ACCEPTED ARTICLE:

Determination of markers associated with important agronomic traits of watermelon (*Citrullus lanatus* L.)

Omer Faruk COSKUN[1*], Osman GULSEN[2]

[1]Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Turkiye

[2]Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Turkiye

*Corresponding Author: omerfaruk.coskun@mku.edu.tr

Abstract

Association analysis using phenotypic information and molecular markers may provide valuable information for molecular breeding and marker-assisted selection. The objectives of this study were to determine markers associated with sugar parameters and important agronomic traits of watermelon and to estimate the level of genetic diversity. Ninety-six watermelon lines were genotyped by combining SSR (Simple Sequence Repeat), ISSR (Inter-Simple Sequence Repeat) and iPBS (Inter-Priming Binding Sites) marker data. These genotypes were also assessed for population structure, linkage disequilibrium (LD), and association mapping (AM) of sugar parameters and other important agronomic traits. In the analysis, 583 markers had LD values to a certain degree. A general linear model using only the Q matrix showing the population structure in association mapping, a complex linear model using a kinship matrix, and a complex linear model using both the Q and K matrix linear models. The regression model explanation rates for the 26 characters varied from 11.3% to 81.3%. The highest rates of regression model explanation were measured for fruit firmness (81.3%) and fruit height (78.2%). It might be e possible to determine the genes associated with these studied characteristics, to contribute to future genetic and breeding studies, and to be used in marker-assisted selection (MAS) studies.

Keywords: Watermelon, sugar parameters, SSR, ISSR, iPBS, association mapping.

Introduction

Watermelon, a member of the Cucurbitaceae family, is an economically important vegetable. Its production in the world was 101.634.720 tons on an area of 3.031.544 ha in 2021 (FAOSTAT, 2021). Watermelon yield and quality are the main parameters assessed in

combination in breeding programmes. The horticultural industry generally focuses on yield. However, in recent years, consumers worldwide have become increasingly interested in the quality of vegetables. Some phytochemicals in watermelon provide significant health benefits (Fraser and Bramley, 2004). Sweetness is one of the most important quality parameters of watermelon fruit. The total sugar content and ratios of glucose, fructose, and sucrose determine the sweetness of watermelon (Brown and Summers, 1985).

Morphological and molecular characterization of watermelon and identification of markers associated with important agronomic traits are valuable for breeding studies. In molecular plant breeding, different marker systems are used for genetic characterization to create genetic maps and linkage groups. Molecular markers are effective method to identify varieties and study their genetic relationships (Du et al., 2019; Zhang et al., 2020; Coskun, 2022; Ebadi et al., 2022; Morilipinar et al.,2022; Sudha et al., 2022; Coskun., 2023). SSR, ISSR, and iPBS markers are effective methods with several advantages, including high levels of polymorphism. SSR and ISSR marker techniques have been used to identify genetic diversity in watermelons (Verma and Arya, 2008). Using iPBS markers, the effectiveness of retrotransposon-based marker techniques in watermelon can be determined, and the possibility of finding new association markers can be increased. The inheritance or high correlation between agricultural traits and molecular markers can be used to predict the phenotypic traits of individuals in the population. This increases the efficiency of the breeding program as it allows the selection of the desired individual before planting in the field.

Molecular characterization, linkage disequilibrium and genetic mapping are critical tools for further genomic studies, as well as for genetic breeding of economically important horticultural species. To create linkage maps, it is necessary to develop mapping populations by crossbreeding between parents with sufficient morphological and molecular polymorphisms. The association mapping approach has an advantage over that obtained using only two parents. Using this technique, all alleles present in a given germplasm can be detected. Linkage disequilibrium studies have been conducted in watermelons (Ocal et al., 2014; Reddy et al., 2014). Association mapping in watermelon is limited, and no markers associated with sugar parameters have been determined. The aim of this study was to determine the genetic diversity of watermelon using different marker techniques, identify markers associated with sugar parameters and other important characteristics of watermelon, and develop regression models. It will be possible to determine the genes associated with these studied characteristics, to contribute to future genetic and breeding studies and to be used in marker-assisted selection (MAS) studies.

Materials and Methods

Material used

In this study, 96 genotypes selected from the watermelon genetic resource collection of Cukurova University, Faculty of Agriculture, Department of Horticulture were used. The samples mainly included selfed (4-6 times) lines of the genotypes. A total of 96 lines consisting of 94 cultivated watermelon (*C. lanatus* var. *lanatus* landrace, a wild form of *C. lanatus* var. *citroides* and one *Praecitrullus fistulosus* line as an outgroup. Morphological and sugar parameter data obtained previously (Coskun and Gulsen, 2023) were used in the association mapping studies. A total of 26 parameter data were used, including two general plant-related traits, three ovary-related traits, eleven fruit-related traits, four seed-related traits, five sugar parameter traits, and additionally yield.

