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# Morphological and Molecular Characterization and Relationships of Turkish Local Eggplant Heirlooms

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**Abstract**

A total of 38 eggplant genotypes, of which 32 were heirloom accessions collected from different regions of Burdur province five were different local genotypes from other provinces, and one was a cultivar, were used as reference in this study. The phylogenetic relationships among these heirlooms were evaluated using 40 morphologic descriptors and five randomly amplified polymorphic RAPD markers. The horizontal dendrograms were created by using UPGMA with both morphologic and molecular data. Burdur heirloom accessions showed high genetic diversity based on morphological and molecular data. The genetic similarity rates ranged from 0.29 to 0.91 according to the morphological data, and ranged from 0.84 to 0.98 according to the molecular data. Molecular data generated by RAPD method, compared to morphological data, were insufficient to reveal genetic diversity. Therefore, in order to confirm genetic variations, studies based on other molecular methods are necessary. The regional genetic populations include a wide eggplant genetic diversity which can be good source for the breeding studies performed in the future.

***Keywords:*** accession, breeding, diversity, genetic variation, phylogenetic relationship, RAPD, UPGMA

## Introduction

Eggplant, which was called as the king of vegetables (Daunay and Janick, 2007), is an indigenous plant of India (Weese and Bohs, 2010). It has been well known since BC III and cultivated for 1,500 years in Asia (Kashyap *et al*., 2003). It is cultivated as a perennial in tropical areas, while it is cultivated as annual in subtropical areas (Kowalska, 2008). Eggplant is placed in *Solanum* genus and includes wide genotypic and phenotypic variation (Fukuoka *et al*., 2010). Eggplant is thought to have been developed from the wild ancestor, *Solanum insanum* has small, round, green, thick-skinned and bitter taste fruits (Barchi *et al*., 2010). Cultivation *S. insanum* of had been performed in China, India and Thailand (Daunay *et al*., 2001). Large fruiting eggplants were cultured in India in early time, and small fruiting was cultured at IV century in China and at IX century in Africa (Sekera *et al*., 2007). First cultivated eggplants were described as high tall plants, with big spines on the calyx, small, bitter fruiting and with high seed content in fruits (Swarup, 1995). Mutation, natural pollination and hybridization, together with selection gave rise to genetic diversity, as well as in decreasing of prickles and bitterness at fruit, changing of fruit shape, size and color (Frary *et al*., 2007). Genetic diversity accumulated and many different heirlooms emerged in countries where it was cultivated (Prohens *et al*., 2003). Entrance of the eggplant to Turkey was carried out by the silk-road. The genetic diversity accumulated in producing areas and by trade of eggplant throughout centuries in Anatolia (Janick, 2001). Eggplant cultivation was done in open field until the second half of the 1970s in Turkey and then cultivation under protected cultivation started. The eggplant cultivation in greenhouse was begun with local varieties. However, the growers preferred hybrid F1 varieties which have the cylindrical and dark purple or black colored fruiting and using of them has become commonly in a short period (Ekiz and Boyaci, 2001). Also, the increase of use F1 hybrids in the open field cultivation was observed in recent years. Steadily decrease was seen in the cultivation of local varieties compared to the hybrids (Cericola *et al*., 2013). The genetic diversity is low among the genotypes which have dark purple-black fruits (Muñoz-Falcón *et al*., 2009). In recent years, one of the important problems faced by eggplant breeding programs, as well as in other species, is a narrowing of the genetic base. To create variations, time-consuming and expensive methods are needed, including mutation breeding, interspecific hybridization and biotechnological approaches. The genetic variation contained by heirlooms among is seen in the previous studies (Demir *et al*., 2010; Muñoz-Falcón *et al*., 2008, 2009; Prohens *et al*., 2003, 2008, 2011). Therefore, collection and characterization of genetic resources is required for the improvement of new varieties. In this respect,

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molecular characterization is more reliable then morphological characterization (Li *et al.*, 2010).

Here we investigate the genetic relationships between Turkish heirlooms using morphological and molecular data is presented here.

## Materials and methods

Morphological and molecular studies were conducted at Bati Akdeniz Agricultural Research Institute, Antalya, Turkey in 2010-2013.

In total 38 materials were used in the study of these. 32 were local heirlooms collected from Burdur province during survey studies. Materials origin, providing method and place/company of origin are given in Table 1. Long purple commercial variety (YRL 68) and heirlooms originating in other province (YRL 1, YRL 3, YRL 6, YRL 57, and YRL 79) were used as reference cultivars for classification of Burdur province heirlooms.

Five RAPD primers OPH-02, OPL-04, OPB-07, OPO-10, OPL-16 (10 mer) reported as highly polymorphic in previously studies (*Demir et al.*, 2010; Nunome *et al.*, 2001) were selected to detect polymorphisms and identify genetic relationship of the heirlooms.

### *Survey*

The survey studies were performed at nine different locations in Burdur province in September both in 2010 and 2011. In total 32 materials were collected from different farms. The locations of collected eggplants in Burdur province were represented on the map (Fig. 1) and their geographic coordinates were defined (Table 2).

