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# Morphologic and molecular assessments of cucumber (*Cucumis sativus* L.) landraces

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# Abstract

In this study, 90 locally grown cucumber (*Cucumis sativus* L.) landraces were collected and morphologically characterized using 20 descriptors derived from UPOV (International Union for the Protection of New Varieties of Plants). Genetic diversity and relationships of the genotypes were revealed using 20 sequence-related amplified polymorphism (SRAP) marker combinations. The discrimination power of each polymorphic marker (estimated by the polymorphism information content) ranged from 0.15 to 0.99 with an average of 0.73. Dice's similarity coefficient ranged between 0.00-1.00. The cluster analysis that was conducted using the unweighted pair group method of arithmetic averages (UPGMA) for both molecular and morphologic data showed that all of the genotypes fell into two main groups and many subdivisions. According to morphological data, fruit length, diameter and weight of the genotypes were determined between 6.5 - 32.5 cm, 25 - 52 mm and, 28 - 625 g respectively. It is clear from the results, a moderate level of genetic diversity, which has the potential for broadening the genetic base, was observed among the Turkish cucumber landraces.

Keywords: breeding; characterization; collection; diversity; NTSYS; SRAP

# Introduction

Cucumber (*Cucumis sativus* L.) is grown widely in open field or glasshouse conditions. It has modest levels of vitamin A, C, and, some phenolic compounds known to have antioxidant properties (Kumar *et al.*, 2010). India is the primary center of diversity for cucumber (Wehner and Robinson, 1991). It has a narrow genetic base with a genetic variability of 3-8% so it is more susceptible to biotic and abiotic stresses (Rao and Hodgkin, 2002).

Nowadays, climate change threatens to reduce crop yield and harming the food security. Local landraces have adaptation skills to shifting climatic conditions. They have been a major resource for crop breeding, due to their tolerance to the extreme climate changes and the resistance to some pests and diseases (Rao and Hodgkin, 2002). Their genetic and phenotypic features need to be characterized carefully for the proper use in breeding programs (Parisi *et al.*, 2017). The success of any plant breeding program depends on the genetic

variability (Bartaula *et al.*, 2019) Establishing a gene pool and determination of their relationships is the first step for plant improvement (Normohammadi *et al.*, 2017).

Morphological markers are good determinants but they are not stable and they influenced by ecologic conditions (Fares *et al.*, 2009). Molecular markers are not affected by the environmental changes and they serve as a tool to overcome the deficiencies of morphological markers in the characterization studies (Agarwal *et al.*, 2008; Gostimsky *et al.*, 2005; Gulsen and Mutlu, 2005). For this reason, using morphological and molecular marker systems together is preferred for the better clarification of the relationships between the genotypes.

In this research, 20 descriptors which were derived from "International Union for Protection of New Plant Varieties" (UPOV) were used for the morphological determinations and the most polymorphic 20 SRAP marker combinations were used for the molecular characterization. SRAP is a PCR-based technique. It depends on the amplification of open reading frames (ORFs) and two-primer amplifications. The primer sequences are random and they have 17-21 nucleotides in length (Agarwal *et al.*, 2008).

Turkey is located on the transition zones. Its geographic position and the variety of agro-climatic conditions caused great biodiversity. This research aimed to establish a characterized gene pool formed by cucumber landraces cultivated in different regions of Turkey. Even though many studies on Turkish cucumber diversity have been completed earlier (Gozen, 2008; Aydemir, 2009), for the first time with this research Turkish landraces were collected and characterized using morphologic and molecular markers together. Therefore, obtained results are significant both for the local and international researchers.

## Materials and Methods

## Material

This study was conducted at Bati Akdeniz Agricultural Research Institute-Antalya located between 36°55'0.98" N 31°05'56.00" E coordinates. Ninety landraces were collected from 52 different areas, used as plant material. The information about their given codes, cultivation areas, and geographic locations were presented in Table 1. Thirty-six accessions came from the Genebank of Aegean Agricultural Research Institute and the other 54 accessions collected from cucumber cultivation areas via agricultural district offices before starting to this study.

## Methods

## <u>Gene pool establishment</u>

Collected genotypes were selfed for two generations to avoid risks because of possible seed mixture. For this reason, all seeds were planted in 2017 spring and autumn season. When the seedlings at the 2-3 leaf stage, they were transplanted to the greenhouse. During the vegetation period, watering, fertilizing, and diseases and pest management were carefully followed. At the fruiting stage, mixed accessions were detected; they were considered as another genotype and coded differentially. Pollination studies were made in each population separately. Hand pollinated flowers were tagged with coloured materials. Following pollination, after 50-55 days, ripen fruits were harvested; seeds were washed for cleaning and dried then kept in cooled storage until the next season.

