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Molecular, morphological and phytochemical characterization of some watermelon (*Citrullus lanatus* L.) genotypes

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Abstract: Watermelon (*Citrullus lanatus* L.) is grown in tropical and temperate regions and an economically important crop. Characterization studies of watermelon may provide valuable information for breeding and research programs. The objectives of this study were to determined of morphological, phytochemical, genetic diversity and population structure among the watermelons. Morphological and phytochemical variations including sugar contents were determined in 96 watermelon genotypes grown in the field. The average number of fruits per plant was determined as 2.52 ± 0.06, and the average yield was determined as 6.2 ± 0.11 kg/m^2. The mean total sugar was determined as 6.27 ± 0.12 %, and the lowest value was measured in genotype 234 (1.1%); the highest value was measured in genotype number 184 (8.66%). A total of 62 SSR (Simple Sequence Repeat) primers were used in the molecular characterization study. The similarity coefficients among the 96 genotypes varied between 0.23 and 0.99. This study indicates that there is a wide morphological and sugar parameters variation among watermelon genotypes but narrow molecular genetic diversity. It also provides useful information for watermelon breeding studies.

Keywords: watermelon; characterization; genetic; SSR; sugar analysis

Watermelon (*Citrullus lanatus*) is grown in tropical and temperate regions and is a member of the Cucurbitaceae family and it is the only cultivated species in the *Citrullus* genus (Chomicki, Renner 2015). Watermelon is an economic crop, accounting for approximately 9.5% of the total global vegetable production (Aslam et al. 2020). Watermelon is among the top five most consumed products worldwide, production in 2021 is approximately 101.62 million tons, and the main producers are countries such as China, Türkiye, India, Brazil, Algeria and Iran (FAOSTAT 2021). It has been cultivated for at least 4 000 years (Schaffer, Paris 2016) and today, hundreds of watermelon varieties have been commercialized in today's seed market, especially F1 hybrids based on the heterosis. Watermelon contains sugar, fiber, vitamins, antioxidants, amino acids, and minerals that can have significant health effects (Collins et al. 2007; Garcia-Lozano et al. 2020). Determining the qualitative and quantitative phenotypic and phytochemical characteristics of the crop is vital for designing market preferred varieties.

Sweetness is an important feature that determines the eating quality of watermelon fruit (Yativ et al.

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2010; Liu et al. 2013) and determining the factors that determine fruit sweetness is important in terms of breeding criteria. There have been several research on the morphological characteristic, but little research on sugar contents of watermelon genetic resources. The total sugar content and the ratios of glucose, fructose and sucrose determine the fruit sweetness of watermelon (Zhu et al. 2017). The variation of sugar content depends on environmental conditions, genotype and analyzed fruit parts of the plant (Yativ et al. 2010). Characterization of watermelon plants for important traits such as disease resistance and plant qualitative and quantitative parameters is needed to support the assembly of superior varieties. In some previous studies, sugar ratios were determined in different watermelon genotypes and it was determined that there were variations (Yau et al. 2010; Yoo et al. 2012).

Watermelon breeding programs are at risk of genetic erosion (Zhang et al. 2016), because they were derived from limited elite breeding lines. During the selection and long-term domestication for desirable qualities, the modern watermelon cultivars have a narrow genetic base and are quite susceptible to different kinds of stress. The first step in bringing together superior watermelon characteristics is to identify and characterize local varieties in watermelon growing centers. Molecular markers are used in molecular marker assisted selection (MAS) studies in terms of shortening the breeding time. Understanding genetic diversity and genotype population structure can accelerate the use of various genetic resources for cultivar development. Molecular markers can be used effectively to identify varieties and study their genetic relationships (Du et al. 2019; Yang et al. 2019; Zhang et al. 2020). Different DNA marker techniques are used for molecular characterization studies (Karaman et al. 2018; Kırac et al. 2022). SSR markers are effective method with several advantages, including high levels of polymorphism, co-dominant inheritance and easy identification of homozygosity and heterozygosity (Zhao et al. 2017). This marker technique has been used to identify genetic diversity in watermelons in some researches (Kwon et al. 2007; Verma, Arya 2008; Nimmakayala et al. 2009). The objectives of this study were to evaluate morphological and sugar content and estimate genetic relationships among watermelon genotypes collected in different geographical regions.

MATERIAL AND METHODS

Plant materials. Studied genotypes are represent a wide range of geographical origins and a range of important agronomic characteristics *C. lanatus* var. *citroides* and *Praecitrullus fistulosus* were included in the study to supply diverse genetic and morphological material for comparison with the amount of relatedness between the domesticated watermelons. A total of 96 watermelon genotypes were used in the study. One genotype is from USA (234-*C. lanatus* var. *citroides*), one genotype from India (331-*Praecitrullus fistulosus*) and one genotype from Uzbekistan (241-*C. lanatus* var. *lanatus*). The remaining genotypes (*C. lanatus* var. *lanatus*) previously collected from 22 different cities representing a large part of Turkey were kindly provided by Prof. Dr. Nebahat Sarı and Prof. Dr. Ilknur Solmaz of Cukuruva University. This study was carried out in Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Turkiye, in 2018 and 2019 years. Seedlings from each genotype were transplanted to the open field at the 2–3 true leaf stage. The soil properties where the watermelon was planted were determined as pH 6.8, EC: 0.75 mmos and $CaCO_3$: 12.0%. Fertilization was performed based on the soil chemical analysis. During the growing period (April–August), the highest average temperature was measured as 26.1 °C and the lowest average temperature was 10.1 °C. The average rainfall in the same period was 31.8 mm. The plants were irrigated regularly based on plant and soil observations by the drip irrigation system. Various observations were recorded in the field conditions.

