 3. 1. Dendrogram showing the genetic similarities of 79 eggplant genotypes that was constructed from SRAP data using DARwin software program

(Cont. on next page)

Figure 3.1. (Cont.)

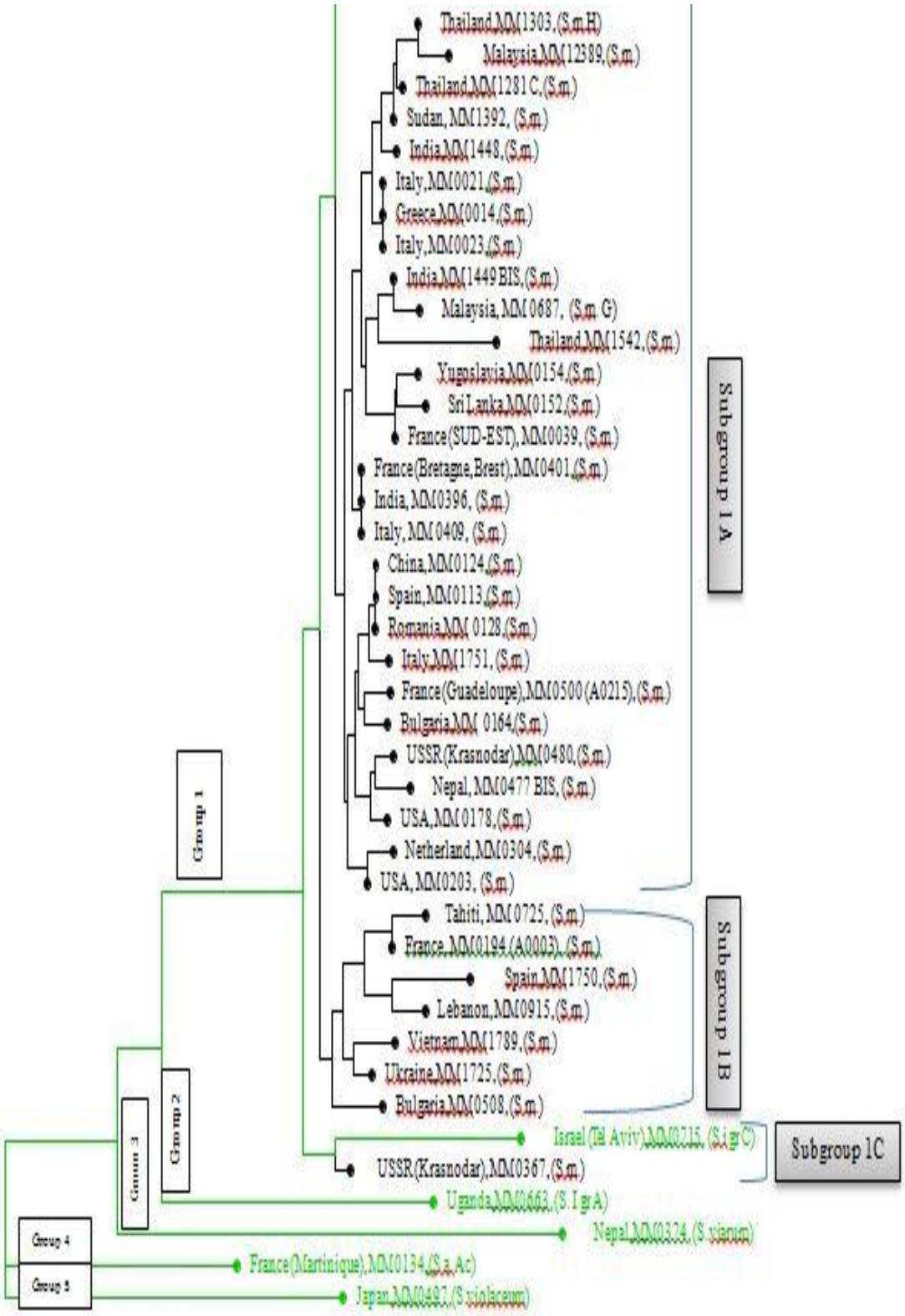

Table 3. 3. Accessions and their groups taken from dendrogram showing the genetic similarities of 73 *S. melongena* and six outgroup genotypes

