THE GENETIC RELATIONSHIP BETWEEN SOME STRAWBERRY CULTIVARS AND F1 POPULATION DERIVED FROM THESE CULTIVARS

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Abstract. In this study, while Osmanli strawberry, which has some superior characteristics like taste, aroma and male sterility that makes breeding studies easier, has been used as maternal parent, three local (Karacilek, Tuylu and Deli) cultivars in Turkey and three standard (Kabarla, Sweet Ann and Sweet Charlie) strawberry cultivars were used as paternal parents. Molecular analyses were conducted for determination of polymorphism levels by using 14 UBC-ISSR primers for the examination of 52 F1 genotypes that included selections as a result of hybridization studies, parents that were used in hybridization studies, and *Fragaria chiloensis*. 76 DNA bands were obtained in total and 60 of them were determined as polymorphic. The resulting data of the evaluation of ISSR-PCR bands, were analysed in Popgene32 and MEGA5.0 packaged software and the dendrogram was obtained according to the UPGMA method. The dendrogram was separated into two major groups at the 20% difference level, one is larger and the other is smaller. While *Fragaria chiloensis*, Tuylu-2 local cultivar, CC48 and CA97 F1 genotypes participated in the small group, the other F1 genotypes and parents participated in the larger group.
Keywords: *hybridization breeding, ISSR, molecular, Osmanli cultivar, strawberry*

Introduction

Strawberry is one of the rare fruit species that can be grown in large ecological zones, and it is known and grown in Turkey for a long time (Konarli, 1986). When the strawberry was classified botanically in the world, it was placed in *Rosales* family and *Fragaria* genus (Hancock and Luby, 1993). Most of strawberries that are grown commercially, belong *Fragaria x ananassa* species that was derived from hybridization of two local American species (*F. chiloensis* X *F. virginiana*) (Hancock, 1999).

More than 20 strawberry species with different ploidy levels were identified. *F. vesca* and *F. viridis* (2n = 2x = 14) are found as diploid species in the flora of Turkey. As well as these wild species, octoploid *F. × ananassa* were used for cultivation in Turkey for a long time, generating strawberry genetic resources. Osmanli and Arnavutkoy local cultivars were started to grown in limited areas because of cultivation of the new cultivars with higher quality and yield from another countries has become more common Osmanli cultivar that is known by its taste, colour and aroma, is only grown in a very small area in Eregli and Bartin regions in the West Black Sea Region of Turkey by few growers (Demirsoy and Serce, 2016). Dokuzoguz (1963) also reported that Eregli, Karsiyaka, and Kestel have specific characteristics comparing to the cultivars of Arnavutkoy, Eregli, Madame Moutot and Mangro, which are all called Osmanli or Arnavutkoy with small, oval-shaped, light-coloured fruits but have different other characteristics.

Modern strawberry cultivation started in the 1960s in Turkey (Konarli and Philippe, 1968). Since the strawberry cultivation started, the main purpose is having a corner on the domestic and foreign market by doing high quality and early-grown cultivation. Besides the superior aromatic characteristics, profitable cultivation with local cultivars is not possible due to low yield, soft fruit flesh and small fruits. Although strawberry cultivars that is originated from another countries have earliness and high yield characteristics, they have middle level aromatic characteristics. Because of these reasons, breeding studies have being conducted that local cultivars have been used as parents since 1960. In the most of these studies, Osmanli strawberry, which have some superior characteristics (taste, aroma, the situation of fruit stalk, etc.) and male sterility that makes breeding studies easier, has been used as maternal parent (Kaska and Paydas, 1986; Ustun and Paydas, 1995; Paydas et al., 1996; Caglar and Paydas, 2002; Kiyga, 2009).

Modern breeding methods were developed especially after the beginning of the 20th century. In the last 30 years, 38 cultivars in Turkey and more than 900 cultivars in the world have been developed and registered (Della Strada and Fideghelli, 2011; Chandler et al., 2012; Turemis and Agaoglu, 2013; Faedi and Baruzzi, 2016).

The knowledge about genetic structure is important, especially when it is worked with wild species (Ahmadi and Bringhurst, 1991). Morphological, physiological and cytological characteristics were used at the beginning for the determination of genetic variation, afterwards, biochemical markers were developed. Furthermore, molecular studies are conducted recently (Scarano et al., 2002).