## Morphological characterization

Spring and autumn period of 2018, morphological characterization of the genotypes was carried out according to the descriptors were given in Table 2. Five plants per accession were characterized, with 20 descriptors, eight of them referred to plant traits and 12 related to the fruit.

Code	Cultivation area	Geographic location	Code	Cultivation area	Geographic location	
YG-2	Çanakkale	40°08'60.00" N	YG-37	Osmaniye	37°04'27.01" N	
YG-4	Bursa	40°11'44.12" N	YG-38	Konya	38°00'28.80" N	
YG-6/1	Edirne	41°40'37.88" N	YG-39	Şanlıurfa	37°08'60.00" N	
YG-6/2	Edirne	41°40'37.88" N	YG-40	Hatay	36°25'29.39" N	
YG-6/3	Edirne	41°40'37.88" N	YG-42	Amasya	40°39'11.99" N	
YG-6/4	Edirne	41°40'37.88" N	YG-44	Manisa	38°36'43.27" N	
YG-7	Zonguldak	41°26'59.99" N	YG-45	Çankırı	40°35'59.82" N	
YG-7/2	Zonguldak	41°26'59.99" N	YG-46	Çorum	40°32'56.00" N	
YG-8	Kocaeli	40°51'7.20" N 29°52'40.80"	YG-47	Niğde	37°57'56.99" N	
YG-12	Tokat	40°18'50.00" N	YG-48	Gaziantep	37°03'33.98" N	
YG-12/1	Tokat	40°18'50.00" N	YG-49	Erzincan	39°44'59.99" N	
YG-13	Trabzon	41°00'18.00" N	YG-50	Kayseri	38°37'17.99" N	
YG-14	Afyon	38°45'24.01" N	YG-51	Samsun	41°17'15.00" N 36°20'0.60"	
YG-14/2	Afyon	38°45'24.01" N	YG-52	Gaziantep	37°03'33.98" N	
YG-15	Rize	40°55'32.39" N	YG-53	Hatay	36°25'29.39" N	
YG-16/1	Amasya	40°39'11.99" N	YG-54	Ankara	39°55'11.53" N	
YG-16/2	Amasya	40°39'11.99" N	YG-55	Kayseri	38°37'17.99" N	
YG-17	Kütahya	39°25'27.01" N	YG-56	Hatay	36°25'29.39" N	
YG-17/2	Kütahya	39°25'27.01" N	YG-57	Zonguldak	41°26'59.99" N	
YG-18	Bilecik	40°08'30.91" N	YG-58	Karaman	37°10'31.20" N	
YG-19	Trabzon	41°00'18.00" N	YG-59	Konya	38°00'28.80" N	
YG-20	Gümüşhane	40°27'22.19" N	YG-60	Mersin	36°47'42.94" N 34°37'4.51"	
YG-21	Aydın	37°50'39.84" N	YG-61	Mersin	36°47'42.94" N 34°37'4.51"	
YG-22	Artvin	41°07'60.00" N	YG-64	Aksaray	38°22'21.18" N	
YG-22/2	Artvin	41°07'60.00" N	YG-65	Mardin	37°18'47.12" N	
YG-23	Muğla	37°13'5.05" N 28°21'59.40"	YG-66	Şanlıurfa	37°08'60.00" N	
YG-23/2	Muğla	37°13'5.05" N 28°21'59.40"	YG-67	Antalya	36°54'29.23" N	
YG-24	Mus	38°43'53.87" N 41°29'5.35"	YG-68	Diyarbakır	37°54'49.07" N 40°13'1.96"	
YG-25	Hakkari	37°34'27.98" N	YG-69	K.Maras	37°34'59.99" N	
YG-26	Kırıkkale	39°50'59.99" N	YG-70	Manisa	38°36'43.27" N	
YG-27	Kırşehir	39°08'60.00" N	YG-71-	Adana	37°00'6.01" N 35°19'44.00"	
YG-28	Balıkesir	39°23'6.00" N 27°31'58.80"	YG-71-	Adana	37°00'6.01" N 35°19'44.00"	
YG-28/2	Balıkesir	39°23'6.00" N 27°31'58.80"	YG-72	Antalya	36°54'29.23" N	
YG-29	Kastamonu	41°22'11.99" N 33°46'9.59"	YG-74	Erzincan	39°44'59.99" N	
YG-30	İzmir	38°25'19.20" N	YG-77	Yozgat	39°49'12.00" N	
YG-31	Sivas	39°31'6.59" N 37°17'25.20"	YG-78	Bursa	40°11'44.12" N	
YG-31/2	Sivas	39°31'6.59" N 37°17'25.20"	YG-	Bursa	40°11'44.12" N	
YG-32	Yozgat	39°49'12.00" N	YG-79	Karaman	37°10'31.20" N	
YG-32/2	Yozgat	39°49'12.00" N	YG-81	Mersin	36°47'42.94" N 34°37'4.51"	
YG-33	Nevşehir	38°37'30.00" N	YG-82	Antalya	36°54'29.23" N	
YG-33/2	Nevsehir	38°37'30.00" N	YG-83	Kilis	36°42'57.96" N	
YG-34	Çanakkale	40°08'60.00" N	YG-85	Osmaniye	37°04'27.01" N	
YG-35	Istanbul	41°00'49.82" N	YG-89	Malatya	38°21'0.65" N 38°19'0.01" E	
YG-36	Samsun	41°17'15.00" N 36°20'0.60"	YG-90	Adana	37°00'6.01" N 35°19'44.00"	
YG-36/2	Samsun	41°17'15.00" N 36°20'0.60"	YG-91	Yalova	40°39'18.04" N	