Morphological, vegetative and fruit quality characteristics. Twenty seeds of each watermelon genotypes were germinated in a greenhouse at the Experimental Station of Erciyes University, Kayseri, Turkey. Seedlings were arranged in a randomized complete block design consisting of three replications with five plants per plot. Some morphological measurements were carried out in plants (Table 1). Pomological measurements were carried out on 3 fruits from each plant. Yield was calculated as yield per plant and was an average of all ripe fruit collected per genotype divided by the total number of live plants. Fruit flesh colour measurements were evaluated by colorimeter (CR-300, Minolta) as *L*, *a**, *b**, C (Chroma), and h° (hue) values.

Sugar determination. Fruits samples, 10–20 g, were collected from the center flesh of the fruit into

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Table 1. Morphological, physiological and yield parameter results of watermelon genotypes

Parameters	Values ± SE	Low data	High data
Seedling emergence rate (%)	92.98 ± 1.42	23	100
Seedling emergence days (day)	9.19 ± 0.3	6	18
Ovarian height (mm)	12.98 ± 0.14	7.36	20.06
Ovarian diameter (mm)	9.84 ± 0.13	4.15	20.02
Fruit weight (kg)	3.5 ± 0.06	0.11	9.32
Fruit diameter (cm)	18.4 ± 0.12	5.5	27.8
Fruit height (cm)	19 ± 0.14	3.0	38.6
Fruit peel thickness (cm)	1.25 ± 0.02	0.1	2.8
Fruit hardness (g)	0.94 ± 0.02	0.26	6.85
Soluble solids content (%)	7.63 ± 0.07	3.9	13.2
Fruit flesh colour *L**	48.38 ± 0.42	9.37	94.85
Fruit flesh colour *a**	19.11 ± 0.49	–34.16	70.3
Fruit flesh colour *b**	2.52 ± 0.06	1.14	7.83
Yield (kg/m^2)	6.2 ± 0.11	0.14	13.97
Seed number (seed/fruit)	391.7 ± 8.5	65	935
Seed width (mm)	6.7 ± 0.03	3.03	9.74
Seed length (mm)	12.88 ± 0.06	7.83	16.15
Seed thickness (mm)	2.61 ± 0.01	1.71	3.78
Weight of 100 seeds (g)	13.24 ± 0.19	4.13	21.16

test tubes and kept on ice. Fruit juice (10 mL) was centrifuged (10 g) for 10 minutes at 4 °C. The juice was diluted with ddH_2O and filtered through a 0.45 mm HPLC nylon filter. Sugars were separated in an analytical HPLC system (Agilent Technologies 1290 Infinity) fitted with a column using a refractive-index detector (model 61362A, Agilent).

Molecular study. Genomic DNA was extracted from plants following the protocol of the cetyltriethylammnonium bromide (CTAB) method. DNA quantity was determined by ultraviolet spectrophotometer (DNA = optical density 260 water volume 50 mg/mL) and diluted in water to a final concentration of 100 ng/mL.

Primer pairs, which generated 62 codominant SSR bands used in this study (Table 2), were from previously reported cucurbit sequences (Katzir et al. 1996; Danin-Poleg et al. 2001; Watcharawongpaiboon ve Chunwongse 2008). Polymerase chain reaction optimized 15-mL reactions contained 50 ng template DNA, 10 nmol dNTPs, 10 nmol SSR primers, 5 U Taq DNA polymerase, 1.5 mL of 10X polymerase chain reaction (PCR) buffer (50 mM KCl, 10M Tris-HCl, 2.5 mM $MgCl_2$, pH 8.3). Typical amplification parameters were used and PCR products (5 mL) were resolved on 6.5% polyacrylamide gels at 50 W for 2.5 hours.

Data analysis. Genotypes were scored as 1, 0 and 9 (for missing data). These data were analyzed using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y., USA) package program (Rohlf 2000). Similarity indexes between individuals were determined (Dice 1945). From the similarity index, UPGMA dendrogram based on DICE similarity matrix and Principal Component Analysis (PCA) based on variance-covariance matrix were performed. Correlation matrix was created by using SIMINT module for Principal Component Analysis. Eigen vectors were determined using this matrix EIGEN module. Three-dimensional graphics were obtained by using Eigen vectors in the PROJ module.

Population structure was analyzed in Structure V2.3 for *K* values ranging from 1 to 10 (Pritchard et al. 2000; Falush et al. 2003). Each running were repeated 10 times with 100.000 burn-in length. The most likely population ancestor was determined by Evanno's correction (Evanno et al. 2005). In addition, estimated allele frequency, effective allele number (*Ne*), Shannon's information index (*I*), expected (*He*) and Unbiased expected heterozygosity (*uHe*) values were determined using the GenAlEx 6.5 program. The amount of polymorphic information (PIC) was determined using Microsoft Excel.