Molecular markers are derived from the DNA in the cell of the plants. Thus, molecular markers are almost 100% reliable for the determination of the diversity between plant populations and plant genotypes. Today, molecular markers are used commonly in plant taxonomy studies, breeding and evaluation of genetic resources (Gulsen and Mutlu, 2005).

DNA markers are more preferred than isoenzyme, biochemical and morphological markers in the studies about the determination of species and cultivars. PCR based DNA markers; AFLP Amplified Fragment Length Polymorphism), CAPS (Cleaved Amplified Polymorphic Sequences), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeats) and RAPD (Random Amplified Polymorphic DNA) were used on many studies about strawberry (Tyrkal et al., 2002; Kunihisa et al., 2003, 2005; Kashyap et al., 2005; Bassil et al., 2006; Milella et al., 2006; Serce et al., 2007). While CAPS, RFLP and SSR are codominant, AFLP, ISSR and RAPD are dominant molecular markers (Kafkas, 2006).

In ISSR (Inter Simple Sequence Repeats) method, one primer is used and it has two, three and four sequence repeat on it. This method is used commonly in the determination of DNA fingerprint and genetic relationships (Cekic et al., 2001; Arnau et al., 2002; Kuras et al., 2004; Debnath et al., 2008; Nunes et al., 2013).

Arnau et al. (2002) scanned eighteen ISSR primers and selected five of them. They used those primers for characterization of genotypes of 30 different strawberry (*F. x ananassa* L.) cultivars and they obtained 113 polymorphic bands.

Serçe et al. (2006) studied with 16 cultivars belong to *F. chiloensis, F. virginiana* and *Fragaria x ananassa*, and Osmanli, Karacilek, Tuylu and Arnavutkoy local cultivars. Although Osmanli strawberry was morphologically similar with *F. chiloensis* clones, it was observed in the dendrogram obtained by 13 SSR primers that Osmanli, Karacilek, Tuylu and Arnavutkoy cultivars were different with the other three species

and generated different group. Moreover, researchers suggested that more primers should be used with the purpose of obtaining more reliable results.

The aim of this study was the determination of genetic relationships by using ISSR markers between 52 F1 genotypes that were selected according to their superior morphological, pomological and physiological characteristics, parents that were used in hybridization studies, and *Fragaria chiloensis* within the scope of 'Osmanli Strawberry Breeding' project that is conducted in University of Gaziosmanpasa. Obtained data were evaluated according to the UPGMA dendrogram and Principal coordinate analysis

Material and method

Material

In this study, within the scope of 'Osmanli Strawberry Breeding' project that is conducted at the University of Gaziosmanpasa in Turkey, 52 selected F1 genotypes, the parents (Osmanli and eleven pollinators) as well as *Fragaria chiloensis* for comparision were used in molecular analysis.

In the hybridization programme, while Osmanli strawberry cultivar which is morphologically male sterile was used as maternal parent, 8 local genotypes ('Osmanli-1', 'Osmanli-2', 'Osmanli-3', 'Osmanli-4', 'Tuylu-1', 'Tuylu-2', 'Karacilek-1', 'Karacilek-2') (*Table 1*), from Eregli district of Zonguldak province and 3 standard cultivars ('Kabarla', 'Sweet Ann' and 'Sweet Charlie') provided from private seedling companies were used as pollinators.

Table 1. Coordinates belongs to local parents, and names of F1 genotypes

Local parent			
Sample Code (ID)	**Altitude (m)**	**Latitude**	**Longitude**
Osmanli-1	220	41° 18.309K	031° 26.666D
Osmanli-2	267	41° 18.528K	031° 26.795D
Osmanli-3	237	41° 17.659K	031° 28.026D
Osmanli-4	152	41° 16.919K	031° 28.879D
Karacilek-1	220	41° 18.309K	031° 26.666D
Karacilek-2	237	41° 17.659K	031° 28.026D
Tuylu-1	237	41° 17.659K	031° 28.026D
Tuylu-2	152	41° 16.919K	031° 28.879D

F1 genotypes					
ID	**Parents**	**ID**	**Parents**	**ID**	**Parents**
CC-4	Osmanli3XSweet Charlie	DB-118	Osmanli4XKabarla	DC-63	Osmanli4XSweet Charlie
CC-35	Osmanli3XSweet Charlie	DB-119	Osmanli4XKabarla	DC-104	Osmanli4XSweet Charlie
CC-42	Osmanli3XSweet Charlie	DB-137	Osmanli4XKabarla	DC-126	Osmanli4XSweet Charlie
CC-48	Osmanli3XSweet Charlie	DA-1	Osmanli4XSweet Ann	DC-136	Osmanli4XSweet Charlie