### *Morphological observations*

For each accession, the seeds were sown in seedling treys containing peat moss. Seedlings in 4-5 leaves stages were planted in glasshouse. Twenty plants were planted for each genotype. Morphological observations were performed according to 40 descriptors chosen among the International Board for Plant Genetic Resources Institute (IBPGRI), International Union for the Protection of New Varieties of


Fig. 1. Geographic map of district and village in Burdur province of Turkey including collection places

Table 1. Origin, providing method and place/company of origin of the materials used in the study

| No | Name of the materials | Origin | Providing method | Place/company of origin |
|---|---|---|---|---|
| 1 | YRL 1 | Antalya-Kumluca District | Agricultural district offices | Grower |
| 2 | YRL 3 | Antalya-Kumluca District | Agricultural district offices | Grower |
| 3 | YRL 6 | Antalya-Kumluca District | Agricultural district offices | Grower |
| 4 | YRL 7 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 5 | YRL 8 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 6 | YRL 9 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 7 | YRL 12 | Burdur/Askeriye Village | Survey in the field | Grower |
| 8 | YRL 14 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 9 | YRL 15 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 10 | YRL 19 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 11 | YRL 20 | Burdur-Aglasun /Cine Village | Survey in the field | Grower |
| 12 | YRL 24 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 13 | YRL 26 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 14 | YRL 27 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 15 | YRL 28 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 16 | YRL 30 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 17 | YRL 34 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 18 | YRL 35 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 19 | YRL 36 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 20 | YRL 43 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 21 | YRL 44 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 22 | YRL 45 | Burdur-Celtikci/Tekke Village | Survey in the field | Grower |
| 23 | YRL 46 | Burdur-Askeriye Village | Survey in the field | Grower |
| 24 | YRL 47 | Burdur-Askeriye Village | Survey in the field | Grower |
| 25 | YRL 49 | Burdur-Askeriye Village | Survey in the field | Grower |
| 26 | YRL 50 | Burdur-Askeriye Village | Survey in the field | Grower |
| 27 | YRL 51 | Burdur-Askeriye Village | Survey in the field | Grower |
| 28 | YRL 52 | Burdur-Askeriye Village | Survey in the field | Grower |
| 29 | YRL 57 | Mugla-Fethiye/Günesli Village | Survey in the field | Grower |
| 30 | YRL 58 | Burdur-Karamanlı/Manca Village | Survey in the field | Grower |
| 31 | YRL 59 | Burdur-Karamanlı/Manca Village | Survey in the field | Grower |
| 32 | YRL 61 | Burdur-Gölhisar/Sorkum Village | Survey in the field | Grower |
| 33 | YRL 62 | Burdur-Yesilova/Kayadibi Village | Survey in the field | Grower |
| 34 | YRL 64 | Burdur-Tefenni District | Survey in the field | Grower |
| 35 | YRL 65 | Burdur-Tefenni District | Survey in the field | Grower |
| 36 | YRL 68 | Long purple | Purchased | Company |
| 37 | YRL 75 | Burdur City Centrum | Agricultural district offices | Grower |
| 38 | YRL 79 | Antalya-Akseki/Uzumdere Village | Survey in the field | Grower |

Table 2. Geographical coordinates of local heirloom eggplant collected in Burdur province of Turkey

| Location | Geographical coordinates |
|---|---|
| Aglasun /Cine Village | 37°35'0.07"N/30°38'1.34"E |
| Celtikci Tekke Village | 37°34'36.86"N/30°26'40.11"E |
| Burdur/Askeriye Village | 37°45'41.88"N/30°21'15.99"E |
| Karamanlı-Manca Village | 37°18'18.74"N/29°53'19.89"E |
| Karamanlı-Centrum | 37°22'2.35"N/29°49'19.71"E |
| Gölhisar-Sorkum Village | 37° 9'54.49"N/29°34'39.38"E |
| Yesilova-Kayadibi Village | 37°31'19.56"N/29°44'35.03"E |
| Tefenni-Centrum | 37°18'27.39"N/29°46'40.57"E |

Plants (UPOV) plant feature criteria and some of the criteria for the breeders. Descriptors include plant, leaf, flower and fruit traits observations and measurements (Table 3). Skin color of the each eggplant specimens was measured with a portable tristimulus reflectance colorimeter Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan), and the parameters were expressed in CIE L*a*b* system, where L* is lightness (brightness-darkness) ranged from 0 to 100 units, a* is light intensity in red (+) or green (–) spectrum, b* is intensity in yellow (+) or blue (–) spectrum. Chroma ($C$: $\sqrt{(a^2+b^2)}$) measures color saturation or intensity and the hue angle ($h° = \tan^{-1}(b^*/a^*)$) determines the red, yellow, green, blue, purple, or intermediate colors between adjacent pairs of these basic colors. The L*, a*, and b*, *C* and *h* ° values obtained from six samples of each eggplant accession. Every record represents the average of three readings which were taken from the equatorial region, spaced equidistantly.

### *DNA extractions and PCR analysis*

DNA extractions from young leaves were performed according to a modified Doyle and Doyle (1990) method by using CTAB protocol (Mutlu *et al*., 2008). RAPD analysis was performed according to Demir *et al*. (2010). The amplification reactions were released containing 20 ng DNA, 0.5 unite *Taq* polymerase, 2.5 μL 10X buffer, 3.5 μL 25 mM $MgCl_2$, 2 μL 2.5 mM dNTPs, 2 μL RAPD primer for RAPD-PCR. DNA was amplified in a thermal cycler. It was programmed for an initial 5 min denaturation step at 94 °C, followed by 35 cycles of a 30 s denaturation step at 94 °C, 1 min annealing at 35 °C, 45 s extension step at 72 °C, followed by a final 8 min extension step at 72 °C.