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Table 2. Measures of genetic diversity based on 62 SSR loci in a collection of 96 watermelon genotypes: total number of bands, number of polymorphic bands, rate of polymorphism, allele frequency (*p* and *q*), number of effective alleles (*Ne*), Shannon's information index (*I*), expected (*He*) and unbiased expected heterozygosity (*uHe*), polymorphic amount of information (PIC)

Primer	Total number of bands	Number of polymorphic bands	Rate of polymorphism	*p*	*q*	*Ne*	*I*	*He*	*uHe*	PIC
CMCT44	8	8	100	0.28	0.71	1.69	0.61	0.42	0.42	0.77
CSTCC813	8	8	100	0.32	0.69	1.77	0.59	0.40	0.41	0.76
CMAG59	2	2	100	0.29	0.71	1.69	0.60	0.41	0.41	0.69
CSJCT14	8	7	87.5	0.30	0.70	1.73	0.61	0.42	0.42	0.62
CMGA104	11	11	100	0.28	0.72	1.68	0.61	0.42	0.42	0.45
CMTC158	6	5	83.3	0.29	0.71	1.7	0.61	0.42	0.42	0.64
CMTC160	5	4	80	0.30	0.70	1.72	0.62	0.43	0.43	0.02
CSCT335	7	7	100	0.30	0.70	1.72	0.61	0.42	0.42	0.61
CSJCT 191	7	6	85.7	0.31	0.69	1.74	0.59	0.41	0.41	0.61
CMACC146	1	0	0	0.32	0.69	1.76	0.61	0.42	0.42	0.63
CMTC168	5	5	100	0.30	0.70	1.73	0.62	0.43	0.43	0.55
CMGT108	7	7	100	0.29	0.71	1.69	0.59	0.40	0.41	0.80
CSTA050	9	9	100	0.30	0.70	1.72	0.60	0.41	0.41	0.69
CSJCT216	6	6	100	0.31	0.69	1.74	0.58	0.39	0.39	0.74
CMCTT144	5	4	80	0.29	0.71	1.69	0.60	0.41	0.41	0.60
CMGA172	6	5	83.3	0.29	0.71	1.7	0.60	0.41	0.41	0.02
CMTC163	7	7	100	0.27	0.73	1.65	0.60	0.41	0.41	0.76
CMTA134a	7	6	85.7	0.29	0.71	1.7	0.62	0.43	0.43	0.67
CSCTTT15a	1	0	0	0.29	0.71	1.69	0.59	0.40	0.40	0.68
CMTC51	4	4	100	0.29	0.71	1.69	0.60	0.41	0.41	0.82
CSLHCPA	3	2	66.7	0.31	0.69	1.75	0.60	0.41	0.41	0.60
CSJCT674	3	3	100	0.28	0.72	1.66	0.59	0.40	0.40	0.52
Cgb4767	6	6	100	0.29	0.71	1.69	0.56	0.37	0.37	0.79
CI.1-06	5	5	100	0.29	0.71	1.69	0.60	0.41	0.41	0.74
CSJCT 315	8	8	100	0.28	0.72	1.68	0.55	0.37	0.37	0.51
CSJCT 641	5	5	100	0.25	0.75	1.59	0.63	0.44	0.44	0.82
CSJCT 720	12	12	100	0.29	0.71	1.69	0.61	0.42	0.42	0.79
CSJCT 904	2	1	50	0.24	0.76	1.58	0.62	0.43	0.43	0.69
ASUW2	6	6	100	0.32	0.68	1.77	0.60	0.41	0.41	0.52
CI.1-120	5	5	100	0.30	0.70	1.73	0.63	0.44	0.44	0.51
CSJCT656	9	9	100	0.31	0.69	1.74	0.58	0.39	0.39	0.45
CSJCT746	8	6	75	0.29	0.71	1.69	0.60	0.41	0.41	0.72
CSJCT950	2	2	100	0.32	0.68	1.77	0.60	0.41	0.42	0.75
ASUW13	7	5	71.4	0.27	0.73	1.64	0.61	0.42	0.42	0.50
CI.2-23	10	10	100	0.29	0.71	1.69	0.64	0.44	0.45	0.66
CSJCT 662	4	4	100	0.29	0.71	1.71	0.60	0.41	0.42	0.61
CSJCT 775	2	1	50	0.31	0.69	1.73	0.57	0.38	0.38	0.02
Cgb4765	3	3	100	0.33	0.67	1.79	0.61	0.42	0.42	0.66
C.I.2-140	5	5	100	0.30	0.71	1.71	0.60	0.41	0.41	0.54