No. represent accessions	Accessions and species	Geographical origin	Group and subgroup
1	MM 0006,(S.m.)	India	1A
2	MM 0014,(S.m.)	Greece	1A
3	MM 0021,(S.m.)	Italy	1A
4	MM 0023,(S.m.)	Italy	1A
5	MM 0039, (S.m.)	France (SUD-EST)	1A
6	MM 0054,(S.m.)	France (SUD-EST)	1A
7	MM 0055 (A0012),(S.m.)	France (SUD-EST)	1A
8	MM 0058,(S.m.)	France (SUD-EST)	1A
9	MM 0061,(S.m.)	Spain	1A
10	MM 0064, (S.m.)	France	1A
11	MM 0069, (S.m.)	USA	1A
12	MM 0091, (S.m.)	USA	1A
13	MM 0101, (S.m.)	Japan	1A
14	MM 0102,(S.m.)	Japan	1A
15	MM 0106,(S.m.)	Spain (Valencia)	1A
16	MM 0113,(S.m.)	Spain	1A
17	MM 0124,(S.m.)	China	1A
18	MM 0125, (S.m.)	USSR	1A
19	MM 0128,(S.m.)	Romania	1A
20	MM 0152,(S.m.)	Sri Lanka	1A
21	MM 0154,(S.m.)	Yugoslavia	1A
22	MM 0164,(S.m.)	Bulgaria	1A
23	MM 0165 (A0067), (S.m.)	Philippines	1A
24	MM 0166 (A0119), (S.m.)	Philippines	1A
25	MM 0178, (S.m.)	USA	1A
27	MM 0198 (A0321), (S.m.)	Netherland	1A
28	MM 0203, (S.m.)	USA	1A
29	MM 0304, (S.m.)	Netherland	1A
30	MM 0357, (S.m.)	China	1A
31	MM 0360, (S.m.)	China	1A
32	MM 0364, (S.m.)	USSR (Moldavia)	1A
34	MM 0377, (S.m.)	India (Kerala)	1A
35	MM 0380 E, (S.m.)	India (Varanasi)	1A

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Table 3.3. (Cont.)

36	MM 0396, (S.m.)	India	1A
37	MM 0400, (S.m.)	Thailand	1A
38	MM 0401,(S.m.)	France (Bretagne, Brest)	1A
39	MM 0409, (S.m.)	Italy	1A
40	MM 0412 (A0209), (S.m.)	Japan	1A
41	MM 0459 (A0060), (S.m.)	India	1A
42	MM 0477 BIS, (S.m.)	Nepal	1A
43	MM 0480, (S.m.)	USSR (Krasnodar)	1A
44	MM 0500 (A0215), (S.m.)	France (Guadeloupe)	1A
46	MM 0563, (S.m.)	Italy	1A
47	MM 0653, (S.m.)	India (Karnataka)	1A
48	MM 0656 (A0129), (S.m.)	Taiwan	1A
49	MM 0687, (S.m. G)	Malaysia	1A
52	MM 12389, (S.m.)	Malaysia	1A
53	MM 1281 C, (S.m.)	Thailand	1A
54	MM 1303, (S.m.H)	Thailand	1A
55	MM 1392, (S.m.)	Sudan	1A
56	MM 1445, (S.m.)	India	1A
57	MM 1448, (S.m.)	India	1A
58	MM 1449 BIS, (S.m.)	India	1A
59	MM 1487, (S.m.)	China (Hangzhou)	1A
60	MM 1498 bis, (S.m.)	India	1A
61	MM 1536, (S.m.)	Malaysia	1A
62	MM 1542, (S.m.)	Thailand	1A
63	MM 1551, (S.m.)	Malaysia	1A
64	MM 1564, (S.m.)	Indonesia	1A
65	MM 1597, (S.m.)	India	1A
66	MM 1677, (S.m.G)	Thailand	1A
67	MM 1713, (S.m.)	USA	1A
68	MM 1724, (S.m.)	Indonesia (Java)	1A
71	MM 1751, (S.m.)	Italy	1A
72	MM 1788, (S.m.)	Italy	1A
79	MM 0195, (S.l.)	Tunisia	1A
26	MM 0194 (A0003), (S.m.)	France	1B
45	MM 0508, (S.m.)	Bulgaria	1B
50	MM 0725, (S.m.)	Tahiti	1B

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Table 3.3. (Cont.)

51	MM 0915, (S.m.)	Lebanon	1B
69	MM 1725, (S.m.)	Ukraine	1B
70	MM 1750, (S.m.)	Spain	1B
73	MM 1789, (S.m.)	Vietnam	1B
33	MM 0367, (S.m.)	USSR (Krasnodar)	1C
77	MM0715, (S.i. C)	Israel (Tel Aviv)	1C
76	MM0663, (S. incanum)	Uganda	2
74	MM0374, (S. viarum)	Nepal	3
78	MM 0134,(S.a. Ac)	France (Martinique)	4
75	MM0497,(S. violaceum)	Japan	5

Note: (S.m.) represent *S. melongena*, (S.m.G) represent *S. melongena* group G, (S.m.H) represent *S. melongena* group H, (S.l.) represent *S. linnaeanum*, (S.a. Ac) represent *S. aethiopicum aculeatum* group, (S.i. C) represent *S. incanum* group c, (S.i. A) represent *S. incanum* group A

Principle Coordinate Analysis (PCoA) was done with outgroups to show the distance of the outgroups in comparison to other accessions. As shown in figure 3, the accessions represented by number 74, 75, 76, 77 and 78 are *S. viarum S. violaceum*, *S. incanum group A*, *S. incanum group C* and *S. aethiopicum Aculeatum group,* respectively, and were found to be most distantly related to other accessions. The first three Eigen vectors accounted for 27.8%, 14.81% and 12.39% of variation. The results showed consistency with the dissimilarity matrix and dendrogram (fig 3.1.). Highest distance was seen in between accessions 74, 75, 76 and 78 as showed in fig 3.2, which also clearly showed in the dendrogram.