CC-60	Osmanli3XSweet Charlie	DA-4	Osmanli4XSweet Ann	CA-3	Osmanli3XSweet Ann
CC-64	Osmanli3XSweet Charlie	DA-6	Osmanli.4XSweet Ann	CA-15	Osmanli3XSweet Ann
DB-7	Osmanli4XKabarla	DA-9	Osmanli4XSweet Ann	CA-23	Osmanli3XSweet Ann
DB-15	Osmanli4XKabarla	DA-40	Osmanli4XSweet Ann	CA-87	Osmanli3XSweet Ann
DB-35	Osmanli4XKabarla	DA-61	Osmanli4XSweet Ann	CA-97	Osmanli3XSweet Ann
DB-41	Osmanli4XKabarla	DA-87	Osmanli4XSweet Ann	CB-26	Osmanli3XKabarla
DB-48	Osmanli4XKabarla	DA-88	Osmanli4XSweet Ann	CB-86	Osmanli3XKabarla
DB-49	Osmanli4XKabarla	DA-95	Osmanli4XSweet Ann	CK1-9	Osmanli3XKaracilek1
DB-57	Osmanli4XKabarla	DC-7	Osmanli4XSweet Charlie	CK1-33	Osmanli3XKaracilek1
DB-68	Osmanli4XKabarla	DC-42	Osmanli4XSweet Charlie	DK1-24	Osmanli4XKaracilek1
DB-73	Osmanli4XKabarla	DC-44	Osmanli4XSweet Charlie	CC-2	Osmanli3XSweet Charlie
DB-88	Osmanli4XKabarla	DC-54	Osmanli4XSweet Charlie	CC-19	Osmanli3XSweet Charlie
DB-97	Osmanli4XKabarla	DC-60	Osmanli4XSweet Charlie	DB-69	Osmanli4XKabarla
DB-84	Osmanli4XKabarla				

Method

DNA isolation

DNA isolation was done from 1.5-2 cm length young leaf samples from each genotype according to the Mini-CTAB method belongs to Doyle and Doyle (1987).

PCR amplification

ISSR method was used because generated bands are reproducible (reliability) and have high polymorphism (scorability), and also, scanning large number of different locus is possible (Prevost and Wilkinson, 1999; Cekic et al., 2001).

For the determination of polymorphism levels, 14 primers were used that were selected from 100 UBC primers, which was used for '*Fragaria vesca*' before (*Table 2*) (Cekic et al., 2001). PCR method with constant annealing temperature that was used by Cekic et al. (2001) and Touchdown PCR method by Sargent et al. (2003) were modified and used. ISSR PCR reaction was done with 2 μL DNA (10 ng/μL) and 23 μL reaction composition [5 μL 10X PCR buffer solution, 2 μL 25 mM Mg^{+2}, 1.25 μL 2.5 mM dNTP, 0.1 μL Taq DNA Polymerase, 1 μL 0.5 μM primer and 13.65 μL PCR water]. PCR products were separated in 100V constant voltage during 2 hours by using 1 X TBE buffer as a gelling agent and the electrode buffer solution, and 2% agarose gel for electrophoresis. Afterwards, DNA bands were imaged under ultraviolet lights.

Data analyses

Data, which were used in the statistical analyses, were obtained by the method that is about scoring one (1) when there was ISSR band and scoring zero (0) when there was not ISSR band. Similarities and differences between genotypes were determined in the molecular level. Principal Coordinates Analysis (PCoA) was done by similarity coefficient. Data were analysed in Popgene32 version 1.32 (Population Genetic

Analysis) and MEGA 5.0 (Molecular Evolutionary Genetic Analysis) packaged software and the dendrogram was obtained according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method (Parmaksiz and Ozcan, 2011). Furthermore, generating similarities/differences matrix between genotypes and Principal Coordinates Analysis (PCoA) were done by using NTSYS-pc ver. 2.10d (Numerical Taxonomy and Multivariate Analysis System) packaged software that was developed by Rohlf (2002).