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Table 2. to be continued

Primer	Total number of bands	Number of polymorphic bands	Rate of polymorphism	*p*	*q*	*Ne*	*I*	*He*	*uHe*	PIC
CSJCT 781	1	0	0	0.26	0.74	1.62	0.60	0.41	0.42	0.56
Cgb5009	3	3	100	0.31	0.69	1.73	0.61	0.42	0.42	0.53
CMTp193	18	18	100	0.29	0.71	1.70	0.61	0.42	0.42	0.50
CMTp201	20	20	100	0.30	0.71	1.71	0.62	0.43	0.43	0.65
CMTmC67	14	14	100	0.31	0.69	1.73	0.59	0.40	0.40	0.41
CMTm120	17	16	94.1	0.30	0.70	1.72	0.61	0.42	0.42	0.68
CMTp46	8	7	87.5	0.31	0.69	1.74	0.59	0.41	0.41	0.50
CMTp174	7	6	85.7	0.28	0.72	1.67	0.60	0.41	0.42	0.74
CMTm130	11	11	100	0.30	0.70	1.72	0.61	0.42	0.43	0.59
CMTm252	11	11	100	0.29	0.71	1.69	0.60	0.41	0.41	0.56
CMTm144	14	14	100	0.29	0.71	1.70	0.61	0.42	0.42	0.71
CMTmC14	13	13	100	0.31	0.69	1.74	0.59	0.40	0.41	0.72
CMTp182	18	18	100	0.29	0.71	1.70	0.60	0.41	0.41	0.62
CMTm261	19	19	100	0.30	0.70	1.72	0.60	0.41	0.41	0.78
CMTm68	10	10	100	0.28	0.72	1.68	0.60	0.41	0.41	0.67
CMTm111	10	10	100	0.29	0.71	1.70	0.61	0.42	0.42	0.56
CMTm206	18	18	100	0.29	0.71	1.69	0.61	0.42	0.42	0.67
CMTmC34	17	17	100	0.29	0.71	1.69	0.60	0.41	0.42	0.69
CMTp158	2	2	100	0.30	0.70	1.72	0.60	0.41	0.42	0.58
CMTp125	17	17	100	0.30	0.70	1.72	0.61	0.42	0.42	0.55
CMTm219	25	25	100	0.29	0.71	1.70	0.60	0.41	0.42	0.64
CMTm83	15	14	93.3	0.29	0.71	1.71	0.61	0.42	0.42	0.77
CMTm207	10	10	100	0.30	0.71	1.71	0.59	0.40	0.41	0.76
Mean	523	502	96	0.29	0.71	1.71	0.60	0.41	0.41	0.69

RESULTS AND DISCUSSION

Morphological, vegetative and fruit quality characterization. The average seedling emergence rate was determined as 92.98 ± 1.42%, and the average number of days when seedling emergence was completed was determined as 9.19 ± 0.3. The mean ovarian height was determined as 12.98 ± 0.14 mm and the mean ovarian diameter was determined as 9.84 ± 0.13 mm. Average fruit weight was determined as 3.5 ± 0.06 kg, the average fruit diameter was determined as 18.4 ± 0.12 cm and average fruit height was determined as 19 ± 0.14 cm. When all genotypes were examined, the average fruit peel thickness was determined as 1.25 ± 0.02 cm. Fruit hardness average was determined as 0.94 ± 0.02 g. The mean of SSC (soluble solids content) was determined as 7.63 ± 0.07 %. Fruit flesh colour L^* mean was determined as 48.38 ± 0.42, fruit flesh colour a^* mean was determined as 19.11 ± 0.49 and fruit flesh colour b^* mean was determined as 20.18 ± 0.21. The average number of fruits per plant was determined as 2.52 ± 0.06 and the average yield was determined as 6.2 ± 0.11 kg/m^2. The average number of seeds per fruit was determined as 391.7 ± 8.5. The mean width of the seed was determined as 6.7 ± 0.03 mm, average seed length was determined as 12.88 ± 0.06 mm and the mean seed thickness was determined as 2.61 ± 0.01 mm. The average weight of 100 seeds was determined as 13.24 ± 0.19 g (Table 1). When the main stem numbers of each genotype of all genotypes are examined, 59.4% of the genotypes have four main stems, 34.4% have five, 1% have six and 5.2% have three main stems. When the hairiness data of all genotypes are examined, 69.8% of the genotypes have moderate hairiness, 11.5% have sparse and 18.8% have frequent hairiness. When the flower structures of all genotypes are examined, 92.7%

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Table 3. Sugar parameter values in water melon fruit samples obtained in this study and other studies

Unit	Glucose	Fructose	Sucrose	Reference
%	0.37–2.78 (1.94 ± 0.04)	0.56–4.60 (3.24 ± 0.06)	0.01–3.94 (1.09 ± 0.1)	This study
%	0.49–4.44	1.0–5.26	0–6.89	Lee et al. 1996
g/100 mL	1.87–4.35	3.05–4.85	1.03–2.68	Pardo et al. 1997
%	1.79 ± 0.3	4.89 ± 0.4	3.92 ± 1.1	Jaskani et al. 2005
g/100 g	1.07–1.526	3.019–4.311	4.583–5.341	Hong et al. 2008
%	1.4–2.0	2.8–3.6	1.8–3.0	Fish et al. 2009
g/100 g	2.6	4.1	1.4	Chareoansiri, Kongkachuichai 2009
%	1.10–1.68	2.04–2.51	0.44–0.92	Yau et al. 2010
g/100 g	0.86–2.97	1.51–5.05	0–4.15	Yoo et al. 2012
g/100 g	0.925	3.694	7.343	Ma et al. 2014

of the genotypes have hermaphrodite flowers, while the rest do not.