Molecular analysis

DNA extraxtion was performed with the DNA isolation method developed by Doyle and Doyle (1990). The total volume for the PCR reaction was prepared as 15 µl: 7.15 µl distilled water, 1.5 µl 10 x DNA polymerase buffer, 2.5 mM dNTPs, 5 mM primer, 1 U Taq Polymerase, and 20 ng DNA. The prepared PCR mix was analysed using 36 iPBS and 12 ISSR primers. Agarose gel was used to display the band profiles of ISSR and IPBS marker studies. Additionally, scoring data for the SSR band profiles were obtained from previous study (Coskun and Gulsen, 2023).

Determination of linkage disequilibrium (LD) and associating mapping (AM)

The LD level between a pair of loci was obtained in Tassel 5.2 program using marker data. Analysis were performed after removing loci with a low number of alleles (f<0.10). In the association mapping study, 26 character-related traits were analysed in 96 watermelon genotypes. General linear model (General Linear Model- GLM+(Q)) using only Q matrix showing population structure in associating mapping, complex linear model using kinship matrix (Mixed Linear Model+K- MLM+(K)) and complex linear model using both Q matrix and K matrix linear model (Mixed Linear Model- MLM+(K+Q)) was used. The web-based Structure Harvester (Earl and Vonholdt, 2012) software was used with the result file to calculate the ΔK value of the populations. The significance level between the marker and phenotypic traits was determined using the Tassel 5.2 program (Bradbury et al. 2007) based on the P values and the F test. The Q matrix showing the population structure used in the mapping was obtained

using the Structure program. The kinship matrix was obtained by analysing 583 polymorphic DNA bands with the A.mat function using the 'rrBLUP'R package (Endelman 2011). The model with the best results was determined by obtaining the QQ (quantile quantile plot) graphs.

Statistic analysis

NTSYS 2.1 and Tassel 5.2 programs were used for molecular analysis. In addition, the estimated allele frequency, effective allele number (Ne), Shannon's information index (I), expected heterozygosity (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. For association mapping, quantile quantile plots and Manhattan plots were obtained using Tassel 5.2. Regression analysis were performed on the related markers obtained using three different statistical methods. For this purpose, backward and forward regression models were used in the SPSS 22.

Results and discussion

Analysis of molecular characterization were performed with a total of 110 primers, 36 iPBS, 12 ISSR and 62 SSR in 96 genotypes. The total number of bands obtained was 1397 and the number of bands per primer was 12.7. A total of 1364 of the 1397 bands obtained were polymorphic, the polymorphism rate was determined to be 97.6% and the band sizes vary is between 45-2100 bp. By combining the iPBS, ISSR and SSR primers into 96 genotypes, similarity coefficients based on the DICE index were determined using the NTSYS package program. The similarity coefficients ranged from 0.25-0.99. The most distant genotypes were 147 and 331, with a similarity coefficient of 0.25. The genotype of the 331 *P. fistulosus* species was closest to the 86, 36 and 62 genotypes, with a similarity ratio of 0.32. There were 87 genotypes in the first main group and 4 (53, 114, 203 and 151) genotypes in the second main group. The similarity coefficient for the genotypes in the first main group was 0.8 and above. The genotypes closest to each other in the UPGMA dendrogram were 45 and 48 genotypes, respectively (Figure 1).