Table 2. ISSR primers

Primer name	5' – 3' sequence	Degree of dissociation (C)	Primer name	5' – 3' sequence	Degree of dissociation (C)
807	AGAGAGAGAGAGAGAGT	50	888	BDBCACACACACACACA	52
808	AGAGAGAGAGAGAGAGC	53	889	DBDACACACACACACAC	52
810	GAGAGAGAGAGAGAGAT	50	890	VHVGTGTGTGTGTGTGT	52
811	GAGAGAGAGAGAGAGAC	53	891	HVHTGTGTGTGTGTGTG	52
835	AGAGAGAGAGAGAGAGYC	54	856	ACACACACACACACACYA	53
841	GAGAGAGAGAGAGAGAYC	54	826	ACACACACACACACACC	52
842	GAGAGAGAGAGAGAGAYG	55	844	CTCTCTCTCTCTCTCTRC	53

Results

Some DNA bands were not clear may be due to PCR gel imaging system or ethidium bromide. Therefore, scoring studies were done with clear DNA bands. 76 DNA bands were determined at the end of the study and 60 of them were polymorphic. Band number obtained from primers varied between 3 and 8, additionally, the average band number was 5.43 and the average polymorphic band number was 4.29. The highest band number was obtained from primer-810 (8 bands) and the lowest band number was obtained from primer-841 (3 bands). DNA bands, which were generated by primer-835, 841, 844 and 888, were observed as polymorphic. The lowest polymorphism rate was determined on primer-842 with 50%. The polymorphic band rate average was determined as 80.14% on 14 primers.

A dendrogram was obtained according to the UPGMA method (*Fig. 1*). The dendrogram was separated into two major groups at the 20% difference level, one is big and other is small. While *Fragaria chiloensis*, Tuylu-2 local cultivar, CC48 (Osmanlı-3 x Sweet Charlie) and CA97 (Osmanlı-3 x Sweet Ann) F1 genotypes participated in the small group, the other F1 genotypes and parents participated in the big group. It was observed that the big major group was separated again into groups with 17% level.

In this study, the highest similarity was determined between Osmanli-3 and Osmanli-4 with 3%, and the second highest similarity was determined between Sweet Charlie and Kabarla. It was also observed that Osmanli-1, Osmanli-2 and Osmanli-3 were in the same subgroup.

It is known that Scatterplot PCo1 and PCo2 analyses support the results of the distribution of dendrogram (Ozyurt, 2011). In this study, it was determined that the distribution of genotypes on the Scatterplot (*Fig. 2*) obtained with Principal

Coordinates Analysis (PCoA), showed similarity with distribution on the dendrogram (*Fig. 1*). 'Osmanli-1 (OsmA)', 'Karacilek-2 (Kara-C)', 'Tuylu-2 (Tuylu-D)', 'CA-97' and '*Fragaria chiloensis* (FC)' were out of the high-density group according to Principal Coordinates Analysis 1 and 2. Furthermore, 'Karacilek-2 (Kara-C)', 'Tuylu-2 (Tuylu-D)', 'Sweet Charlie', '*Fragaria chiloensis* (FC)*', 'CC-48', 'CA-97' and 'DB-97' were out of the high-density group according to Principal Coordinates Analysis 1 and 3.

Discussion and conclusion

76 DNA bands were determined at the end of the study and 60 of them were polymorphic. Cao et al. (2000) stated that 50 polymorphic bands are sufficient to determine similarities and differences between genotypes successfully. It was observed that 60 obtained polymorphic bands are sufficient to work with. The polymorphic band rate average was determined as 80.14% on 14 primers. Arnau et al. (2002) used 5 ISSR primers on characterization of 30 different strawberry (*F. x ananassa* L.) genotypes and it was stated that 113 polymorphic bands were obtained.

Kuras et al. (2004) used ISSR and RAPD marker methods for determination of genetic relationships between 24 strawberry cultivars, which were obtained by breeding program conducted in Poland. Moreover, these two marker methods were compared in the same study. 41 of 90 ISSR primers and 23 of 48 RAPD primers were found as polymorphic. As a result of the UPGMA analyses, genetic relationships were determined and the family tree was generated. As a result of comparison of marker methods, ISSR was found better than RAPD in terms of genetic relationships.