**Table 1.** The given codes, their cultivation areas, and geographic locations

## DNA extraction

Selfed seeds of 90 genotypes were sown again in the spring of 2019. When the seedlings were at 2 - 4 leaf stage, plant samples were taken to perform DNA extraction. The DNAs of plants were isolated according to the modified CTAB protocol by Doyle and Doyle (1990). After the extraction, the DNA concentrations were determined in 1% agarose gel. All extracted genomic DNAs were stored at -20  $^{\circ}$ C in TE buffer for SRAP analysis.

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Features	Explanations	Features	Explanations	
Growth habit	(1) Weak, (3) Medium, (5) Strong	Intensity of skin colour	(1) Light, (3) Medium, (5) Intense	
Length of first 15 internodes	(1) Very short, (3) Short, (5) Medium, (7) Long	Prickliness on fruit	(1) Absent, (3) Present	
Length of internodes	(1) Short, (3) Medium, (5) Long	Colour of prick	(1) Black, (3) White	
Size of blade	(1) Small, (3) Medium, (5) Big	Groove on fruit	(1) Absent, (3) Medium, (5)	
Intensity of green	(1) Light, (3) Medium, (5) Very	Length of peduncle	(1) Short, (3) Medium, (5) Long	
Sex expression	(1) Monocie, (3) Gynocie, (5) Andromonocie	Fruit netting distribution at seed harvest stage	(1) Absent, (3) Present	
Number of female flowers per node	(1)1, (3) 1/3, (5) More than 3	Fruit skin colour at physiological ripening	<ul><li>(1) Yellow, (3) Orange (5) Brown,</li><li>(7) White, (9) Yellowish-green,</li><li>(11) Green</li></ul>	
Parthenocarphy	(1) Absent, (3) present	Average fruit weight	(1) Light, (3) Medium, (5) Heavy	
Fruit Length	(1) Short, (3) Medium, (5) Long, (7) Very long	Flowering time	(1) Early, (3) Medium, (5) Late	
Fruit diameter	(1) Narrow, (3) Medium, (5) Broad	Number of lateral shoots in first 15 nodes	(1) Few or absent, (3) Medium, (5) Very	

**Table 2.** Morphologic descriptors derived from UPOV by Gozen (2008)

## SRAP analysis

The most polymorphic 20 SRAP primer combinations were determined first before starting this stage. The primers were obtained from the previous studies on cucumber conducted by Ferriol *et al.* (2003; 2004). SRAP primers and their sequences that were used to create 20 combinations were presented in Table 3. The PCR reaction solution was composed of 1  $\mu$ l DNA (20 ng DNA), 15  $\mu$ l 1 x PCR master mix, 1  $\mu$ l each primer (0.3  $\mu$ M each primer) and 7  $\mu$ l ddH2O. PCR amplification was 5 minutes for initial denaturation at 94 °C, then 5 cycles composed of denaturing at 94 °C for 1 minute, annealing at 35 °C for 1 minute, extension at 72 °C for 1 minute and followed by 35 cycles; heating at 94 °C for 1 minute, annealing at 55 °C for 1 minute, extension at 72 °C. PCR amplifications were repeated twice for each primer combination.