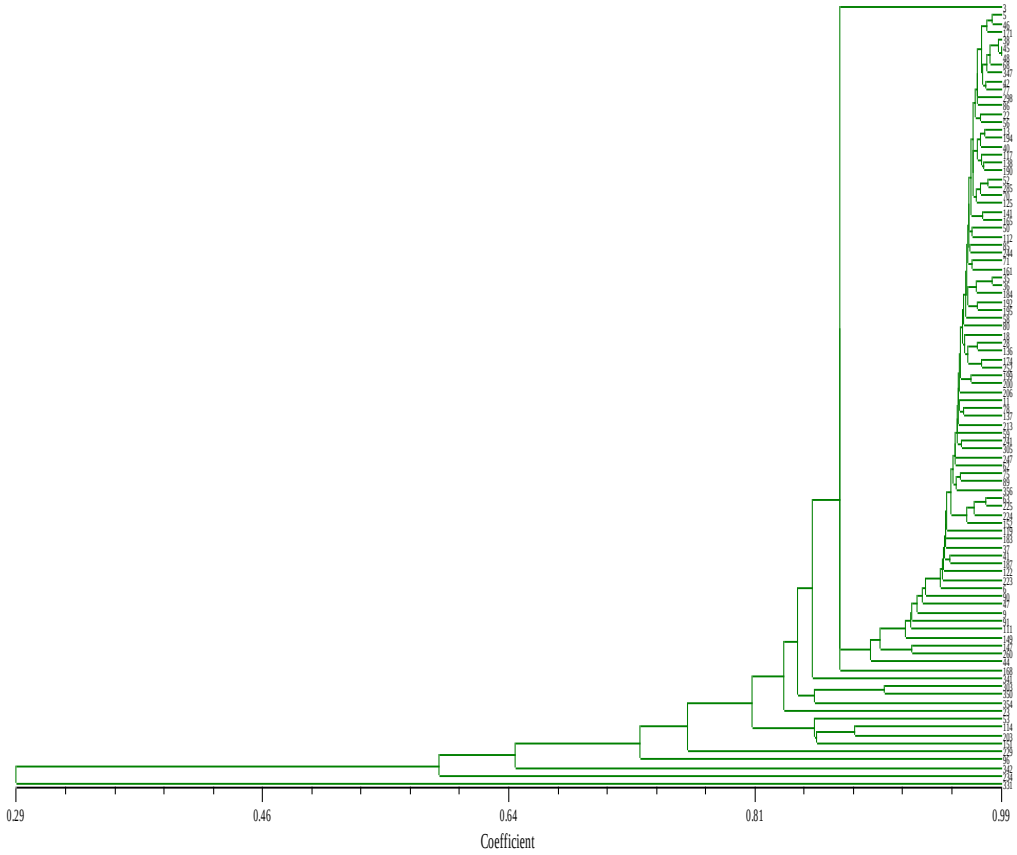

Figure 1. UPGMA dendrogram constructed with iPBS+ISSR+SSR primers using the DICE similarity index in ninety-six watermelon genotypes.

In the principal component analysis obtained using 96 genotypes, the cumulative sum of the first three eigenvalues for the two- and three-dimensional graphs was determined as 89.1. In the three-dimensional PCA graph, the 85 genotypes took place together and formed the first cluster. Genotypes 331, 234, 342, 303, 354, 229, 96, 350, 34, 62 and 36 were located separately and independently of the others. Genotypes 331 and 234 were located the farthest away (Figure 2).

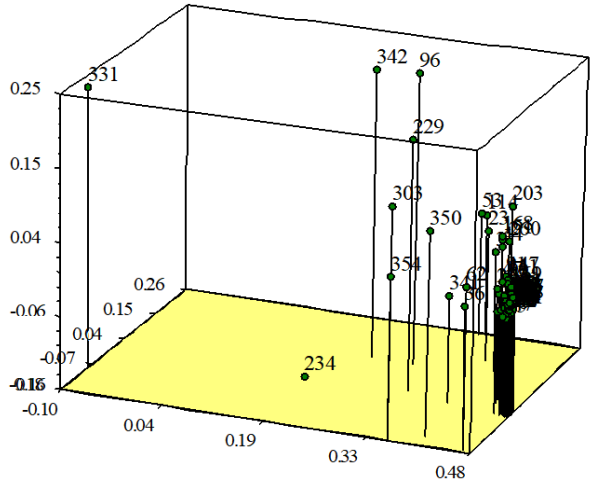

Figure 2. Three-dimensional graph obtained as a result of principal component analysis with iPBS+ISSR+SSR primers in 96 watermelon genotypes.

Considering the K values obtained with iPBS+ISSR+SSR data using the Structure Harvester program, it was determined that the 96 watermelon genotypes consisted of two subpopulations. There were 8 pure individuals in the first subpopulation and 73 pure individuals in the second population. Fifteen genotypes had mixed genetic structures. Genotypes included in the first population were ETAE origin 53, Diyarbakir origin 96, Mardin origin 114, Usak origin 203, USA origin 234 and *C. lanatus* var. *citroides*, Hatay origin 229, India origin 331, *P. fistulosus*, and Antalya origin 342 (Figure 3).