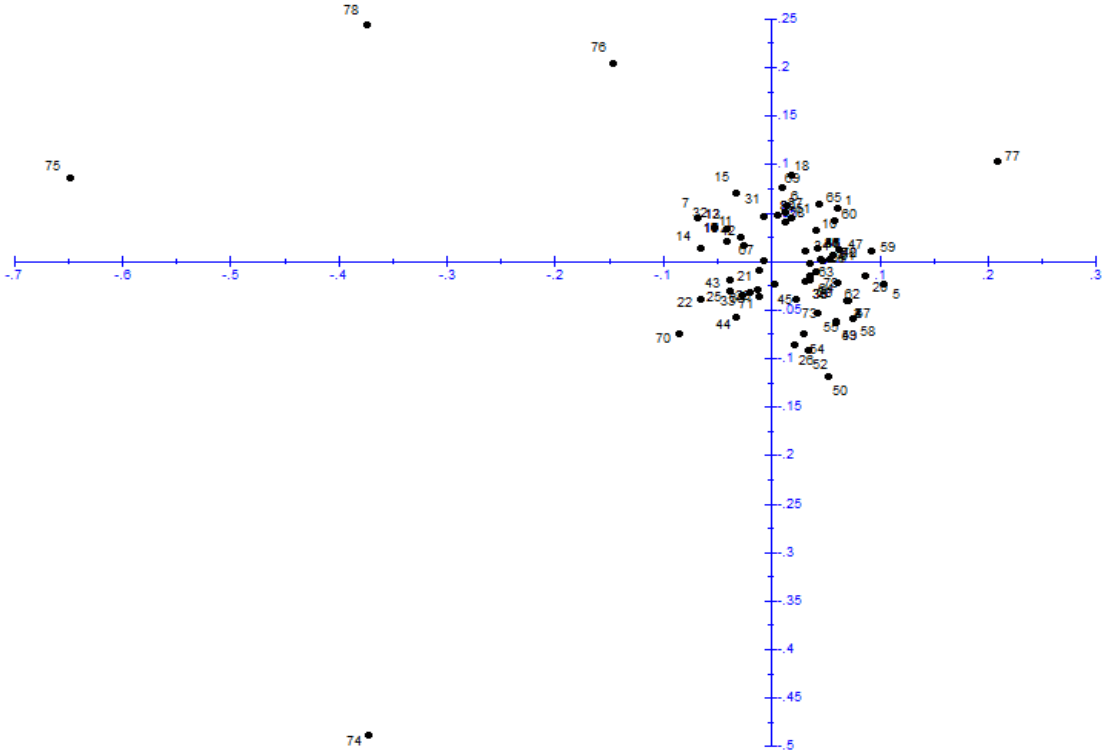

Figure 3. 2. Factorial analysis (Principle Coordinate Analysis) of all accessions, accessions are labeled according to table 2.1

Factorial analysis was also done without outgroups. This analysis showed two main clusters (fig 4). The PCoA also isolated subgroup 1B found in the dendrogram (fig 3.1), which dispersed out of the two circles.. Eigen vector values varied when the outgroups were removed. The first three eigen vectors represented 20.0%, 11.9% and 11.1% of variance, respectively. The result indicated that the contribution of outgroup loci on the dissimilarities of a given axes is significant. The red dotted and green dotted circle in figure 4 matched with the tree seen in the dendrogram of subgroup 1A. Subgroup 1B accessions of the dendrogram were dispersed in the PCoA, supportively had an average similarity of 0.89 which is lower than the average similarity registered under subgroup 1A. The tree constructed showed similarity with the principal coordinate analysis. Accessions found in the red, green and outside the two circles consist of accessions from different continents and diverse geographical locations. However, most of the accessions found in the green circle are from Asia.