The PCR products were fractionated on 2.5% high-resolution agarose gel in 1XTAE buffer at 110 V for 3 hours and photographed (Kodak Gel Logic 200) under UV light for further analysis. A 100 bp DNA ladder was used as a molecular standard.

## Data analysis

Genetic similarity and phylogenetic relationships were analysed by the UPGMA (Un-weighted pairgroup method, arithmetic average) clustering procedure using the software NTSYS (Numerical Taxonomy Multivariate Analysis System) version pc 2.2 (Rohlf, 1998). For morphological data, each genotype was characterized with descriptors were shown in Table 2. For molecular analysis data, each genotype was identified for each primer based on the presence (1) and absence (0) of bands. Similarity indices were calculated according to Dice (1945). Polymorphism rates (Pr) = (Polymorphic allel / Total allel) X 100 and polymorphism information content (PIC) =  $1-\Sigma$  fi2 (Smith et al., 1997) were calculated.

Primers	Sequences	Primers	Sequences
Me1	TGA GTC CAA ACC GGA TA	Em2	GAC TGC GTA CGA ATT TGC
Me2	TGA GTC CAA ACC GGA GC	Em4	GAC TGC GTA CGA ATT TGA
Me3	TGA GTC CAA ACC GGA AT	Em5	GAC TGC GTA CGA ATT AA
Me4	TGA GTC CAA ACC GGA CC	Em6	GAC TGC GTA CGA ATT GCA
Me5	TGA GTC CAA ACC GGA AG	Em7	GAC TGC GTA CGA ATT CAA
Me6	TGA GTC CAA ACC GGA CA	Em9	GAC TGC GTA CGA ATT CAG
Me8	TGA GTC CAA ACC GGA CT	Em10	GAC TGC GTA CGA ATT CAT
Me9	TGA GTC CAA ACC GGA GG	Em11	GAC TGC GTA CGA ATT CTA
Me10	TGA GTC CAA ACC GGA AA	Em13	GAC TGC GTA CGA ATT CTG
Me11	TGA GTC CAA ACC GGA AC	Em14	GAC TGC GTA CGA ATT CTT
Me12	TGA GTC CAA ACC GGA GA	Em15	GAC TGC GTA CGA ATT GAT
Me15	CGA ATC TTA GCC GGA TA	Em16	GAC TGC GTA CGA ATT GTC

Table 3. SRAP primers and their sequences which were used to create combinations

## **Results and Discussion**

In this study collected local cucumber genotypes were determined morphologically different. Morphological observations of 47 genotypes using 10 UPOV descriptors were presented in Table 4 as an example. According to this data, genotypes were classified in terms of their growth habit, 28 of them (31%) determined as strong, 49 of them (54%) were medium and 13 of them (14%) were reported as weak. Poor growth leads to plants susceptible to diseases, pests, and harsh environmental conditions. Therefore, while a breeding program set up, plant growth habits should be considered. Seventy-eight of used genotypes were detected as monoecious, regarding the flowering time, lateness was observed on 54 genotypes and 19 of them have no prickliness.

Although cultivated cucumbers are mostly similar in terms of root and stem development, when the fruit characteristics, especially fruit shape is considered they could be quite diverse (Bisognin, 2002). In the current study, fruit length, diameter, and weight of the genotypes were determined between 6.5 - 32.5 cm, 25-52 mm, and 28-625 g respectively. Features such as fruit length, diameter, and color are used to determine the economic importance of a variety (Kennard and Havey, 1995; Ahmed *et al.*, 2004).

It was understood from the study, while the cucumber is native to India where its wild forms still exist (Sebastian *et al.*, 2010), examined landraces are morphologically quite diverse because of Turkey's location between the transition zones (Figure 1). Genotypes were also observed regarding their number of lateral shoots in the first 15 nodes. While 11 genotypes have no lateral shoots, 79 genotypes have lateral shoots between 4 and 14. In a study, Wehner and Guner (2002) reported that, some genotypes achieve high yielding with branching ability while others reach it with more nodes. Also, the higher yield of some genotypes in their study was associated with both extra branches and nodes.