The average seedling emergence rate was determined as 92.98 ± 1.42% in all genotypes of this study. Maggs-Kölling et al. (2000) determined the germination percentage as 61.76% in some watermelon genotypes. Gichimu et al. (2009) determined fruit weights of between 1.43–3.01 kg. The fruit weight variation (0.11–9.32) obtained from this study was determined in a wider range. The average fruit weight of this study was found to be lower than the value determined by Maggs-Kölling et al. (2000) (4.56 kg). The reason why the average fruit weight values determined in this study were different from other studies may be due to different environmental conditions and the genetic material used. The average fruit diameter is 18.4 ± 0.12 cm in all genotypes. The average fruit diameter of this study was found to be lower than the value (25.1 cm) determined by Maggs-Kölling et al. (2000). The fruit diameter of 5.5–27.8 cm, which we obtained from this study, showed more variation than these values. This is due to differences between environmental conditions and genotypes. The fruit height values obtained from this study, ranging from 3–38.6 cm, showed more variation than the values determined by Hajiali et al. (2016). The fruit peel thickness values obtained from this study, ranging from 1–28 mm, showed more variation than the values determined by Gichimu et al. (2009) and Hajiali et al. (2016). Gusmini et al. (2004) examined the fruit skin thickness of a total of 112 watermelon cultivars in the USA, and found that most of the tested cultivars had a rind thicker than 10 mm and were suitable for pickle production. In this study, the mean of 81 of the 96 genotypes was higher than 10 mm. This shows that related genotypes can be evaluated in terms of pickle production.

Brix content is commonly used to estimate fruit sugar content and is strongly correlated with sugar content (Hashizume et al. 2003). The mean soluble solid content (SSC) values were 7.63 ± 0.07% in all genotypes. The fruit SSC average we determined was higher than those determined in other studies (Maggs-Kölling et al. 2000). The SSC values obtained from this study (3.9 to 13.2%) showed more variation than reported by Walters (2009) and Hajiali et al. (2016). This may be due to environmental and genotypic differences.

L^*, a^* and b^* measurements were made in order to determine the fruit colours quantitatively. Among the colour values, L^* indicates light-darkness, $-a^*$ towards green, $+a^*$ towards red, $-b^*$ towards blue, $+b^*$ towards yellow. The a^* and b^* measurement averages of this study were higher than the values determined by Maggs-Kölling et al. (2000). The average number of fruits per plant was determined as 2.52 ± 0.06. The fruit number we determined in this study were higher than the values determined by Gichimu et al. (2009) (0.89–5.67). The average yield we determined in this study were (6.2 ± 0.11 kg/m^2) higher than the values determined by Hajiali et al. (2016) (2.11–2.49 kg/m^2).

Seed number, seed width, seed length, seed thickness and seed weight were determined regarding the seeds. The average weight of 100 seeds of this study was higher than the value (11.47 g) determined by Maggs-Kölling et al. (2000). The number of seed/fruit we determined in this study was higher than the average value (126–372.3) de-

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termined by Gichimu et al. (2009). The seed width (3.03–9.74 mm) determined in this study was found in a wider range than the values determined by Hajiali et al. (2016) (5.1–9.9). The seed length (7.83–16.15 mm) we determined in this study was found in a similar range with the values determined by Hajiali et al. (2016) (9.76–17.4). The reason for the differences in seed parameters may be due to the different genotypes and the study carried out on a wider variety. Qualitative and quantitative parameters in watermelon are greatly affected by environmental conditions. It is expected that different results will be obtained in watermelon genotypes grown in different ecological conditions.

Sugar content analyses. When all genotypes were examined, the fructose average was determined as 3.24 ± 0.06%. The smallest value was measured in genotype 234 (0.56%), and the highest value was measured in genotype 40 (4.60%). The mean glucose was determined as 1.94 ± 0.04%. The smallest value was measured in genotype 234 (0.37%), and the highest value was measured in genotype number 199 (2.78%). The mean sucrose was determined as 1.09 ± 0.1%. The smallest value was measured in genotype 341 (0.01%), and the highest value was measured in genotype number 184 (3.94%). The mean total sugar was determined as 6.27 ± 0.12%. The lowest value was measured in genotype 234 (1.1%), the highest value was measured in genotype number 184 (8.66%). When all genotypes were examined, the average fructose/glucose ratio was determined as 1.69 ± 0.03. The lowest value was calculated for genotype 223 (1.13) and the highest value was calculated for genotype number 303 (2.6).

In fruit sugar analysis, glucose, fructose and sucrose amounts and total sugar and fructose/glucose ratios were determined. There appears to be a wide variation in sugar subparameter values. In previous studies, glucose was determined in the range of 0.452–4.44%, fructose 0.36–5.26% and sucrose 0–0.743% (Table 3). While the mean glucose value (1.94 ± 0.04) determined in this study was higher than the values determined by Jaskani et al. (2005) (1.79 ± 0.3) and Ma et al. (2014) (0.925), it was lower than the mean determined by Chareoansiri and Kongkachuichai, (2009). The glucose range determined in this study (0.37–2.78%) showed wider variation than other studies (Hong et al. 2008; Fish et al. 2009; Yau et al. 2010). The mean fructose value (3.24 ± 0.06%) determined in this study was lower than that found in some other studies (Jaskani et al. 2005; Chareoansiri, Kongkachuichai 2009; Ma et al. 2014). However, in terms of fructose values, a wider range was determined than many studies (Hong et al. 2008; Fish et al. 2009; Yau et al. 2010). While the mean value of sucrose determined in this study (1.09 ± 0.1%) was lower than some studies (Jaskani et al. 2005; Chareoansiri, Kongkachuichai 2009; Ma et al. 2014), it was found in many studies (Fish et al. 2009; Yau et al. 2010) was detected in wider variation. However, the lowest and highest sucrose intervals determined in this study were found to be narrower than some studies (Lee et al. 1996; Yoo et al. 2012). In a study, high differences were observed between genotypes in the relative ratios of three sugar sub-parameters (Yativ et al. 2010). In this study, large variations were determined in terms of the distributions of sugar sub-parameters in total sugar contents. Fructose was represented at the lowest 33% (genotype 18) and the highest 66% (genotypes 247 and 252). Glucose was represented at the lowest rate of 20% (genotype 174) and the highest rate of 47% (genotype numbered 223). Sucrose was represented at the highest rate of 45% (genotype 184). Similar to Yativ et al. (2010) results, fructose ratio was determined as the highest rate among other sugar parameters. The reasons why sugar subparameter values determined in this study are different from other studies are the differences between genotypes and the use of more genotypes. The fact that the genotypes used in this study showed plant gene source characteristics also increased the variation.