### *Statistical analysis*

Genetic similarity was analyzed by the UPGMA (Unweighted pair-group method, arithmetic average) clustering procedure using the software NTSYS (Numerical Taxonomy Multivariate

Table 3. Descriptors used for characterization and evaluation of eggplant accessions used in the study

| Traits | Description |
|---|---|
| Plant habit | Score range (1=Open, 3=Bushy, 5=Semi open) |
| Plant height | Score range (1=Long, 3=Intermediate, 5=Short) |
| Stem thickness | Score range (1=Thick, 3=Intermediate, 5=Thin) |
| Stem hairiness | Score range (1=Dense, 3=Intermediate, 5=Tenuous) |
| Stem color | Score range (1=Grayish, 3=Green, 5=Green-purple, 7=Grayish-green-purple, 9=Grayish-green, 11= Grayish-purple, 13=Purple) |
| Shoot tip color | Score range (1=Grayish, 3=Green, 5=Green-purple, 7=Grayish-green-purple, 9=Grayish-green, 11=Grayish-purple, 13=Purple) |
| Length of internodes | Score range (1=Long, 3=Intermediate, 5=Short) |
| Leaf color | Score range (1=Light green, 3=Green, 5= Dark green) |
| Leaf size | Score range (1=Large, 3=Intermediate, 5=Small) |
| Leaf hairiness | Score range (1=Dense, 3=Intermediate, 5=Tenuous) |
| Presence of spine on petiole | Score range (1=Many, 3=Intermediate, 5=Few, 7=Absent) |
| Bud size | Score range (1=Large, 3=Intermediate, 5=Small) |
| Bud hairiness | Score range (1=Dense, 3=Intermediate, 5=Tenuous, 7=Absent) |
| Presence of spine on bud | Score range (1=Many, 3=Intermediate, 5=Few, 7=Absent) |
| Flower color | Score range (1=Light purple, 3=Purple, 5= Dark purple) |
| Flower size | Score range (1=Large, 3=Intermediate, 5=Small) |
| Calyx size | Score range (1=Large, 3=Intermediate, 5=Small) |
| Fruit shape | Score range (1=Long, 3=Intermediate, 5=Short, 7=Ovoid, 9=Pear shaped) |
| Dominated fruit color | Score range (1=White, 3= Green, 5=Purple, 7=Black) |
| Range of dominated fruit color | Score range (1=Regular, 3=Mottled, 5=Stripe, 7=Mealy) |
| Fruit stalk length | Score range (1=Long, 3=Intermediate, 5=Short) |
| Presence of spine on fruit stalk | Score range (1=Few, 3=Intermediate, 5=Many, 7=Absent) |
| Fruit calyx prickles | Score range (1=Few (1-5 pricks), 3=Mid (6-20 pricks) , 5=Many (more than 20) |
| Fruit brightness | Score range (1=Bright, 3=Matt) |
| Fruit end shape | Score range (1=Flat, 3= Pointed, 5=Round) |
| Fruit curvature | Score range (1=Present, 3=Absent) |
| Fruit end button size | Score range (1=Large, 3= Intermediate, 5=Small) |
| Fruit length (cm) | The average measurement of ten fruits |
| Fruit diameter (cm) | The average measurement of ten fruits |
| Average fruit weight (g) | The average measurement of ten fruits |
| Presence of groove on fruit | Score range (1=Present, 3=Absent) |
| Fruit flesh firmness | Score range (1=Tightly, 3= Floppy, 5=Spongy) |
| Fruit flesh color | Score range (1=Greenish, 3=Greenish-cream, 5=White, 7=White- cream, 9=Greenish-white, 11=Cream) |
| Presence of hole in fruit | Score range (1=Present, 3=Absent) |
| Degree of fruit curvature | Score range (1=Slight, 3= Mid, 5=Much) |
| Soluble solids | The average measurement of five fruits juice samples |
| The length of fruit coated by calyx | Score range (1=Less than 20%, 3= between 20-70%, 5=More than 70%) |
| Tendency to parthenocarpy | Score range (1=Present, 3=Absent) |
| Presence of seed in fruit | Score range (1=Few, 3=Intermediate, 5=Many) |
| Seed maturity | Score range (1=Immature, 3=Mature) |

Analysis System) pc 2.2 version (Rohlf, 1998), using morphological and molecular data. For morphological data, each genotype was characterized with description number (Table 3). For molecular analysis data, each genotype was identified for each primer based on the presence (1) and absence (0) of bands. In order to show the variations STANDARDIZATION module was used. Correlation matrix adapted to SIMINT module was used to determine of correlation coefficient. The dendrograms were drawn using the clustering method UPGMA via the SHAN module. The cophenetic correlation coefficient was calculated with Mantel method to evaluate the efficiency of clustering.

## Results

A high morphological diversity was observed among eggplant genotypes characterized by quantitative and qualitative descriptors. Some of the phenotypic observation data related with important variable traits (calyx prickles, fruit size, weight, shape and curvature, skin color, groove etc.) are reported in Table 4. The fruits calyx of all genotypes had prickles. However, nearly 50% of them had lower numbers of prickles. Most of the genotypes had between 20-70% of their fruit length covered by the calyx. The degree of fruit curvature in 50% of the genotypes was slight. Nearly 30% percent of the genotypes had fruits with grooved appearance. Dominant fruit color was mostly purple and distribution of this color was 50% regular, 22% mealy, 18% mottled, and 2% striped according to the genotypes. Fruit flesh color was generally white and hole in the fruit was mostly absent. The lowest total soluble solid content (brix value) was 2.8. The highest brix value (6.1) was determined in YRL 15 which was collected from Cine village The average diameter of the fruits ranged from 32.67 to 73.22 mm. Fruit length and fruit weight average ranged between 10.9 and 23.3 cm, 0.100 and 0.235 kg, respectively.