Figure 1. Different fruit types of germplasm

		Langeb of			Number			A	Number	
	C	Length of	Length of	Size of	Number	Fruit	Fruit	Averag	of lateral	Intensity
Genotype	Growth	first 15	internode	blade	of remaie	Length	Diameter	e rruit	shoots in	of skin
	habit	inter nodes	(cm)	(cm)	nowers	(cm)	(mm)	weight	first 15	colour
		(cm)			per node			(g)	nodes	
YG-2	Medium	92,7	9,7	32,0	1	14,7	37,9	242	11,3	Medium
YG-4	Medium	80,0	6,3	18,6	1	14,6	39,2	185,5	5,6	Medium
YG-6/1	Medium	93,0	6,2	20,3	1	6,5	53,5	230,0	6,5	Light
YG-6/2	Medium	95,0	6,5	23,0	1	6,5	55,8	250,0	8,5	Medium
YG-6/3	Medium	93,5	6,0	22,6	1	6,5	54,9	200,0	8,5	Medium
YG-6/4	Strong	94,5	6,1	24,3	1	7,8	52,7	222,0	10,0	Light
YG-7	Medium	72,0	7,7	30,6	1	14,7	40,8	165,5	7,0	Medium
YG-7/2	Weak	72,0	7,1	24,3	1	8,3	32,8	200,0	9,5	Medium
YG-8	Medium	89,7	6,6	26,3	1	14,8	39,5	91,6	11,0	Light
YG-12	Medium	105,2	8,1	25,5	1	12,3	38,2	152,0	11,5	Medium
YG-12/2	Medium	100,2	8,0	23,5	1	10,3	38,2	132,0	11,5	Medium
YG-13	Medium	94,0	8,6	29,6	1	17,1	39,35	88,0	14,0	Light
YG-14	Weak	89,0	7,2	27,0	1	13,6	38,5	121,0	11,0	Medium
YG-14/2	Weak	84,0	8,3	25,0	1-3	13,3	36,7	237,0	9,5	Medium
YG-15	Weak	105,0	10,6	19,0	1-3	18,0	39,2	223,0	9,0	Light
YG-16	Medium	95,0	8,3	26,3	1-3	12,6	30,5	123,0	6,5	Medium
YG-16/2	Weak	112,5	7,7	29,3	1	15,8	34,5	191,0	10,0	Light
YG-17	Medium	101,0	9,1	28,0	1-3	13,1	35,6	135,5	12,5	Medium
YG-17/2	Medium	98,0	9,7	26,3	1-3	15,0	37,5	185,0	12,5	Medium
YG-18	Weak	85,0	7,7	24,6	1-3	10,5	34,2	172,0	12,5	Medium
YG-19	Medium	83,0	8,8	28,6	1-3	10,1	34,5	49,0	7,5	Light
YG-20	Strong	112,5	9,8	26,6	1-3	14,0	34,7	99,3	10,5	Medium
YG-21	Weak	94,0	8,8	27,6	1	32,5	42,05	585,0	9,5	Light
YG-22	Strong	102,5	9,6	29,3	1-3	22,1	37,15	407,5	4,0	Light
YG-22/2	Medium	71,0	6,2	31,0	1	29	37,6	560,0	6,5	Medium
YG-23	Medium	80,5	8,2	27,6	1-3	11	35,6	183,0	10,5	Light
YG-23/2	Medium	94,5	7,2	31,3	1	13,6	35,35	54,6	11,0	Light
YG-24	Strong	98,0	8,0	31,6	1-3	12,6	34,95	119,0	11,5	Medium
YG-25	Medium	106,5	8,0	28,3	1-3	9,8	38,4	109,0	12,5	Intense
YG-26	Strong	89,5	10,0	29,3	1-3	18	39,5	28,0	11,5	Light
YG-27	Strong	97,0	9,25	28,0	1-3	9	30,2	109,0	10,5	Light
YG-28	Medium	107,5	10,6	26,6	1-3	11	30,4	47,5	9,5	Light
YG-28/2	Medium	91,0	7,3	29,6	1	10,6	33,7	104,0	6,0	Medium
YG-29	Medium	80,5	8,2	27,3	1-3	10,5	29,65	81,0	11,0	Light
YG-30	Medium	77,0	7	27,6	1-3	12,5	33,6	176,0	5,5	Medium
YG-31	Weak	86,0	8,3	24,3	1-3	15,3	31,45	143,0	8,0	Light
YG-31/2	Medium	83,5	8,5	27,3	1-3	11,1	30,4	37,3	12,0	Light
YG-32	Medium	96,5	10,0	26,3	1-3	13,6	34,8	81,0	9,5	Light
YG-32/2	Medium	98,0	8,2	29,0	1-3	21	25,2	184,0	6,5	Light
YG-33	Medium	98,5	9,0	17,0	1	8,5	27,7	36,3	7,5	Light
YG-33/2	Medium	80,2	7,5	27,3	1-3	20,1	39,5	166,0	8,5	Medium
YG-34	Medium	73,5	8,4	30,0	1-3	21,8	34,9	245,0	8,5	Medium
YG-35	Medium	95,0	8,5	27,6	1	15,8	34,7	270,0	10,0	Light
YG-36	Medium	106,5	10,2	28,3	1-3	16,8	35,9	135,5	8,0	Medium
YG-36/2	Medium	89,0	7,5	28,0	1-3	15,1	35,6	56,0	8,5	Light
YG-37	Medium	101,0	8,5	26,0	1-3	22,6	38,2	381,0	11,0	Medium
YG-38	Strong	130,2	11,3	27,6	1	32,5	42,05	585,0	9,5	Intense