Genetic analyses. A total of 62 primers were used in SSR studies in 96 genotypes. The lowest number of bands (1) (CSJCT 781, CSCTTT15a and CMACC146) and the highest number (25) (CMTm219) bands were obtained from the primers. The total number of bands obtained is 523 and the number of bands per primer is 8.4. No polymorphism was observed in primers CSJCT 781, CSCTTT15a and CMACC146. Polymorphism was obtained at the rate of 93.3% in the CMTm83 primer, 94.1% in the CMTm120 primer and 100% in the other primers. 502 of the 523 bands obtained were polymorphic and the polymorphism rate was determined as 96%. Band sizes vary between 45–1 700 bp. Effective allele counts in SSR analyzes ranged from 1,582 (CSJCT 904) to 1.794 (Cgb4765) (mean 1.706). Shannon's knowledge index ranged from 0.554 (CSJCT 904) to 0.628 (CSTCC813)

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(mean 0.602). Expected heterozygosity values range from 0.368 (CSJCT904) to 0.443 (Cgb4765) (mean 0.412). Polymorphic information content was determined between 0.021 and 0.868 (mean 0.638). Primers for which the amount of polymorphic information was determined below 0.5 were CSCT335, CMACC146, CSCTTT15a, ASUW13, CSJCT 781 and CMTp174 (Table 2).

The similarity coefficients depending on the DICE index were determined with the NTSYS package program using SSR primers in 96 genotypes. The similarity coefficient range in 96 genotypes varied between 0.23 and 0.99. The most distant genotypes were the genotypes 35 and 331 with a similarity coefficient of 0.23. The similarity ratio of 331 (*Praecitrullus fistulosus*) genotype to other genotypes was 0.39. Genotype 234 belonging to the subspecies *C. lanatus* var. *citroides* was found closest to the 165 and 200 genotypes with a similarity ratio of 0.65. The similarity coefficient between the *Praecitrullus fistulosus* and *C. lanatus* var. *citroides* genotypes is 0.27. The similarity coefficient in the UPGMA dendrogram was determined between 0.26 and 1.0. Genotypes 331 and 234 were separated from the others at cluster analyses. Cluster analyzes of other genotypes revealed two main groups. There were 87 genotypes in the first group and 7 genotypes (62, 96, 342, 350, 354, 229 and 303) in the second group. The similarity coefficient of the genotypes in the first group is above 0.8. In the UPGMA dendrogram, the closest genotypes are 48 to 356 and 13 to 36 (Figure 1).