Skin color characteristics of the eggplant heirlooms are shown in Table 5. Fruit color varied greatly among different genotypes. Regarding the skin Lightness (L*), higher values were obtained from genotypes which were mostly, cream, yellowish, orange-yellowish or goldenred in color (L* > 70). When L* values were ranged from 48 to 58 together with $h°$ values ranged from 80 to 112, it is observed that these genotypes' skin color were generally greenish-yellow, chartreuse or green in color. Regarding the b* values, very low values near or below zero mostly indicated that the skin color was cyan (if $h° > 285$); blue (if $h° > 240$); purple (if $h° > 285$); magenta (if $h° > 350$). It is observed from the research, that fruits which had lower $h°$ values ($1.05 < h° < 12.60$) were found to be red in color. Chroma values were varied from 3.82 to 24.26.

Totally 40 basic morphological descriptors were used for to determine the phylogenetic relationships among the Burdur local heirlooms. The Eigen value was 84. A 2-way Mantel test (Mantel, 1967) method was performed. Approximate Mantel t- statistic test were t = 10.0925, p = 1.0000. The matrix correlation (r) was 0.72. The similarity rates according to the coefficient similarity of genotypes ranged between 0.29 and 0.91.

Two major groups were revealed using the dendrogram generated by the UPGMA method using morphological data (Fig. 2). First group (Group A) was consisted of YRL 75, YRL 65, YRL 61 and YRL 59. These genotypes showed low genetic similarity with reference genotypes and cultivar (Long purple). Second group was divided into three subgroups. Group B consist of YRL 19 which showed high genetic similarity with reference cultivar YRL 68 (Long purple). The highest genetic similarity was observed in Group D between genotypes YRL 47 and YRL 51. The fruits of eggplant Burdur heirlooms belonging to the Group C are shown in Fig. 3.

Table 4. Some of the phenotypic descriptors related important variable traits of eggplant