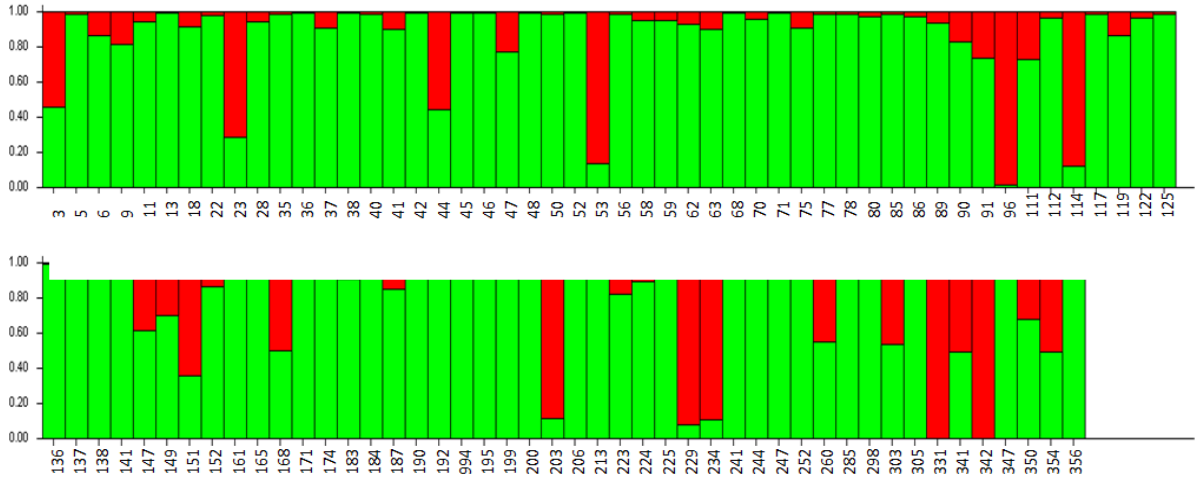

Figure 3. Graphical representation of the iPBS+ISSR+SSR data and the membership coefficients obtained from the Structure program.

The number of bands obtained per primer (12.7) was higher than Alsohim and Motawei (2014), Elias (2016), Soghani et al. (2018). In this study, the polymorphism rate (97.6%) obtained from the 96 genotypes was lower than that detected by Elias (2016) and Dje et al. (2010). This was found to be higher than those of Alsohim and Motawei (2014) and Soghani et al. (2018). Although similarity coefficient values (0.29-0.99) obtained in 96 genotypes in this study showed a wider variation than the values determined by Dje et al. (2010), similarity coefficients were higher. The biggest reason for the current differences is the number and diversity of genetic resources examined. Retrotransposon-based marker systems have been successfully used in genetic diversity studies of some plants (Mardi et al., 2011; Nasri et al., 2013). This marker technique has been studied in some cucurbit species (Khoei et al., 2014; Khoei et al., 2015) but not in watermelon. In this study, the genetic characterization efficiencies of iPBS primers for watermelon genotypes were determined. In this study, 96% of the polymorphisms were obtained. The obtained polymorphism rate and similarity coefficient values show that this primer technique is suitable for genetic characterization of watermelon genotypes. In this study, the average effective allele numbers in iPBS analysis were found to be 1.706 and Shannon's knowledge index was 0.602.

The expected value (He) averages obtained in this study were 0.412, 0.416 and 0.415 for iPBS, ISSR and SSR primers, respectively. The expected values of the three marker primers were higher than those reported in other studies on watermelon (Mujaju et al., 2013; Mujaju and Nybom, 2011) and others (Mashilo et al., 2016; Mashilo et al., 2017). Mujaju et al. (2011) and Singh et al. (2017) obtained expected values similar to those obtained in this study. The polymorphic information amount (PIC) averages obtained in this study were 0.679, 0.498 and 0.638 for iPBS, ISSR and SSR primers, respectively. The PIC values determined by Mujaju et al. (2013) and Kwon et al. (2010), working with SSR primers, were found to be lower than the values obtained from iPBS and SSR primers and higher than the values obtained from ISSR primers. The data obtained in this study were high (Elias, 2016; Singh et al., 2017). Only 10 of the 110 primers had PIC value <0.5. Differences in PIC values may be partly due to polymorphism of the primers used and partly due to genetic differences between the studied materials.

In the analysis conducted using the Tassel 5.2 program, it was determined that 583 markers had LD values at certain degrees. Out of a total of 103927 marker pairs, 28795 showed LD at the 0.05 level (27.7%), 17782 at the 0.01 level (17.1%), and 7915 at the 0.001 level (7.6%). The mean LD value (D') among the loci showing a statistically significant LD was 0.54. LD is usually evaluated using r^2, which summarizes both recombination and mutation histories (Flint-

Garcia et al., 2003). The mean r^2 value (square of the correlation coefficient between the two loci) for all marker pairs was 0.105. Approximately 16.2% of the r^2 values were above 0.2, whereas 28.7% were above 0.1. The LD blocks obtained for the 583 markers are shown in Figure 4 as "heat map".