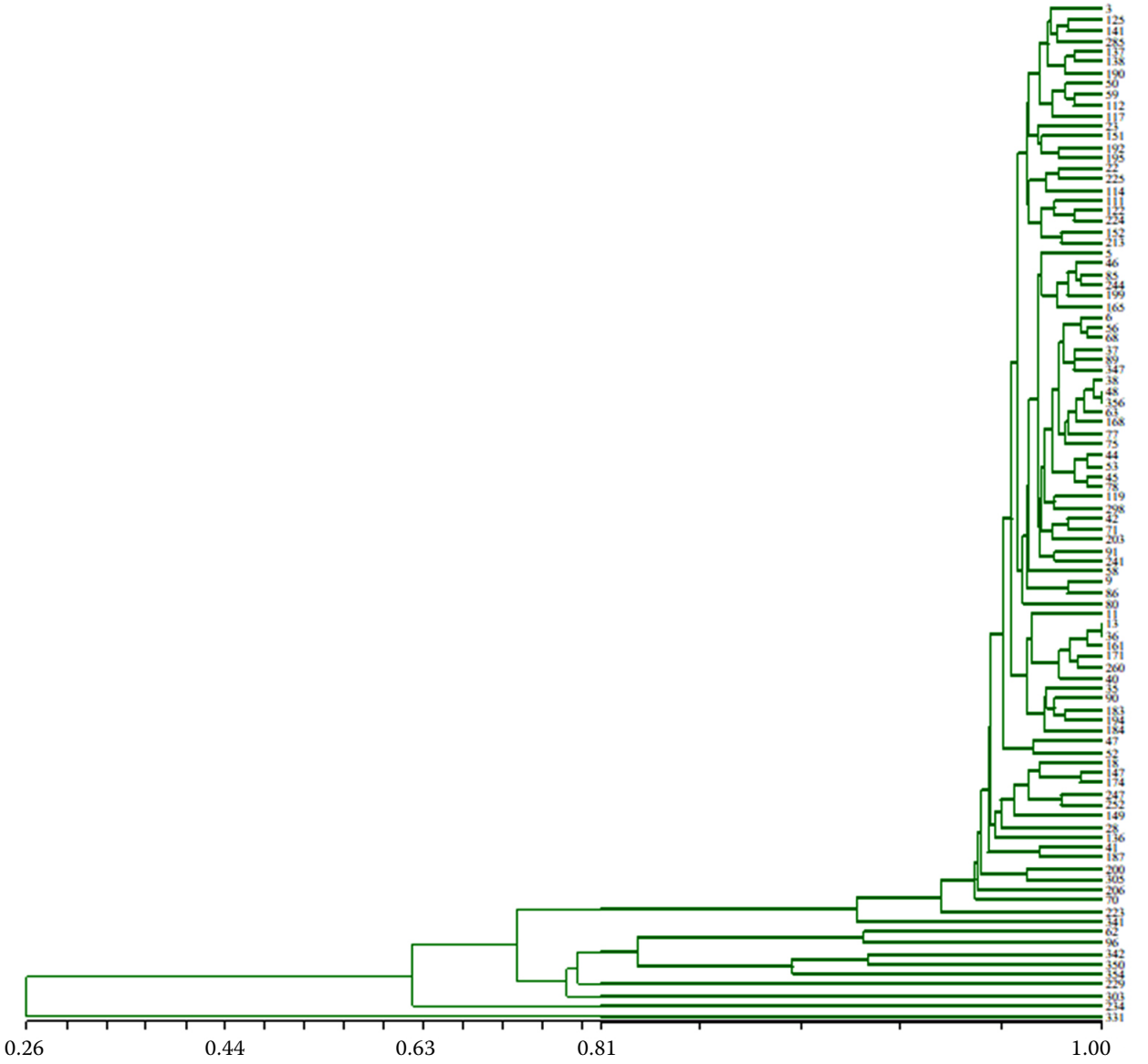

Figure 1. Dendrogram created in SHAN module using similarity indexes of DNA data

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In the three-dimensional PCA graph, 84 genotypes took place together and formed the first cluster. The genotypes 36, 341 and 223, which form the second cluster closest to these genotypes. Genotypes 234, 229, 350, 354, 342, 96, 62 and 36 separated from other genotypes and formed the third cluster. Genotype 331 was located farthest from other genotypes. There are differences in the determined clusters, especially in terms of 3 dimensions and genotype 234 in the second group is located differently from the others (Figure 2).

Considering the *K* values obtained with SSR data using the Structure Harvester program, it was determined that 96 watermelon genotypes consisted of 2 subpopulations. The belonging rate of 85 genotypes in the first sub-population and 9 genotypes in the second population was found to be 80% and above. Included in the second population are genotypes 62, 96, 229, 234, 303, 331, 342, 350 and 354 (Figure 3). two genotypes have mixed type genetic structure. Those with mixed genetic structure are genotypes 223 from Mardin and 341 from Antalya. Genotypes with mixed genetic structure numbered 223 and 341 are closer to the first subpopulation in terms of belonging ratios.

Sixy-two primers were used in SSR studies. In previous studies, Levi et al. (2009) performed analysis with 100 EST-SSR primers and in other studies, less than 50 primers were used. The number of SSR primers used was sufficient to analyze sufficient number of loci. The highest numbers were obtained in terms of the total and polymorphic bands obtained. Probably this is due that all scorable bands were recorded in this study. The polymorphism rate obtained in 96 genotypes in this study was higher than the polymorphism rate found by Levi et al. (2009), Mujaju et al. (2010), Hwang et al. (2011a), Wang et al. (2015). It was found to be lower than the polymorphism values determined by Hwang et al. (2011b). The similarity coefficient values (0.26–0.99) obtained in 96 genotypes in this study showed a narrower variation than the values determined by Kwon et al. (2010), but compared to some other SSR studies (Hwang et al. 2011a; Sheng et al. 2012; de S. Gama et al. 2013; Kim et al. 2015; Mashilo et al. 2017a) wider variation was determined. The biggest reason for the current differences is the number and variety of genetic resources examined. Different watermelon subspecies and varieties added to the data file cause an increase in diversity. Diversity is also high in different watermelon genotypes taken from distant geographical regions. At the same time, the number of primers used and their efficiency can also be effective on polymorphism and genetic distance calculation. The rate of polymorphism detected in this study higher than that found in oth-