The dendrogram obtained from genotypes was separated into two major groups, one is big and other is small, and the separation was done successfully with 14 ISSR primers. Debnath et al. (2008) determined genetic relationship of 27 strawberry cultivars by using 17 ISSR primers, and they obtained 225 polymorphic bands. The results were evaluated with UPGMA analysis, and it was stated that genetic relationship between cultivars varied between 63-77%.
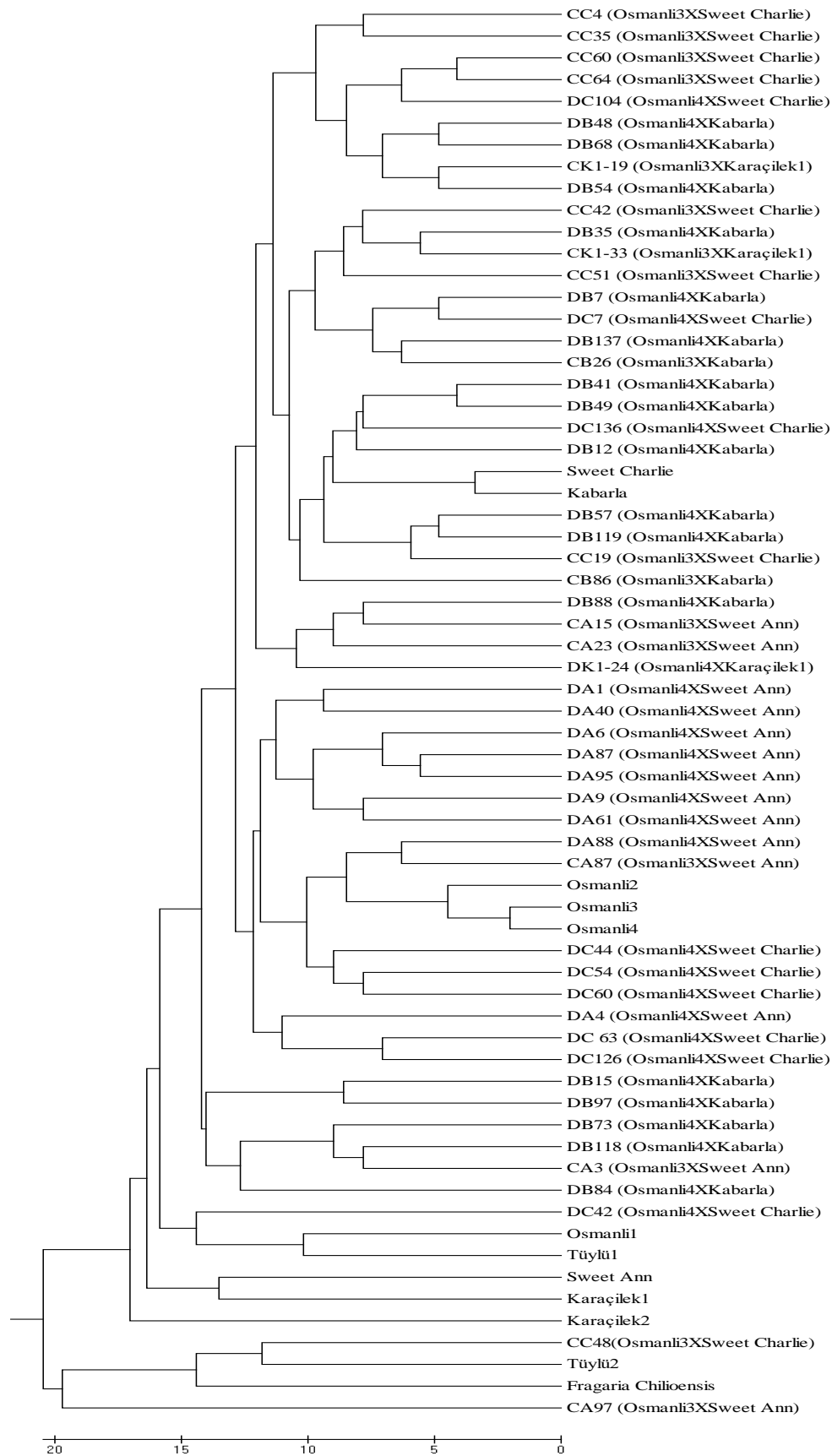
***Figure 1.** Dendrogram obtained with cluster analysis*
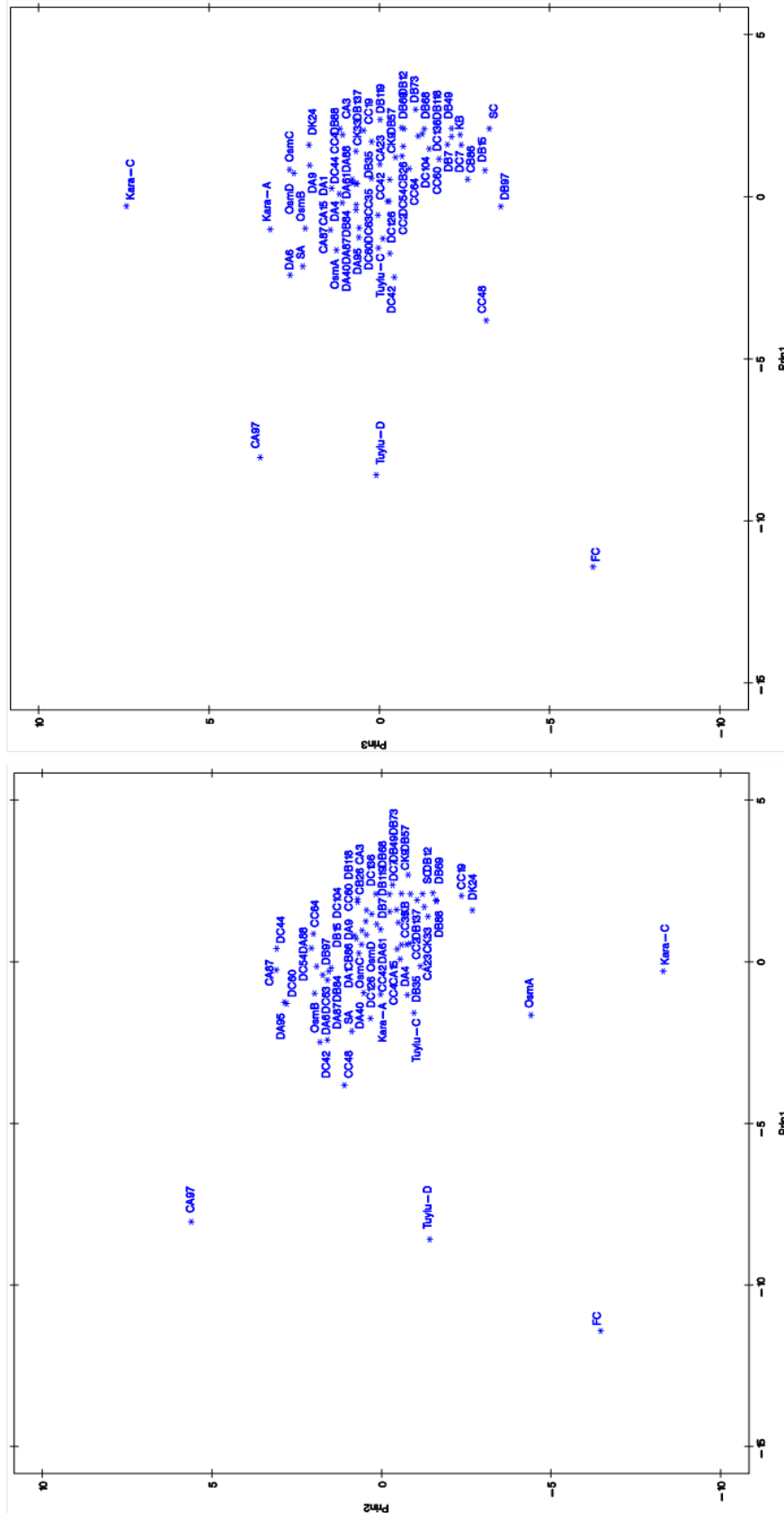
Figure 2. *Scatterplot of PCo2 vs PCO1 and scatterplot of PCo3 vs PCO1 obtained by principal coordinate analysis*

In the literature, it was stated that 'Osmanli' local cultivar and *F. chiloensis* show similarity, however, Tuylu-2 genotype and *F. chiloensis* showed similarity with 14.00% in this study. All the local genotypes were collected from Eregli district in Black Sea Region, also, it was observed that Tuylu and Osmanli local cultivars showed similarity in terms of fruit structure. *F. chiloensis* and Tuylu local cultivars were in the same group, thus, it is thought that Osmanli local cultivar changed by the time due to cross pollination.

Selected F1 genotypes will be used in the further studies leading to obtain new strawberry cultivars. Specific molecular markers could also be generated using these F1 genotypes.

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