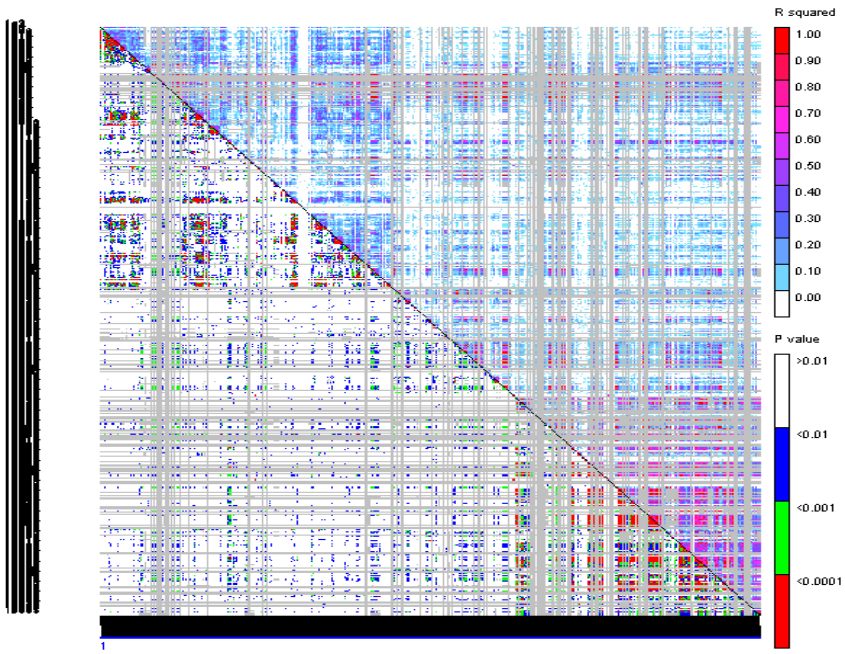

Figure 4. LD measurements (values above the diagonal, r^2) and probability values (values below the diagonal, P) for the iPBS, ISSR and SSR markers.

In the association mapping study, 26 important characteristics, including morphological and sugar parameters, were used for 96 watermelon genotypes. To eliminate false-positive results, the results were compared according to the models using a model containing three different statistical approaches. The Kinship matrix was obtained by analysing 583 polymorphic DNA bands with the A. mat function using the "rrBLUP" R package (Endelman 2011). Considering the Q-Q plot graphs for associating mapping in five characters, it was determined that MLM (K) and MLM (K+Q) analysis were appropriate for all characters.

In the model obtained with ovarian height values, nine significant independent variables at $p<0.05$ level (iPBS-2277.1400, iPBS-2277.1500, iPBS-2217.1600, iPBS-2228.350. iPBS-2244.920, iPBS-2249.220, ISSR-DBDACA7.440, SSR-CGB4767.175 and SSR-CMTp46.360). The rate of explanation of the ovarian height of the model based on these markers was 63.4%. The model obtained using ovarian diameter values included six independent variables (ISSR-DBDACA7.540, iPBS-2239.1130, iPBS-2393.660, SSR-CI.1-120.185, SSR-CGB4767.175 and iPBS-2074.530). The rate of explanation of the ovarian diameter of the model based on these markers was 46.3%. In the model obtained with ovarian

hairiness values, there were four independent variables at $p<0.05$ level (iPBS-2217.450, iPBS-2239.950, iPBS-2249.420 and ISSR-GACA4.720). The rate of explanation of the hairiness value in the ovary of the model based on these markers was 37.4%. In the model obtained from the hermaphrodite flower status data, there were 2 independent variables at the $p<0.05$ level (SSR-CSTA050.560 and SSR-CMTp125.600). The hermaphrodite flower state of the model based on this marker was explained 47%. In the model obtained with the main stem number data, there were two independent variables at $p<0.05$ level (SSR-CMTp182.120 and iPBS-2381.1280). The rate of explaining the number of main bodies of the model depending on this brand was 27.2%.