**Table 4.** Some of the phenotypic data related to important variable traits of first 47 accessions (remaining43 accessions' data wasn't presented here to keep the article shorter)

In this study, 20 morphologic descriptors which were derived from UPOV by Gozen (2008) used for the definition of the phylogenetic relationships among the 90 Turkish cucumber landraces. Obtained morphological data was used to draw the dendrogram (Figure 2) generated by the UPGMA method.



Figure 2. UPGMA dendrogram showing phylogenetic relationships of local cucumber genotypes using morphological data

According to the dendrogram presented in Figure 2, two major and four subgroups were revealed with the similarity rate of %25. Although the most distant genotypes were determined YG-2 and YG-34 gathered from Canakkale, the most similar ones were YG-61 from Mersin, YG-67 from Antalya (Figure 3). Thus, the fact that, the origins of genotypes were close or far apart did not affect their morphological similarity in the research.



Figure 3. The most distant and similar genotypes according to UPGMA dendrogram which was drawn using morphological descriptors

Determination of the genetic diversity of a gene pool is crucial for the success of the breeding programs. Because they are more reliable and consistent, using neutral markers is essential in such studies (Al-Rawahi *et al.*, 2011). Therefore, morphological and molecular markers were preferred in the current study and the discrimination power of each polymorphic marker (estimated by the polymorphism information content) ranged from 0.15 to 0.99 with an average of 0.73. Dice's similarity coefficient ranged from 0.00 to 1.00. The dendrogram provided from the molecular data was given in Figure 4. Accordingly, 90 genotypes used in the study divided into 2 main clusters which were subdivided into themselves. Genetic diversity and relationships of the genotypes were revealed using 20 sequence-related amplified polymorphism (SRAP) marker combinations.

Genotypes namely YG-15 (from Rize), 6/1, 6/2, 6/3, 6/4 (from Edirne), 33 (from Nevsehir), 35 (from Istanbul) and 83 (from Kilis) in the cluster C created a very different group than the other 82 genotypes (Figure 4). Among the genotypes included in cluster C; YG-15, YG-33, YG-35 were understood to be *Cucumis melo* var. *flexuosus* and YG-6/1-2-3-4 and YG-83 were thought as wild cucumber genotypes (Figure 5).



Figure 4. UPGMA dendrogram showing similarity of local cucumber genotypes using molecular data



Figure 5. Genotypes in cluster C in terms of the SRAP analysis

In addition to this, YG-15 and YG-83 were defined as the most distant genotypes of the Cluster C. In terms of the morphologic data, while the YG-6/1-3, YG-15, YG-33, and YG-35 were in the group 1, YG-6/2-4 and YG-83 were found in group 2. The phenomenon of hybrid vigour occurring from the crosses between genetically distinct genotypes forms a prominent meaning of crop improvement (Tiwari, 2015). Therefore, morphologically similar and molecularly distinct genotypes could be used as parent lines in the further cucumber breeding studies.