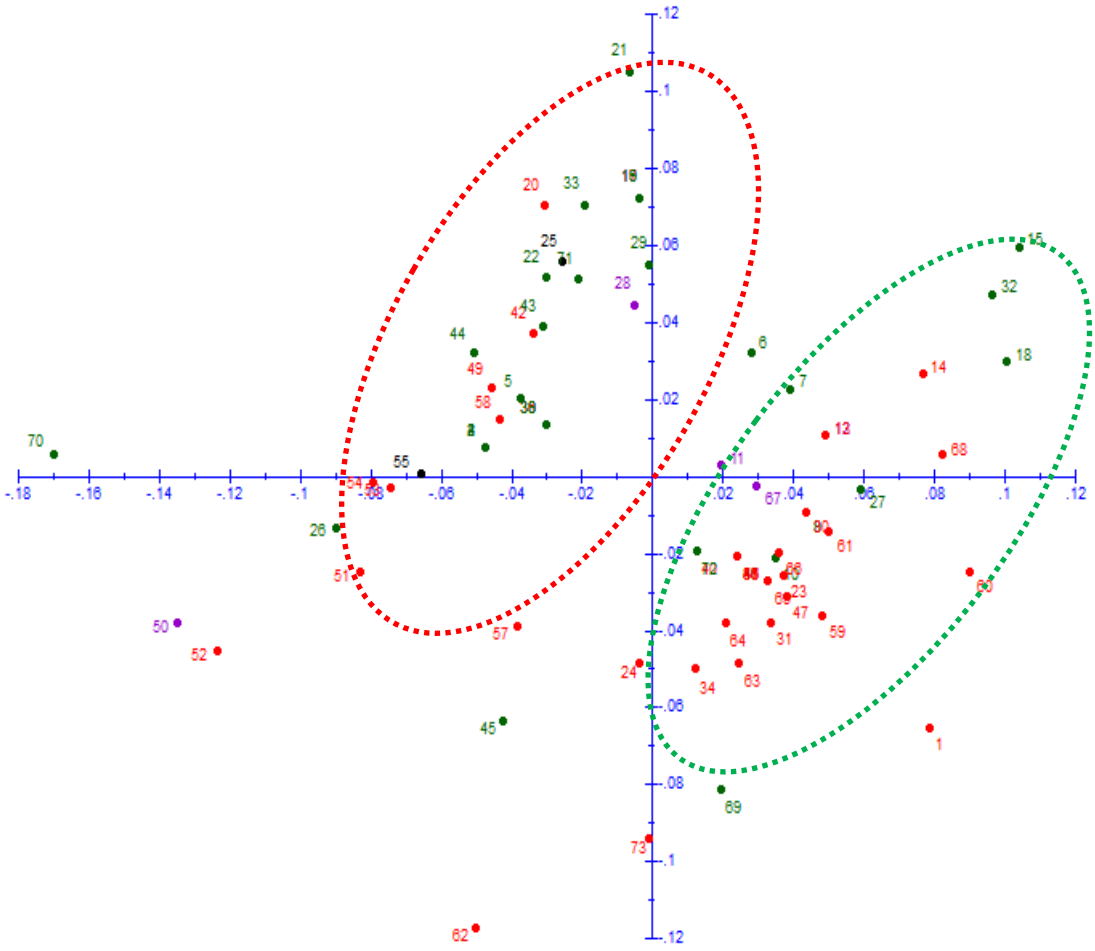

Figure 3. 3. Factorial analysis (Principal Coordinate Analysis) without outgroups, accessions are labeled according to table 2.1.

3.1.3. Population Structure Analysis

Population structure of the 79 accessions from four continents was also determined using the SRAP data. According to STRUCTURE software and STRUCTURE HARVESTER analysis, the accessions grouped into three clusters (Table 3.4). Thus, the delta K value reached its maximum value when the K was 3 (fig 5). Each accession was assigned to subpopulation group A, B, or C when its level of membership was higher than 70%. The first cluster consisted of five accessions, where all the outgroups were found except *S. linnaeanum*.

The first cluster did not contain any *S. melongena* accessions. The second (B) and third clusters (C) comprises 31 and 36 accessions, respectively. The remaining seven accessions were not assigned to any of the groups and were classified as admixed. Population subgroup A, B and C consist 6.3%, 39.2% and 45.6% of the total

germplasm, respectively. The remaining 8.9% was admixed germplasm. The fixation index was 0.0065 for subgroup A, 0.7233 for subgroup B and 0.8346 for subgroup C, which indicated the total genetic variance in the populations. Population subgroup A consisted of accessions with high genetic variance; similarly, the dendrogram assigned these accessions in different groups (table 3.3).

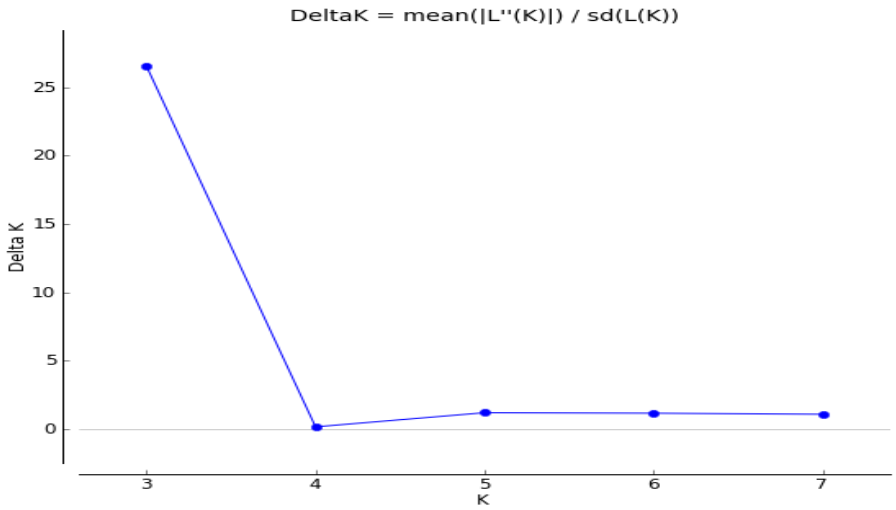

Figure 3. 4. Plot for detecting the number of Delta K with different values of K.