In the model obtained with fruit weight values, there were three independent variables ($p<0.05$) (iPBS-2389.350, SSR-CMTm207.350, and SSR-CMTmC67.500). The rate of explaining fruit weight variation in the model based on these markers was 47.8%. In the model obtained with fruit diameter values, there were four independent variables at $p<0.05$ level (iPBS-2074.290, iPBS-2384.750, iPBS-2393.820 and SSR-CMTmC67.500). The rate of explanation of the fruit diameter of the model based on these markers was 52.4%. In the model obtained with fruit height values, there were four independent variables at $p<0.05$ level (iPBS-2384.500, SSR-ASUW2.170, iPBS-2077.460 and iPBS-2400.1350). The rate of explanation of the fruit height of the model based on these markers was 78.2%. In the model obtained with fruit peel thickness values, there were four independent variables at $p<0.05$ level (SSR-CGB4767.170, iPBS-2228.500, ISSR-DBDACA7.490 and iPBS-2383.720). The rate of explanation of the fruit peel thickness of the model based on these markers was 29.8%. In the model obtained with fruit firmness values, there were four independent variables at $p<0.05$ level (iPBS-2074.290, iPBS-2217.1450, SSR-CMTp182.120 and SSR-CMTm207.350). The rate of explaining the fruit firmness of the model based on these markers was 81.3%. In the model obtained with TSS values, there were three independent variables at $p<0.05$ level (ISSR-AG8T.500, SSR-CGB5009.200 and SSR-CMTp182.160). The rate of explanation of the TSS value of the model based on these markers was 23.9% (Table 1).

In the model obtained with fruit color L* values, there were three independent variables at $p<0.05$, that is (iPBS-2074.290, SSR-CMTiPBS-2077.4609.500, ISSR-CAC6.220). The rate of explanation of the fruit color L* value of the model based on these markers was 45.7%. The model obtained with fruit color a* values included six independent variables at $p<0.05$ level (ISSR-CT8TG.860, iPBS-2375.750, ISSR-CAC6.220, ISSR-HVHCA7T.480, SSR-CMTp182.160 and SSR-CMTC160.600). The rate of explanation of the fruit color a* value of the model based on these markers was 38.8%. In the model obtained with fruit color b* values,

there were five independent variables at $p<0.05$ level (ISSR-AG8T.560, iPBS-2391.1150, iPBS-2400.405, iPBS-2226.250 and ISSR-TAA8.1450). The rate of explaining the fruit color b* value of the model based on these markers was 32.5%. In the model obtained with fruit number values, there were five independent variables at $p<0.05$ level (iPBS-2217.1450, iPBS-2217.1600, ISSR-CAC6.220, SSR-CMTiPBS-2077.4609.500 and SSR-CMTm207.350). The rate of explanation of the number of fruits in the model based on these markers was 59.9%. The model obtained with the yield values included two independent variables ($p<0.05$) (iPBS-2077.490 and iPBS-2383.1250). The rate of explanation of the efficiency values of the model based on these markers was 68.6% (Table 1).

In the model obtained with seed number values, there were two independent variables at $p<0.05$ level (SSR-CMTm144.550 and SSR-CMTp158.1050). The rate of explanation of the number of seeds in the model based on these markers was 11.3%. In the model obtained with seed width values, there were two independent varies at ($p<0.05$) (iPBS-2217.450 and SSR-CGB4767.170). The seed-width disclosure rate of the model based on these markers was 29.2%. In the model obtained with seed height values, there were two independent variables at $p<0.05$ level (SSR-CSJCT 191.240 and SSR-CMTm207.350). The seed size explanation rate of the model based on these markers was 34.1%. In the model obtained with seed thickness values, there were four independent variables at $p<0.05$ level (ISSR-GACA4.350, iPBS-2074.53043, SSR-CMTp182.160 and ISSR-CAC6.1250). The rate of explanation of the seed thickness of the model based on these markers was 37.4%. In the model obtained with seed weight values, there were 4 independent variables at $p<0.05$ level (ISSR-GACA4.350, SSR-CGB4767.170, SSR-CSJCT 191.230 and ISSR-TAA8.920). The rate of explanation of the seed weight of the model based on these markers was 49.2% (Table 1).