| Genotype | Fruit calyx prickles | Fruit length covered by the calyx | Degree of fruit curvature | Fruit grooved | Dominated fruit color | Distribution of dominant color | Fruit flesh firmness | Fruit flesh color | Seed content | Seed maturity | Hole in the fruit | Brix | Fruit diameter (mm) | Fruit lenght (cm) | Fruit weight (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| YRL 1 | few | between 20-70% | slight | absent | green | mottled | floppy | greenish-cream | intermediate | mature | absent | 5.2 | 34.80 | 19.8 | 0.116 |
| YRL 3 | few | between 20-70% | slight | present | purple | striped | tightly | white | many | mature | present | 4.2 | 49.79 | 14.2 | 0.140 |
| YRL 6 | mid | between 20-70% | slight | absent | green | mottled | floppy | greenish-cream | intermediate | immature | absent | 4.2 | 36.60 | 23.3 | 0.140 |
| YRL 7 | mid | less than 20% | mid- | absent | black | regular | floppy | cream | intermediate | mature | absent | 5.1 | 40.33 | 18.7 | 0.133 |
| YRL 8 | mid | less than 20% | slight | present | purple | regular | tightly | greenish-cream | intermediate | mature | present | 4.7 | 46.86 | 15.7 | 0.133 |
| YRL 9 | few | between 20-70% | slight | absent | black | regular | tightly | greenish-cream | intermediate | mature | absent | 3.8 | 45.38 | 18.5 | 0.138 |
| YRL 12 | few | less than 20% | slight | absent | purple | regular | spongy | white | intermediate | mature | present | 2.8 | 40.98 | 20.5 | 0.125 |
| YRL 14 | mid | between 20-70% | none | absent | black | regular | tightly | greenish-cream | few | mature | present | 4.3 | 43.60 | 17.2 | 0.133 |
| YRL 15 | mid | between 20-70% | slight | present | black | regular | tightly | greenish-cream | few | immature | present | 6.1 | 35.07 | 18.8 | 0.110 |
| YRL 19 | few | between 20-70% | slight | absent | black | regular | tightly | greenish-cream | intermediate | mature | absent | 4.1 | 47.32 | 17.5 | 0.146 |
| YRL 20 | mid | between 20-70% | slight | absent | black | regular | spongy | greenish-cream | many | immature | present | 5.2 | 42.17 | 20.6 | 0.160 |
| YRL 24 | few | less than 20% | slight | absent | purple | regular | spongy | greenish-cream | few | immature | present | 5.2 | 38.96 | 18.3 | 0.100 |
| YRL 26 | few | between 20-70% | mid- | absent | green | mealy | floppy | white | intermediate | mature | present | 3.2 | 45.73 | 16.7 | 0.143 |
| YRL 27 | mid | between 20-70% | slight | absent | purple | mealy | floppy | white | intermediate | mature | present | 4.2 | 58.13 | 18.0 | 0.213 |
| YRL 28 | few | less than 20% | mid- | absent | black | regular | floppy | white | intermediate | mature | present | 3.4 | 53.13 | 16.3 | 0.177 |
| YRL 30 | mid | less than 20% | mid- | absent | purple | mealy | spongy | white | intermediate | immature | present | 3.1 | 47.43 | 14.5 | 0.133 |
| YRL 34 | mid | between 20-70% | slight | present | purple | mealy | spongy | white | few | mature | present | 3.1 | 54.11 | 22.1 | 0.232 |
| YRL 35 | mid | between 20-70% | mid- | absent | purple | mealy | spongy | white | few | mature | present | 3.0 | 53.66 | 17.5 | 0.207 |
| YRL 36 | mid | between 20-70% | mid- | absent | purple | mealy | floppy | white | intermediate | mature | present | 3.2 | 52.05 | 14.8 | 0.163 |
| YRL 43 | mid | between 20-70% | none | present | purple | mottled | spongy | white | intermediate | immature | present | 3.2 | 57.87 | 15.1 | 0.187 |
| YRL 44 | mid | between 20-70% | slight | absent | purple | mealy | spongy | white | intermediate | mature | present | 3.4 | 51.29 | 15.2 | 0.168 |
| YRL 45 | mid | between 20-70% | slight | present | purple | mealy | tightly | white | few | mature | present | 3.9 | 50.50 | 14.6 | 0.173 |
| YRL 46 | mid | less than 20% | mid- | present | purple | regular | floppy | white | intermediate | mature | absent | 4.1 | 42.79 | 16.4 | 0.125 |
| YRL 47 | few | less than 20% | mid- | present | purple | regular | floppy | white | intermediate | immature | absent | 4.1 | 35.05 | 18.0 | 0.127 |
| YRL 49 | few | less than 20% | mid- | absent | purple | regular | tightly | white | intermediate | immature | present | 3.8 | 44.98 | 19.7 | 0.153 |
| YRL 50 | mid | less than 20% | mid- | absent | purple | regular | tightly | white | intermediate | mature | present | 3.1 | 43.15 | 19.2 | 0.143 |
| YRL 51 | few | less than 20% | mid- | present | purple | regular | floppy | white | intermediate | mature | absent | 3.1 | 44.82 | 17.4 | 0.147 |
| YRL 52 | few | less than 20% | mid- | absent | purple | regular | tightly | white | intermediate | mature | present | 2.9 | 42.73 | 17.5 | 0.133 |
| YRL 57 | few | between 20-70% | mid- | absent | green | mottled | tightly | greenish-cream | intermediate | immature | present | 5.1 | 32.67 | 19.1 | 0.100 |
| YRL 58 | few | less than 20% | mid- | absent | purple | regular | tightly | white | few | mature | present | 4.2 | 47.03 | 18.5 | 0.148 |
| YRL 59 | mid | between 20-70% | mid- | absent | purple | mottled | floppy | white | intermediate | mature | present | 4.7 | 51.40 | 13.0 | 0.155 |
| YRL 61 | mid | between 20-70% | none | present | purple | regular | tightly | white | intermediate | mature | present | 4.1 | 64.76 | 10.9 | 0.183 |
| YRL 62 | few | between 20-70% | slight | absent | black | regular | tightly | greenish-cream | few | immature | present | 4.9 | 46.09 | 16.3 | 0.143 |
| YRL 64 | few | less than 20% | slight | absent | purple | regular | floppy | white | intermediate | mature | present | 3.8 | 51.86 | 22.9 | 0.228 |
| YRL 65 | mid | less than 20% | slight | present | purple | mottled | tightly | white | intermediate | mature | present | 3.8 | 73.22 | 11.6 | 0.235 |
| YRL 68 | mid | between 20-70% | slight | present | black | regular | tightly | greenish-cream | medium | immature | present | 3.1 | 50.76 | 14.2 | 0.150 |
| YRL 75 | mid | between 20-70% | none | present | black | regular | tightly | greenish-cream | intermediate | mature | present | 4.2 | 65.97 | 11.9 | 0.137 |
| YRL 79 | few | between 20-70% | slight | present | green | mottled | floppy | greenish-cream | intermediate | immature | present | 4.2 | 47.71 | 13.6 | 0.122 |