Compared to the dendrogram, the population structure analysis split out the outgroups that were found in the group 2, 3, 4, and 5 of the dendrogram; 83.9% of cluster B comprised accessions from subgroup 1A, four accessions from subgroup 1B and one accession from subgroup 1C. Accessions assigned in cluster C were from subgroup 1A except Ukraine accession (MM1725) from subgroup 1B. Five and two accessions assigned in the admixture were from subgroup 1A and subgroup 1B of the dendrogram, respectively. All three clusters and the admixed accessions were from different continents. Thus, thee accessions found in each cluster were from different geographical origins.

Table 3. 4. Accession assigned in the three population subgroups and admixture as determined by STRUCTURE

No. represent accessions	Geographic origin	Accessions	Population sub group
74	Nepal	MM0374, (S. viarum)	A
75	Japan	MM0497,(S. violaceum)	A
76	Uganda	MM0663, (S. incanum)	A
77	Israel (Tel Aviv)	MM0715, (S.i. C)	A
78	France (Martinique)	MM 0134,(S.a. Ac)	A
2	Greece	MM 0014,(S.m.)	B
3	Italy	MM 0021,(S.m.)	B
4	Italy	MM 0023,(S.m.)	B
5	France (SUD-EST)	MM 0039, (S.m.)	B
16	Spain	MM 0113,(S.m.)	B
17	China	MM 0124,(S.m.)	B
19	Romania	MM 0128,(S.m.)	B
20	Sri Lanka	MM 0152,(S.m.)	B
21	Yugoslavia	MM 0154,(S.m.)	B
22	Bulgaria	MM 0164,(S.m.)	B
25	USA	MM 0178, (S.m.)	B
26	France	MM 0194 (A0003), (S.m.)	B
28	USA	MM 0203, (S.m.)	B
29	Netherland	MM 0304, (S.m.)	B
33	USSR (Krasnodar)	MM 0367, (S.m.)	B
36	India	MM 0396, (S.m.)	B
38	France (Bretagne, Brest)	MM 0401,(S.m.)	B
39	Italy	MM 0409, (S.m.)	B
42	Nepal	MM 0477 BIS, (S.m.)	B
43	USSR (Krasnodar)	MM 0480, (S.m.)	B
44	France (Guadeloupe)	MM 0500 (A0215), (S.m.)	B
49	Malaysia	MM 0687, (S.m. G)	B
50	Tahiti	MM 0725, (S.m.)	B
51	Lebanon	MM 0915, (S.m.)	B
52	Malaysia	MM 12389, (S.m.)	B
53	Thailand	MM 1281 C, (S.m.)	B
54	Thailand	MM 1303, (S.m.H)	B
55	Sudan	MM 1392, (S.m.)	B
57	India	MM 1448, (S.m.)	B
58	India	MM 1449 BIS, (S.m.)	B
70	Spain	MM 1750, (S.m.)	B
1	India	MM 0006,(S.m.)	C
7	France (SUD-EST)	MM 0055 (A0012),(S.m.)	C
8	France (SUD-EST)	MM 0058,(S.m.)	C

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Table 3.4. (Cont.)

9	Spain	MM 0061,(S.m.)	C
10	France	MM 0064, (S.m.)	C
12	USA	MM 0091, (S.m.)	C
13	Japan	MM 0101, (S.m.)	C
14	Japan	MM 0102,(S.m.)	C
15	Spain (Valencia)	MM 0106,(S.m.)	C
18	USSR	MM 0125, (S.m.)	C
23	Philippines	MM 0165 (A0067), (S.m.)	C
24	Philippines	MM 0166 (A0119), (S.m.)	C
27	Netherland	MM 0198 (A0321), (S.m.)	C
30	China	MM 0357, (S.m.)	C
31	China	MM 0360, (S.m.)	C
32	USSR (Moldavia)	MM 0364, (S.m.)	C
34	India (Kerala)	MM 0377, (S.m.)	C
35	India (Varanasi)	MM 0380 E, (S.m.)	C
37	Thailand	MM 0400, (S.m.)	C
40	Japan	MM 0412 (A0209), (S.m.)	C
41	India	MM 0459 (A0060), (S.m.)	C
46	Italy	MM 0563, (S.m.)	C
47	India (Karnataka)	MM 0653, (S.m.)	C
48	Taiwan	MM 0656 (A0129), (S.m.)	C
56	India	MM 1445, (S.m.)	C
60	India	MM 1498 bis, (S.m.)	C
61	Malaysia	MM 1536, (S.m.)	C
63	Malaysia	MM 1551, (S.m.)	C
64	Indonesia	MM 1564, (S.m.)	C
65	India	MM 1597, (S.m.)	C
66	Thailand	MM 1677, (S.m.G)	C
67	USA	MM 1713, (S.m.)	C
68	Indonesia (Java)	MM 1724, (S.m.)	C
69	Ukraine	MM 1725, (S.m.)	C
72	Italy	MM 1788, (S.m.)	C
79	Tunisia	MM 0195, (S.l.)	C
6	France (SUD-EST)	MM 0054,(S.m.)	Admixed
45	Bulgaria	MM 0508, (S.m.)	Admixed
62	Thailand	MM 1542, (S.m.)	Admixed
73	Vietnam	MM 1789, (S.m.)	Admixed
11	USA	MM 0069, (S.m.)	Admixed
59	China (Hangzhou)	MM 1487, (S.m.)	Admixed
71	Italy	MM 1751, (S.m.)	Admixed

Population structure isolated the outgroups except *S. linnaeanum* and grouped in population subgroup A. The PCoA executed without outgoups (fig 4) and STRUCTURE cluster showed the difference of accessions found in group one of the dendrogram. Accordingly the result found in the structure clustering showed consistent results with PCoA. All the accessions found inside the red dotted line of PCoA assigned in subgroup B of the population analysis. Cluster C comprises most of the accession found inside green dotted line of PCoA and the admixed accession found in between the vicinity of the red and green dotted lines.