According to the fructose character and MLM (K+Q) model, there were 2 independent variables at the $p<0.05$ level (ISSR-AG8T.1140 and iPBS-2387.480). The rate of explanation for the fructose value of the model based on these markers was 41.5%. In the model obtained according to the MLM (K+Q) model with the glucose character, there were five independent variables at $p<0.05$ level (iPBS-2226.1600, iPBS-2077.740, iPBS-2376.470, iPBS-2376.1060 and SSR-CMTp174.850). The rate of explanation of the glucose value of the model based on these markers was 61.1%. In the model obtained according to the MLM (K+Q) model with the sucrose character, there were five independent varies at ($p<0.05$) (SSR-CSJCT 191.230, iPBS-2076.900, ISSR-HVHTCC7.500, ISSR-DBDACA7.600 and iPBS-2239.950). The rate of explanation of the sucrose value of the model based on these markers was 47.2%. In the model obtained according to the MLM (K+Q) model with total sugar character, there were 2

independent variables at the $p<0.05$ level (SSR-CMTm252.1150 and iPBS-2387.480). The rate of explaining the total sugar value of the model based on this marker was 41.9%. In the model obtained according to the MLM (K+Q) model with fructose/glucose character, there were four independent variables at $p<0.05$, that is (ISSR-GACA4.720, iPBS-2077.740, ISSR-DBDACA7.780 and ISSR-DBDACA7.1080). The rate of explaining The fructose/glucose ratio of the model based on this marker was 46.1% (Table 1).

Table 1. Marker counts and annotation rates associated with important agronomic traits.

Character	Method	Number of Associated Markers	Number of Markers Remaining in the Model	Model Description Ratio
Ovary height	GLM (Q)	29	9	% 63.4
Ovary diameter	GLM (Q)	19	6	% 46.3
Fruit weight	MLM (K+Q)	22	3	% 47.8
Fruit width	MLM (K+Q)	8	4	% 52.4
Fruit size	MLM (K+Q)	46	4	% 78.2
Fruit skin thickness	MLM (K+Q)	19	4	% 29.8
Fruit firmness	MLM (K+Q)	64	4	% 81.3
TSS	GLM (Q)	32	3	% 23.9
Fruit color L*	MLM (K+Q)	18	3	% 45.7
Fruit color a*	MLM (K+Q)	13	6	% 38.8
Fruit color b*	MLM (K+Q)	10	5	% 32.5
Number of fruits	MLM (K+Q)	13	5	% 59.9
Yield	MLM (K)	69	2	% 68.6
Number of seeds	MLM (K+Q)	16	2	% 11.3
Seed width	MLM (K+Q)	36	2	% 29.2
Seed size	MLM (K+Q)	20	2	% 34.1
Seed thickness	MLM (K+Q)	16	4	% 37.4
Seed weight	MLM (K+Q)	13	4	% 49.2
Ovarian hairiness	GLM (Q)	16	4	% 37.4
Hermaphrodite flower status	MLM (K+Q)	67	2	% 47
Number of main body	MLM (K+Q)	18	2	% 27.2
Fructose	MLM (K+Q)	66	2	% 41.5
Glucose	MLM (K+Q)	78	5	% 61.1
Sucrose	MLM (K+Q)	31	5	% 47.2
Total sugar	MLM (K+Q)	92	2	% 41.9
Fructose/glucose	MLM (K+Q)	36	4	% 46.1

AbdoliNasab and Rahimi (2020) determined the number of markers associated with important traits in watermelon to be 13 for 2015 data and 12 for 2016 data. A higher number of associated markers were determined in this study. The number of markers related to fruit weight, fruit diameter, fruit height, fruit skin thickness, fruit firmness and fruit number and the regression model explanation rate were found to be higher than those determined by Yagcioglu's (2016) GLM method. The model with two markers associated with the number of seeds explained the number of seeds by 11.3%, and the model with two markers associated with seed width explained the seed width at a rate of 29.2%. In some other studies, linkage mapping studies were conducted on seed characteristics (Prothro et al., 2012). The model with two markers

related to seed size explained 34.1% of the seed size. The significance level of the markers associated with seed and fruit characteristics in this study was higher than that determined by AbdoliNasab and Rahimi (2020). In this study, the number of related markers determined by the MLM (K) method was lower than that determined by the GLM method by Yagcioglu (2016). The reason for determining a larger number of markers and having a higher significance value in this study in relation to some morphological features may be the differences in the number and types of analysed markers and genotypes.

Association mapping has not been previously performed with sugar parameters in watermelons. However, three studies have done link mapping. Ren et al. (2014) nine and Cheng et al. (2016) identified four QTLs for sugar parameters. In our study, 18 markers remained in the model for all sugar parameters. The relationship rates varied from 41.5% to 62.8%. The detection of sugar parameters, one of the most important criteria in terms of quality, and markers related to this level will be important in terms of shortening the breeding period. The use of DNA markers associated with important agronomic traits can increase the efficiency and accuracy of classical plant breeding through marker-assisted selection (MAS).