Table 5. Skin color characteristics of eggplant

| Genotype | L* | a* | b* | C | h° | color |
|---|---|---|---|---|---|---|
| YRL 1 | 48.33 ± 6.38 | -0.67 ± 4.80 | 18.38 ± 4.04 | 19.04 ± 3.88 | 88.77 ± 15.99[X] | chartreuse |
| YRL 3 | 73.36 ± 4.06 | 9.05 ± 2.52 | 6.31 ± 1.09 | 11.27 ± 1.52 | 36.39 ± 11.67 | yellowish-orange |
| YRL 6 | 51.54 ± 8.50 | -6.18 ± 5.43 | 22.47 ± 6.03 | 23.75 ± 6.71 | 101.46 ± 13.81 | chartreuse |
| YRL 7 | 26.21 ± 0.66 | 6.03 ± 1.76 | 0.36 ± 0.51 | 6.05 ± 1.78 | 62.44 ± 131.52 | yellowish |
| YRL 8 | 31.83 ± 3.32 | 11.44 ± 2.19 | 2.69 ± 1.07 | 11.78 ± 2.31 | 12.60 ± 4.48 | redish |
| YRL 9 | 29.28 ± 3.64 | 10.22 ± 3.40 | 1.25 ± 1.45 | 10.38 ± 3.48 | 5.85 ± 6.76 | redish |
| YRL 12 | 31.71 ± 3.20 | 21.64 ± 2.52 | -0.98 ± 0.75 | 21.67 ± 2.54 | 357.56 ± 1.74 | magenta |
| YRL 14 | 25.83 ± 0.99 | 4.97 ± 0.79 | 0.36 ± 0.88 | 5.03 ± 0.93 | 182.80 ± 175.41 | cyan |
| YRL 15 | 25.23 ± 0.22 | 4.07 ± 1.31 | 0.002 ± 0.26 | 4.08 ± 1.31 | 299.07 ± 131.60 | purple |
| YRL 19 | 25.48 ± 0.51 | 3.81 ± 0.78 | -0.03 ± 0.22 | 3.82 ± 0.79 | 238.99 ± 167.40 | blue |
| YRL 20 | 25.82 ± 0.92 | 4.84 ± 1.15 | 0.003 ± 0.28 | 4.85 ± 1.15 | 299.43 ± 131.95 | purple |
| YRL 24 | 29.02 ± 3.24 | 17.85 ± 4.63 | -0.42 ± 0.54 | 17.86 ± 4.65 | 243.94 ± 146.42 | blue |
| YRL 26 | 55.22 ± 5.86 | -7.43 ± 2.45 | 17.56 ± 3.60 | 19.10 ± 4.20 | 112.31 ± 3.60 | greenish |
| YRL 27 | 68.54 ± 5.60 | 8.83 ± 2.37 | 5.71 ± 1.76 | 10.88 ± 1.00 | 34.29 ± 14.89 | orange |
| YRL 28 | 29.89 ± 2.86 | 9.70 ± 0.84 | 1.57 ± 1.36 | 9.93 ± 0.72 | 9.29 ± 8.33 | red |
| YRL 30 | 66.80 ± 2.88 | 11.23 ± 1.66 | 6.30 ± 1.17 | 12.99 ± 1.04 | 29.80 ± 7.71 | orange |
| YRL 34 | 77.73 ± 3.23 | 4.64 ± 1.69 | 9.76 ± 1.00 | 10.98 ± 0.16 | 64.53 ± 10.30 | cream-yellowish |
| YRL 35 | 75.07 ± 2.53 | 6.01 ± 1.29 | 8.28 ± 1.20 | 10.38 ± 0.33 | 53.91 ± 9.74 | yellow |
| YRL 36 | 72.34 ± 4.46 | 8.41 ± 2.74 | 6.96 ± 1.55 | 11.30 ± 1.24 | 41.16 ± 14.66 | orange-yellow |
| YRL 43 | 56.55 ± 9.09 | 19.35 ± 5.14 | 2.09 ± 2.76 | 19.81 ± 4.51 | 68.93 ± 128.77 | yellow |
| YRL 44 | 72.50 ± 8.32 | 7.51 ± 4.42 | 7.49 ± 2.90 | 11.58 ± 2.54 | 48.10 ± 21.20 | goldenrod |
| YRL 45 | 76.29 ± 4.16 | 5.00 ± 2.26 | 10.97 ± 2.10 | 12.40 ± 1.05 | 64.49 ± 13.53 | yellow |
| YRL 46 | 37.41 ± 5.48 | 22.90 ± 1.68 | -0.49 ± 1.07 | 22.94 ± 1.68 | 298.96 ± 131.62 | purple |
| YRL 47 | 32.80 ± 4.44 | 20.59 ± 3.19 | -0.50 ± 1.36 | 20.64 ± 3.23 | 357.03 ± 2.04 | magenta |
| YRL 49 | 32.04 ± 3.54 | 23.02 ± 2.99 | -0.51 ± 0.47 | 23.04 ± 3.00 | 240.48 ± 166.70 | blue |
| YRL 50 | 36.77 ± 6.11 | 22.17 ± 2.18 | -0.07 ± 1.29 | 22.20 ± 2.15 | 179.90 ± 177.00 | cyan |
| YRL 51 | 37.55 ± 4.73 | 21.85 ± 3.29 | -0.89 ± 0.93 | 21.88 ± 3.30 | 285.88 ± 142.39 | purple |
| YRL 52 | 35.51 ± 2.61 | 23.76 ± 1.51 | -1.37 ± 0.48 | 23.81 ± 1.49 | 356.67 ± 1.24 | magenta |
| YRL 57 | 44.44 ± 8.19 | 4.04 ± 6.62 | 9.70 ± 5.12 | 13.35 ± 1.43 | 63.87 ± 38.34 | yellowish |
| YRL 58 | 37.80 ± 5.11 | 23.66 ± 2.46 | -1.61 ± 0.66 | 23.72 ± 2.43 | 356.05 ± 1.90 | magenta |
| YRL 59 | 56.43 ± 6.66 | 18.22 ± 4.99 | 1.86 ± 2.45 | 18.55 ± 4.72 | 80.11 ± 137.94 | greenish-yellow |
| YRL 61 | 51.83 ± 4.36 | 14.86 ± 2.14 | 0.41 ± 3.95 | 15.45 ± 1.56 | 242.80 ± 156.44 | blue |
| YRL 62 | 31.03 ± 5.72 | 10.50 ± 2.59 | 2.53 ± 2.32 | 10.96 ± 2.90 | 12.19 ± 9.68 | redish |
| YRL 64 | 37.51 ± 4.79 | 24.23 ± 0.59 | 0.02 ± 1.29 | 24.26 ± 0.61 | 285.99 ± 142.37 | purple |
| YRL 65 | 73.19 ± 4.48 | 8.17 ± 2.89 | 7.90 ± 0.91 | 11.66 ± 1.61 | 45.71 ± 12.66 | orange-yellow |
| YRL 68 | 35.81 ± 5.99 | 9.95 ± 2.41 | 6.17 ± 3.81 | 12.01 ± 3.63 | 27.50 ± 15.13 | dark-orange |
| YRL 75 | 25.39 ± 0.72 | 6.03 ± 1.01 | 0.12 ± 0.14 | 6.03 ± 1.01 | 1.05 ± 1.04 | redish |
| YRL 79 | 57.34 ± 3.69 | 0.12 ± 2.26 | 19.50 ± 2.14 | 19.64 ± 2.08 | 88.98 ± 6.91 | chartreuse |