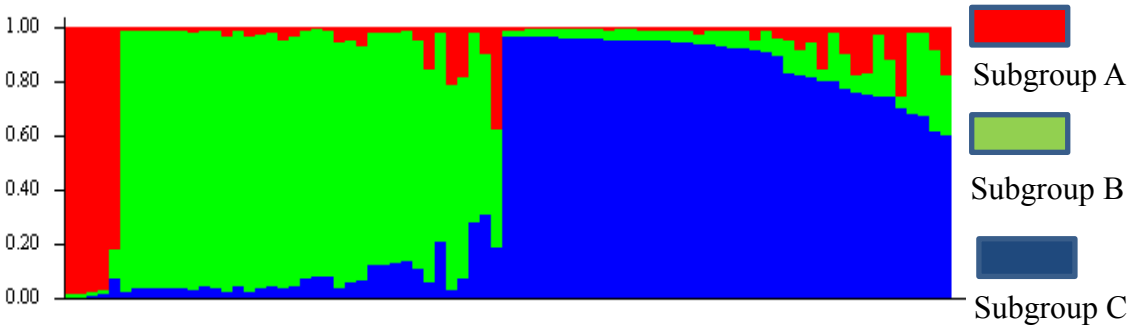

Figure 3. 5. Population structure bar graph using eggplant (S. melongena) germplasm.

3.2. Discussion

3.2.1. Polymorphism of SRAP Markers

This study investigated genetic diversity and population structure of a worldwide collection of eggplant using SRAP markers for the first time. SRAP primers are routinely used for genetic analysis of plants. They were first used in eggplant for studying genetic variation of Chinese accessions and showed high efficiency and applicability (H.Li et al., 2010). Expectedly, a higher percentage of polymorphism of SRAP markers were observed in outgroups. The average PIC of *S. melongena* accessions was 0.28 in 0 to 1 scale, which is approximately similar to the values reported by others. Nunome et al. (2009); Mu˜noz-Falcón et al. (2011) and H.Ge et al. (2013) reported an average PIC of 0.27, 0.248 and 0.285, respectively using genomic SSR markers. A PIC value greater than 0.5 indicates the loci of high polymorphism, PIC value in between 0.25 and 0.5 indicates loci of intermediate polymorphism and PIC value less than 0.25 indicate loci of low polymorphism (H.Ge et al., 2013). The result showed moderate PIC which a good result for markers targeting conserved sequence in

genome (Li and Quires, 2001) and limited number of primers used. Markers associated to non-coding regions are usually polymorphic than coding region (Lee et al., 2004; Martin et al., 2010).

3.2.2. Genetic Diversity and Relationships

The highest number of average alleles detected in the Li et al. (2010) study was 11.5 alleles per locus using both 55 SRAP and AFLP primers in 56 eggplant accessions collected from China. In this study the average alleles detected per primer combination were 4.7 using ten SRAP combinations, which is smaller than the result obtained from (H. Li et al. 2010) study. The variation observed in between these two SRAP studies on the average alleles detected was possibly due to the difference in number of primer combinations used and strength of the band we scored. Compared to the average alleles detected in other markers studies (Naegele R. et al., 2014; T.K. Behera et al., 2006; Nunome et al., 2003b and Karihaloo et al., 1995), SRAP marker showed high effectiveness and reproducibility. T.K. Behera et al., 2006 and Nunome et al., 2003b used 23 STMS primers and produced an average alleles per primer of 4.4 and 3.1, respectively. In addition, Karihaloo et al., 1995 detected an average allele of 3.6 per locus from 22 RAPD primers and Naegele R. et al., 2014 detected an average allele of 3.8 alleles per locus from 22 SSRs. Indeed, the result showed consistency with Burdak et al, (2004a; 2004b; 2005) report that SRAP detect number of alleles and abundant than ISSR and SSR markers. Cericola F. et al. (2013) reported average alleles of 5.8 per locus higher than the average alleles detected by Naegele R. et al.(2014) , which supported our conclusion that the average alleles variation detected using similar markers might be because of number and similarity of germplasm and number of primers used.