According to the dendrogram presented in Figure 4, there were 37 genotypes in cluster B, remaining 45 genotypes were found in cluster A. According to Dice similarity and distance indexes group A consisted of genetically the most similar genotypes with the smallest similarity value of 0.88. Mliki *et al.* (2003) studied the genetic diversity of 26 African cucumber accessions. Their data suggest that some genotypes from Egypt have unique genetic diversity and this germplasm has the potential for broadening the genetic base of cucumber breeding studies. Gaikwad (2011) reported that genotypic variation was slightly low as compared to phenotypic variation in cucumber. The highest genotypic, as well as phenotypic variation was observed on fruit length, fruit number, and fruit weight. With the current study, it was cleared that Turkey has reasonable genetic diversity in terms of cucumber and the 20 SRAP primer combinations established here could be used for germplasm identification in further classification studies. Today management of biodiversity for sustainable food has become a significant subject. Landraces of every country represent a major source of diversity because of the adaptation capability to the habitats of origin. These resources could be used in the plant breeding programs for the constitution of more resilient and qualitatively improved varieties (Parisi *et al.*, 2017).

In Table 5, used 20 SRAP primer combinations and their number of total alleles, polymorphic alleles, polymorphism rates (%), and polymorphism information content (PIC) values were presented. Considering these, the most allelic primer combination was found as me11-em9 with the total 12 alleles. The least allelic primer combinations were determinate as me12-em10 and me6-em16 with the 5 alleles. Polymorphism rate (%) was ranked between %16- %100.

Primers	Total alleles	Polymorphic alleles	Polymorphism rate (%)	PIC*
me1-em4	9	6	66.67	0.69
mel-em11	6	1	16.67	0.15
me2-em10	8	4	50.00	0.48
me2-em7	6	1	16.67	0.99
me3-em7	8	3	37.50	0.43
me3-em15	9	7	77.78	0.98
me4-em13	7	3	42.86	0.85
me4-em14	11	7	63.64	0.99
me5-em6	9	2	22.22	0.99
me5-em13	11	5	45.45	0.82
me6-em16	5	4	80.00	0.99
me8-em16	8	4	50.00	0.99
me8-em11	6	2	33.33	0.90
me9-em2	6	6	100.00	0.99
me9-em11	9	5	55.56	0.82
me10-em9	6	3	50.00	0.38
mell-em9	12	7	58.33	0.59
me12-em2	8	2	25.00	0.52
me12-em10	5	4	80.00	0.45
me15-em5	7	3	42.86	0.63
Total	156	79	-	-
Mean	7.8	3.9	50.73	0.73

Table 5. Total alleles, polymorphic alleles, polymorphism rate (%), and PIC values of used SRAP primer combinations

\*Polymorphism information content

## Conclusions

With this project, 90 of the local cucumber genotypes grown in different regions of Turkey were collected and morphologically characterized considering the 20 UPOV descriptors. Later, genetic similarity of the genotypes was identified between the 0.00 - 1.00 using 20 SRAP primer combinations showing polymorphism. Used marker systems also allowed the efficient management of the gene pool by eliminating redundant accessions. It was cleared that Turkey has reasonable diversity regarding cucumber. It was also understood that the obtained gene pool has a potential for broadening the genetic base of cucumber for further breeding studies. According to the clusters achieved in the study, morphologically similar and molecularly distinct genotypes could be used as parent lines in the further cucumber breeding studies.

## **Authors' Contributions**

LK and VG collected the cucumber landraces. AY did molecular studies in the laboratory. EC did all the field works and morphological observations, measurements etc., in addition she evaluated the data and wrote the manuscript. All authors read and approved this final version of the manuscript.

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## **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