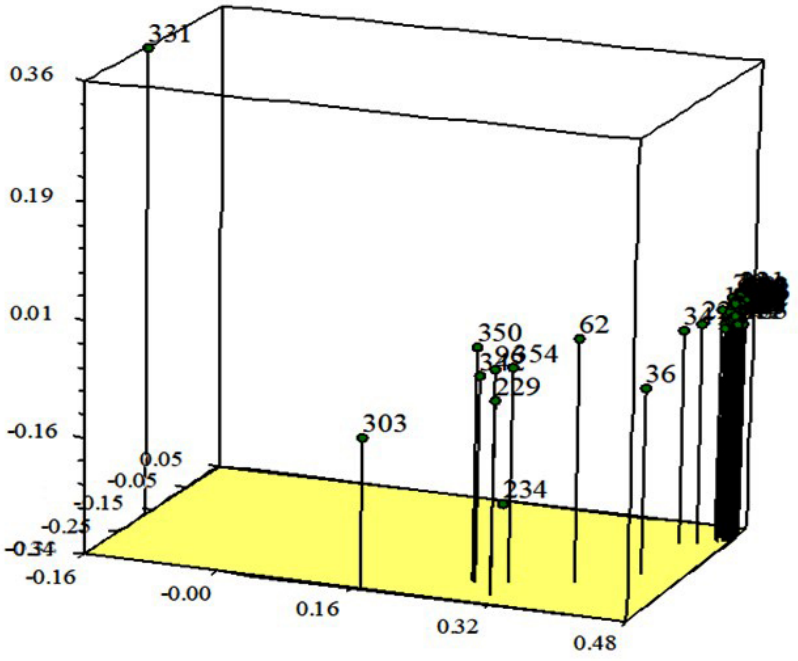

Figure 2. Three-dimensional graph obtained as a result of principal component analysis with SSR

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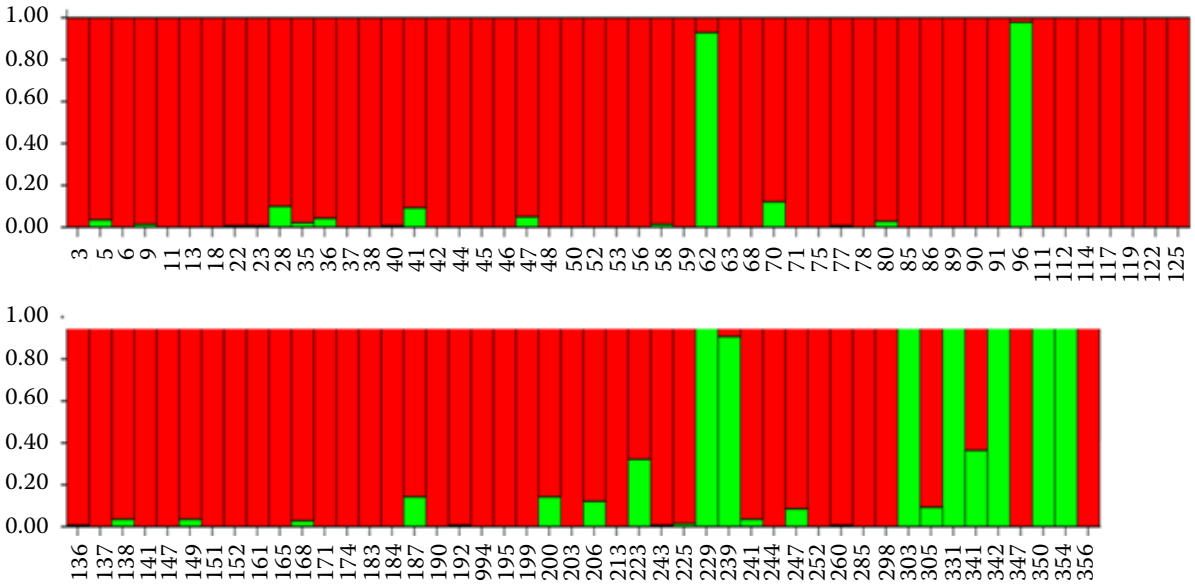

Figure 3. Graphical representation of the SSR data and the membership coefficients obtained from the Structure program

er studies (Lee et al. 1996; Xu et al. 2004; Solmaz et al. 2010). The rate of polymorphism detected only by Levi et al. (2001) was higher than the value we obtained in the SSR technique of this study.

The mean of polymorphic information amount (PIC) obtained in this study is 0.638 in SSR primers. Only 6 of the 62 primers had a PIC value below 0.5. While the PIC values were higher than the values determined by Mujaju et al. (2010) and Mujaju et al. (2011), they were found to be lower than those determined in some other studies (Mujaju et al. 2013; Kwon et al. 2010; Joobeur et al. 2006). In this study, PIC values obtained from SSR primers were found to be similar to those determined by Mashilo et al. (2017a;b). Differences in PIC values may be due partly to the polymorphism of the primers used and partly to genetic differences between the studied material.

CONCLUSION

High yield and high fruit quality are the main goals of today's watermelon growers. For watermelon breeding studies, it is desirable to use existing genetic resources and to have high genetic diversity. These study indicate that there is a wide morphological and sugar parameters variation among watermelon genotypes. However, molecular characterization studies show that watermelons have a narrow genetic diversity. This bottleneck in genetic diversity is the result of the very similar origins of watermelon genotypes distributed throughout the world. Low genetic diversity in watermelon genotypes may result in decreased heterosis power or inaccurate heterosis estimates. Conservation of watermelon genetic resources and increasing genetic diversity are methods that will ensure the preservation and development of current production levels. In this respect, it is important to protect watermelon genetic resources and determine their morphological, sugar content and genetic structures. Genotypes that stand out in terms of yield and quality can be used in breeding studies as variety/parent candidates. The data obtained as a result of the study will contribute to future genetic and breeding studies and it will be possible to use them in marker assisted selection (MAS) studies.

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