Pair-wise similarity index measures within *S. melongena* ranged from 0.697 to 1 and an average similarity of 0.89, which varied from the study did on *S. melongena* accessions collected from Asia with an average similarity coefficient of 0.78 (H. Li et al., 2010). Normally, the genotypes collected from different geographical locations are expected to have higher variation than genotypes collected from one location, even though the similarity of genotype is not restricted to geographical origin (Skroch et al., 1998). *S. melongena* cultivated throughout the world, while Asia (India, China and Southeast Asia) consists of the greatest diversity followed by Middle East (Knapp S. et

al., 2013). In our work, Asian *S. melongena* accessions showed a minimum and maximum similarity of 0.75 and 1, respectively, which showed almost similar diversity with *S. melongena* accessions assigned in Europe. In our study we had 36 S. melongena accessions from Asia and the diversity was moderate. In consistence with our SRAP study considerable level of genetic diversity in chines eggplant collections reported by H.Li et al. (2010). Karihaloo et al. (1995), and Weese and Bohs (2010) also reported low polymorphism in between *S. melongena* accessions in Asia using RAPD and ITS markers, respectively. Whereas, other studies such as (Behera et al., 2010) and (Nunome et al., 2003b) discovered the wide diversity of *S. melongena* in Asia using STMS and SSR markers, respectively. Khan (1979) and Duanay et al. (2001) reported that the high diversity registered in Asia might be an indication of primary center of diversification of *S. melongena* was in Southeast Asia. These different results reported by different scientists might be because of sampling bias. Furthermore, wide range of variation registered in between *S. melongena* accessions might be because of broad genetic background and germplasm exchange. In some areas narrow range of variation registered in between *S. melongena* accessions might be because of farmer's selection for specific traits.

Based on the constructed tree and PCoA, germplasm from the same location did not cluster together. Accessions from Asia, Europe, Africa and North America were distributed in every sub group and accession from Malaysia, China and India were found in all three main groups. This result supports the extensive gene flow among different geographic regions (Meyer et al, 2012). According to the dendrogram (fig 3.1fig) and PCoA (fig 3.2 and 3.3), genetic variation was not correlated with geographical distribution also approved by other studies (Meyer et al, (2012). Nevertheless, genetic variation in between different area of china also not correlated (H. Li. et al., 2010). Therefore, diverse geographical origins of different accessions cannot be considered as a parameter to describe actual genetic diversity.

All of the outgroups except *S. linnaeanum* were found to be distantly related to *S. melongena* accessions and they were grouped together with most of the accession tested. *S. linnaeanum* and *S. violaceum* were from the outgroups that showed the highest and lowest similarity with *S. melongena. S. incanum* had an average similarity of 0.627 with *S. melongena*. A strong relationship between the wild species *S. incanum* and the cultivated *S. melongena* was reported in different studies using morphology, nuclear marker, chloroplast and allozyme markers (T.K Behera 2006, Sakata and Lester, 1997;

Isshiki 1994, Sakata et al 1991, Thang 1988, and Pearce and Lester 1979). In a recent study using morphological and molecular approaches, Meyer et al, (2012) reported *S. incanum* as the strong sister to clad of *S. melongena* that supports this study and previous reports. In addition, our and Meyer et al(2012) result supported the strong similarity of *S. linnaeanum* and *S. melongena* reported by Furini A. and Wunder J. (2004). *S. linnaeanum* had an average similarity of 0.913 with *S. melongena*, which is the highest similarity registered with outgroups. In contrary, Doganlar et al. (2002) showed over 81% of polymorphism in between *S. linnaeanum* and *S. melongena,* which indicated the minimum similarity of the species. Moreover, Y. Tumbilen (master thesis, 2009) reported *S. linnaeanum* as a closest species to *S. melongena* accessions from the outgroups used. *S. linnaeanum* (Letser and Hasan 1991) and *S. incanum* are described as possible progenitors of the modern eggplant. *S. linnaeanum* and *S. incanum* have 63% similarity that might support the evolutionary relation of the two species.

S. violaceum, *S. viarum*, *S. aethiopicum*, and *S. incanum* distantly related to other accessions. *S. incanum* collected from Uganda was found to be distantly related to the one from Israel. In other studies *S. incanum* accessions from Asia had close genetic distance with S. *melongena*, whereas *S. incanum* accessions from Israel and Mediterranean basin were comparatively distant to *S. melongena* (Karahaloo, 2009: Meyer et al, 2012 and Vilanove et al., 2011). Next to *S. violaceum*, S. *viarum* was distantly related to other accessions, which supported Meyer et al, (2012) report that *S. viarum* is one of the most distant taxa from *S. melongena.* The degree of relationship of these species could depend on crossability. All the outgroups included in the study have crossability with *S. melongena* (Daunay et al., 1991). Similarly, *S. melongena* hybridization with *S. incanum* (Lester & Hasan, 1991), *S. violaceum* (Rao & Kumar, 1980), and *S. aethiopicum* (Omidiji, 1986), were described. The crossability of these species might be one of the reasons for close relationships. Furthermore, hybridization has likely occurred frequently among domesticates and progenitors, but these latter hybrids may be morphologically indistinguishable from true wild forms (Meyer et al, 2012). The degree of relation of the *S. incanum* accessions from different regions with the cultivated *S. melongena* support the history of eggplant in that it is known that Arab traders brought Indian eggplants into western Asia, Europe and Africa by the 14th century (Lewicki and Johnson, 1974). On the contrary, Lester and Hasan (1919), Sakata and Lester (1994) and Daunay et al. (2001) explained that origin of *S. incanum* was in Africa, and that it migrated to Southeast Asia and subsequently evolved into *S.*

melongena group F and then group G. The accessions included in our study not comprised of different species and groups to show the evolutionary and domestication relationships of eggplant.