#### References

- Agarwal M, Shrivastava N, Padh H (2008). Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports 27(4):617-631. *https://doi.org/10.1007/s00299-008-0507-z*
- Ahmed M, Abdul H, Zarqa A (2004). Growth and yield performance of six cucumber (*Cucumis sativus* L.) cultivars under agro-climatic conditions of Rawalakot, Azad Jammu and Kashmir. International Journal of Agriculture and Biology 6:396-399.
- Al-Rawahi MF, Al-Said A, Khan IA, Al-Khanjary S (2011). Diversity of cucumber accessions in Oman. International Journal of Agriculture and Biology 13:505-510.
- Aydemir I (2009). *Determination of genetic diversity in cucumber (Cucumis sativus L.) germplasms*. MSc thesis, İzmir Institute of Technology, pp 40.
- Bartaula S, Adhikari A, Panthi U, Karki P, Timalsena K (2019). Genetic variability, heritability and genetic advance in cucumber (*Cucumis sativus* L.). Journal of Agriculture and Natural Resources 2(1):215-222.
- Bisognin DA (2002). Origin and evolution of cultivated cucurbits. Ciencia Rural 32:715-723.
- Dice LR (1945). Measures of the amount of ecologic association between species. Ecology 26:297-302.
- Doyle JJ, Doyle, JL (1990). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bullettin 19(1):11-15.
- Fares K, Guasmi F, Touil L, Triki T, Ferchini A (2009). Genetic diversity of pistachio tree using inter simple sequence repeat markers (ISSR) supported by morphological and chemical markers. Biotechnology 8(1):24-34. https://scialert.net/abstract/?doi=biotech.2009.24.34
- Ferriol M, Pico B, Nuez F (2003). Genetic diversity of some accessions of *Cucubita maxima* from Spain using RAPD and SRAP markers. Genetic Resources and Crop Evolution 50(3):227-238.
- Ferriol M, Pico B, de Cordova PF, Nuez F (2004). Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. Crop Science 44:653-664.
- Gaikwad AG, Musmade AM, Dhumal SS, Sonawane HG (2011). Variability studies in cucumber (*Cucumis sativus* L.). Ecology Environment and Conservation 17(4):799-802.
- Gozen V (2008). Morphologic characterization in hybrid greenhouse cucumber breeding and determination of appropriate hybrid combinations and hybrid seed quality. Phd thesis, Ankara pp 185.
- Gulsen O, Mutlu N (2005). Bitki biliminde kullanılan genetic markırlar ve kullanım alanları. Alatarım 4(2):27-37.
- Gostimsky SA, Kokaeva ZG, Konovalov FA (2005). Studying plant genome variation using molecular markers. Russian Journal of Genetics 41(4):378-388.
- Kennard WC, Havey MJ (1995). Quantitative trait analysis of fruit quality in cucumber: QTL detection, confirmation and comparison with mating-design variation. Theoretical and Applied Genetics 91:53-61.
- Kumar D, Kumar S, Singh J, Narender, Rashmi, Vashistha BD, Singh N (2010). Free radical scavenging and analgesic activities of *Cucumis sativus* L. fruit extract. Journal of Young Pharmacists 2:365-368. https://doi.org/10.4103/0975-1483.71627

- Mliki A, Staub JE, Zhangyong S, Ghorbel A (2003). Genetic diversity in African cucumber (*Cucumis sativus* L.) provides potential for germplasm enhancement. Genetic Resources and Crop Evolution 50(5):461-468.
- Normohamadi S, Solouki M, Heidari F (2017). Diversity in cucumber genotypes based on morphological traits and SSR molecular markers. Biosciences Biotechnology Research Asia 14(2):775-782. http://dx.doi.org/10.13005/bbra/2507
- Parisi M, Di Dato F, Ricci S, Mennella G, Cardi T, Tripodi P (2017). A multi-trait characterization of the 'Friariello' landrace: a Mediterranean resource for sweet pepper breeding. Plant Genetic Resources 15(2):165-176. https://doi.org/10.1017/S1479262115000490
- Rao VR, Hodgkin T (2002). Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue and Organ Culture 68:1-19.
- Rohlf FJ (1998). NTSYS-PC: Numerical taxonomy and multivariate analysis system. Release 2.20j. Exeter Software, Setauket, N.Y.
- Sebastian P, Schaefer H, Telford IR, Renner SS (2010). Cucumber and melon have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. Proceedings of the National Academy of Sciences 107(32):14269-14273.
- Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, ... Ziegle J (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPS and pedigree. Theoretical and Applied Genetics 95:163-173.
- Staub JE, Serquen FC, Horejsi T, Chen JF (1999). Genetic diversity in cucumber (*Cucumis sativus* L.): IV. An evaluation of Chinese germplasm. Genetic Resources and Crop Evolution 46(3):297-310.
- Tiwari R (2015). Molecular characterization and genetical studies in cucumber (*Cucumis sativus* L.) (Doctoral dissertation, Ph.D. (Agri.) Thesis, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, pp 129.
- Wehner TC, Robinson RW (1991). A brief history of the development of cucumber cultivars in the US. Cucurbit Genetics Cooperative 14:1-4.
- Wehner TC, Guner N (2002). Growth stage, flowering pattern, yield, and harvest date prediction of four types of cucumber tested at 10 planting dates. In: XXVI International Horticultural Congress: Advances in Vegetable Breeding 637:223-230.



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