[X]: Means represent three 10-fruit samples ± SD.

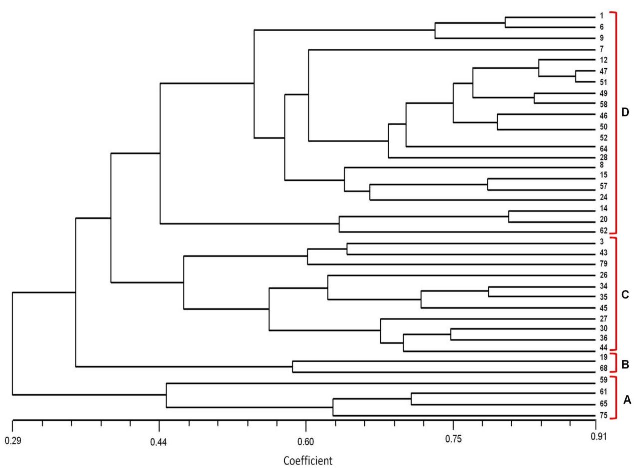


Fig. 2. UPGMA dendrogram showing phylogenetic relationships of local Burdur eggplant heirlooms together with reference cultivars using morphological data


Fig. 3. The fruits of Burdur eggplant heirlooms belonging to the Group C

A total of 65 amplified RAPD bands were generated. Twenty nine bands were polymorphic and the mean percentage of polymorphism was 44.61%. OPO-10 primer produced the maximum numbers of bands (18). Although the OPB-07 primer produced the minimal number of bands (8), it revealed a 100% polymorphism (Table 6). The OPH-2, OPB-07, OPO-10 and OPL-16 primers' PCR products and their band patterns are shown in Fig. 4a-d.

Table 6. Primer code, sequence, GC content, number of polymorphic bands and percent polymorphism of each RAPD primer used

| Primer code | Nucleotide sequence | No. of amplified bands | No. of polymorphic bands | Polymorphic bands (%) |
|---|---|---|---|---|
| OPH-02 | 5'-TCG GAC GTG A-3' | 14 | 7 | 50 |
| OPL-04 | 5'-GAC TGC ACA C-3' | 16 | 4 | 25 |
| OPB-07 | 5'-GGT GAC GCA G-3' | 8 | 8 | 100 |
| OPO-10 | 5'-TCA GAG CGC C-3' | 18 | 5 | 27.77 |
| OPL-16 | 5'-AGG TTG CAG G-3' | 9 | 5 | 55.55 |
| **TOTAL** | | **65** | **29** | **44.61** |

The phylogenetic relationships among 38 genotypes were evaluated using 29 polymorphic loci of the genomic DNA generated using randomly amplified polymorphic DNA (RAPD) technique. The approximate Mantel t-test statistic were t = 8.9263, p = 1.0000. The matrix correlation (r) was found to be 0.64. The similarity rates according to the coefficient similarity of genotypes ranged between 0.84 and 0.98.

Using molecular data two major groups emerged in the dendrogram generated with UPGMA method (Fig. 5). First group branched into two subgroups and were showed in brackets as Group A and Group B. Group A was consisted of YRL 15 and YRL 27. Group E included most of the genotypes had high similarities with reference cultivar. The highest genetic similarity was observed in Group D with 98% percentage similarity index between genotypes YRL 6 and YRL 44. YRL 1 and YRL 27 were detected as the most distantly genotypes from each other in the cluster. Fruits of Burdur eggplant heirlooms YRL 6 and YRL 44, which showed highest genetic similarity with 98% percentage are shown in Fig. 6. Also, the fruits of Burdur heirlooms which grouped together in cluster E in the UPGMA dendrogram using molecular data and reference culture are shown in Fig. 7.

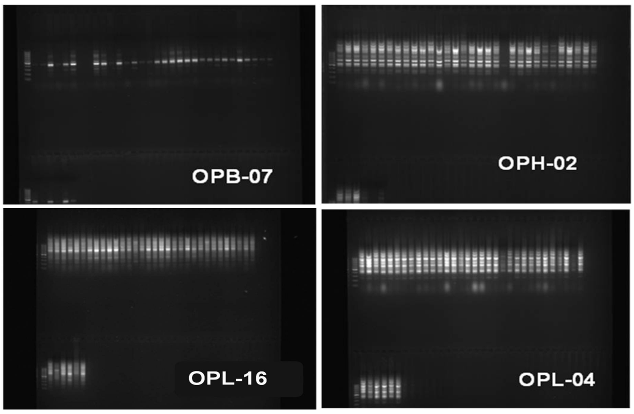


Fig. 4. PCR products of Burdur eggplant heirlooms generated using RAPD primers a:OPB-07, b:OPH-02, c:OPO-016, d:OPL-04