3.2.3. Population Relationships

All the accessions found in population subgroup A, B and C were distributed to all continents and indicates the possible movement of germplasm. Admixed germplasm covered 8.9%, which indicated the migration or interbreeding between genetic clusters. The fixation indices (Fst) value ranged from 0 to 1, a value of zero means that we sampled within a panmictic unit and at the other extreme, a value of one means that there is no diversity within subpopulations (F. Balloux and N. Lugon-Moulin, 2002). Hence, high fixation indices were registered in population subgroups B and C which were 0.7233 and 0.8346, respectively. These values indicated a minimum difference in the population subgroups. Whereas, population subgroup A had an Fst value of 0.0065 that showed high difference in the population subgroup and importance of more structuration. The registered genetic differentiation among population was 0.1005, 0.1257, and 0.0484 in between A and B, A and C, and B and C, respectively. Population sub group A was distant to other subgroups, similar to the result of dendrogram and PCoA. A previous work by (Wright, 1978; Hartl & Clark 1997 and F. Balloux and N. Lugon-Moulin, 2002) stated that the genetic differentiation among populations would be minimum when the value of Fst was between 0.05 and 0.15, moderate when the value of Fst was between 0.15–0.25 and high when Fst was greater than 0.25. In this study, the value of Fst was in between 0.05 and 0.25 in between A and B; and A and C, which indicates that the structure between the population subgroups was moderate. Minimum genetic differences also registered in between subgroup B and C.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

Genetic diversity and population structure of eggplant accessions collected from five continents were evaluated. SRAP markers showed a consistent result with diversity studies using other markers that showed the effectiveness and reproducibility of the marker. Moreover, SRAP can produce reliable result with limited facility. Eggplant contains wide variants and diversity has been studied at the morphological, biochemical and molecular levels. Inconsistent results have been reported in different studies comparing morphological and molecular characterization (Hurtadao et al., 2012; Prohens et al., 2005). Using the advantage of the SRAP marker system that targets exons in the ORF, studying a wide collection of eggplant across the world with abundant SRAP primers and morphology may help to resolve the complexity of characterization. The morphology and molecular result may be significantly correlated and there will not a need of collecting complementary data, which minimize the cost and time. Our result gave consistent result on species similarity with other molecular studies using limited facilities and minimum costs. Selected works indicate that SRAP markers have the potential to enhance diversity study by providing an easy-to-use, highly variable marker with inherent biological significance (Robarts & Wolfe, 2014). SRAP products are invaluable for discovery of polymorphism and diversity study. Understanding the genetic diversity of those eggplant collections using markers are more precise than morphological characterization. Morphological characterization is not absolute because of genetic and environmental interaction (Prohens et al., 2004).

The result found in our study supported the close relation of *S. incanum* and *S. linnaeanum* to the cultivated *S. melongena* than the cultivated *S. aethiopicum.* Other studies also supported our result, which gave insight for revising the previous taxonomic classification of eggplant. The result obtained from the dendrogram, principal coordinate analysis and population structure approximately similar. High similarity registered in different geographical region was an indication for movement of germplasm. The diversity registered in Asia and Europe nearly similar, but it was impossible to justify the overall eggplant diversity of the continents using underrepresented sample.

Most of the research including the recent study on taxonomy of eggplant inferred the data published 23 years ago by Lester & Hasan (1991). The study covered a limited number of accessions and did not include wild Asian prickly accessions (Jin-Xiu-Wang et al., 2008), whereas many scientists reported Asia as the possible origin and domestication center of eggplant. To solve this dependency on limited information Knapp S. et al. (2012) and R.S. Meyer et al. (2012) recently assessed eggplants using morphological and molecular similarities. These new studies improved the species level names of eggplant relatives that were under informal classification and identified similar species named differently. However, these works was executed with a number of challenges like the lack of exact provenance and confusion of naming accession from different regions. According to Andow (2010), many of the species names have complex synonymies and some have been known in several different names, which is also supported by the report of R.S. Meyer et al. (2012). Different regions have different accession names and it is very difficult to identify. Some of the accessions used in this study are known by other accession numbers in other studies. In our dissimilarity pairwise matrix we found accessions with 100% similarity that might be the same germplasm collected from different geographic region with different names. Alternatively, it could be the result of using a limited number of primers. Ideally, further molecular marker analysis using worldwide accessions is recommendable. Therefore, collecting worldwide accessions from different eggplant germplasm centers may clarify the ongoing confusions of eggplant classification.

The accumulation of genomic information about eggplant will not only facilitate genetics research and molecular breeding of eggplant itself, but will also make this species a valuable and unique member of the Solanaceae for comparative biological studies of genetics, physiology, development, and evolution. Eggplant breeding should include worldwide germplasm to maximize diversity, improve adaptation and increase productivity of the plant. Because, the gene of important agronomic characters of eggplant were orthologs in tomato, potato and pepper. So, germplasm management is fundamental for providing well characterized material for crop improvement.

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