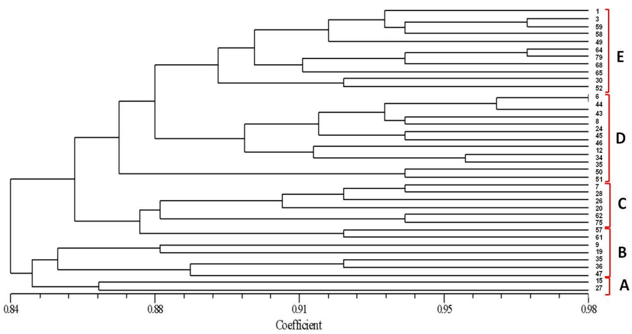


Fig. 5. UPGMA dendrogram showing phylogenetic relationships of local Burdur eggplant heirlooms together with reference cultivars using molecular data


Fig. 6. Fruits of Burdur eggplant heirlooms YRL 6 and YRL 44, which showed highest genetic similarity with 98% percentage


Fig. 7. Fruits of eggplant Burdur heirlooms with reference cultivar (YRL 68) situated in GROUP E generated by UPGMA using molecular data

## Discussions

Eggplant has a wide genetic diversity in the regions where it is cultivated, although they are not native to the region (Muñoz-Falcón *et al.,* 2008). In spite of the fact that Turkey is not the place origin of eggplant, wide genetic diversity has been reported in Turkey (Demir *et al.*, 2010; Tümbilen *et al.*, 2011a, 2011b). It is clearly evident in this study data that Burdur, which is a small geographical region, had a rich genetic diversity. Also, a wide genetic variability was determined in both Spain and Jordan local genotypes (Prohens *et al.*, 2003). Local genotypes can contribute to enhancing the gene pool used in breeding studies and to help increase heterosis (Muñoz-Falcón *et al.*, 2009). In recent years, some factors like cultivation of commercial varieties instead of heirlooms, construction of buildings on agricultural land, and innovation in cultivation methods have led to erosion of plant genetic resources (Cericola *et al.*, 2013). Therefore, there is a need collecting and identification of local heirlooms before they disappear (Muñoz-Falcón *et al.*, 2008).

Some characters that contributed to genetic diversity were as flowering dates, the number of seeds per fruits, fruit features, and the growth pattern of plants. These features are controlled by several genes in eggplant (Frary and Doğanlar, 2003). *Solanum melongena* accessions could characterize these descriptors like bigger and flabby fruits, less flowers/inflorescence, few fruits/plant and higher acidity etc. compared to the wild relatives (Polignano *et al.,* 2010). Consistent with previously works, a higher diversity for most morphological descriptors was recorded in the collection of Burdur local heirlooms identified in this study. Fruit color can be cream, green, red, reddish-purple, dark purple or black, and some varieties produce fruit which is where the genetic variation necessary for future varietal improvement and for addressing future breeding challenges will be found.

Molecular markers linked with agronomic traits are useful tools for marker assisted selection and mapping candidate genes studies in breeding programs (Nunome *et al.*, 2009;

Wang *et al.*, 2010). Some of the RAPD markers used were determined to have relationship with coloring of stem, calyx and fruiting in eggplant (Frary *et al.*, 2003). This provides a great advantage for identifying features affecting by ecological conditions (Biswas *et al.*, 2009; Nunome *et al., 2001).* Relationships among eggplant materials have been studied by molecular studies for use by eggplant breeders (Furini and Wunder, 2004). As mentioned above the RAPD markers revealed as a potential useful tool for determination of genetic diversity. It was found that RAPD analysis in eggplant with four primers had been adequate to identify of genetic diversity (Biswas *et al.*, 2009). Also, RAPD markers were more effective than ISSR for revealing genetic diversification as reported by Ali *et al.* (2011). *Tiwari et al.* (2009) previously reported that even two of the 29 RAPD primers were found to be sufficient for identification of local cultivars. All RAPD markers used in the study have produced polymorphic bands as expected. However, if fruit criteria are taken into consideration for distinguishing in the dendrogram and grouping, it is not enough for the breeders. More informative DNA markers can be used to provide better progress in eggplant breeding studies (Stàgel *et al.,* 2008). Simple Sequence Repeats (SSRs) methods were found more successful for distinguishing closely related eggplant cultivars (Hurtado *et al.,* 2012; Muñoz-Falcón *et al.,* 2009; Prohens *et al.,* 2008). The approach of using SSR markers instead of RAPD markers can provide better results in the phylogenetic relationships studies. Muñoz-Falcón *et al.* (2009) reported that if the morphological and molecular data are considered together, they can be provide sufficient and useful information for the breeders. Similarly to the findings of other studies, we suggest that the molecular evidences need to be supplemented by morphological data to validate the phylogenetic relationships among the genotypes. It is very important to note that the genetic variations can't be detected by only showing a dendrograms generated by molecular data.

## Conclusions

The local populations are of great importance for the breeders so that they adapted well to their cultivated areas. There is a need collecting and identification of these heirlooms before integrated to the breeding programs. The aim of the study was to investigate the diversity among heirlooms cultivated in Burdur province. A high genetic diversity was determined widely among them. These materials can be of a potential value for the breeders.

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