The regression model explanation rates for the 26 characters varied from 11.3% to 81.3%. This could be due to the choice of markers. The highest rates of regression model explanation were measured for fruit firmness (81.3%) and fruit height (78.2%). The lowest regression disclosure rates were determined for the number of seeds (11.3%) and main stems (27.2%) of the SSC (23.9%). In previous studies, some genetic mapping studies related to fruit characteristics in watermelon have been carried out. Genetic mapping studies are generally conducted in the form of linkage mapping. Therefore, fewer characteristics were examined than those determined in the present study. In this study, markers associated with 26 characteristics were identified. Chi et al. (2017) six, Sandlin et al. (2012) six, Li et al. (2018) three, Cheng et al. (2016) seven, Ren et al. (2014) twelve characters The QTL has been determined. Compared to other watermelon linkage maps in this study, other researchers identified fewer associated markers because other studies used populations with different genetic diversity. Populations obtained by crossover have a much more limited genetic diversity than natural populations. This reduces the number of associated markers identified.

These findings suggest that there is narrow genetic variation among watermelon genotypes. Within the scope of this study, DNA markers associated with important characteristics were determined by association mapping analysis using different marker techniques in watermelon. The results obtained in this study showed the importance of association mapping in terms of determining marker-trait relationships in watermelon breeding. This study combined different

mapping models and provided information on the suitability of watermelon genotypes for association mapping analysis. It is possible to determine the effect of genetic variation on the results of the associating mapping study with the data obtained from the study to determine the genes associated with these studied characters, to contribute to future genetic and breeding studies, and to be used in marker-assisted selection (MAS) studies.

Acknowledgements

This study was funded by the Scientific Research Unit of Erciyes University (FDK-7724).

References

AbdoliNasab, M. and Rahimi, M. 2020. Association analysis of traits in watermelon genotypes using molecular markers. *Iran J. Sci. Technol. Trans. Sci*., **44:** 361-369. https://doi.org/10.1007/s40995-020-00837-z

Alsohim, A.S. and Motawei, M.I. 2014. Genetic diversity and presence of DREB gene in watermelon cultivars and wild type of watermelon based on molecular markers. *J. Food Agric. Environ*., **12** (3-4): 281-284. https://doi.org/10.1234/4.2014.5398

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y. and Buckler, E.S. 2007. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatic*s, **23**(19): 2633-2635. https://doi.org/10.1093/bioinformatics/btm308

Brown, A.C. and Summers, W.L. 1985. Carbohydrate accumulation and color development in watermelon. *J. Am. Soc. Hortic. Sci*., **110**(5): 683-687.

Cheng, Y., Luan, T., Wang, X., Gao, P., Zhu, Z., Liu, S., Baloch, A.M. and Zhang, Y. 2016. Construction of a genetic linkage map of watermelon (*Citrullus lanatus*) using CAPS and SSR markers and QTL analysis for fruit quality traits. *Sci. Hortic*., **202**: 25-31. https://doi.org/10.1016/j.scienta.2016.01.004

Chi, Y.Y., Peng, G., Zhu, Z.C., Luan, F.S., Guiying, L.I. and Peng, Y.U. 2017. The QTL analysis of fruit and seed associated traits in watermelon based on CAPS markers. *Sci. Agric. Sin*., **50**(7): 1282-1293. https://doi.org/10.3864/j.issn.0578-1752.2017.07.011

Coskun O.F. 2023. Determination of genetic diversity in some pumpkin genotypes using SSR marker technique. *Erzincan Uni. J. Sci. Techn*., **15**(3): 942-952. https://doi.org/10.18185/erzifbed.1113553

Coskun O.F. 2023. Molecular characterization, population structure analysis, and association mapping of Turkish parsley genotypes using iPBS markers. *Horticulturae*, **9**(3):336. https://doi.org/10.3390/horticulturae9030336

Coskun, O.F. and Gulsen, O. 2023. Molecular, morphological and phytochemical characterization of some watermelon (*Citrullus lanatus* L.) genotypes. Unpublished manuscript.

Dje, Y., Tahi, C.G., Bi, A.I.Z., Baudoin, J.P. and Bertin, P. 2010. Use of ISSR markers to assess genetic diversity of African edible seeded *Citrullus lanatus* landraces. *Sci. Hortic*., **124**:159-164. https://doi.org/10.1016/j.scienta.2009.12.020

Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**(1): 13-15.

Du, H.S., Yang, J.J., Chen, B., Zhang, X.F., Zhang, J., Yang, K., Geng, S.S. and Wen, C.L. 2019. Target sequencing reveals genetic diversity, population structure, core-SNP markers, and fruit shape-associated loci in pepper varieties. *BMC Plant Biol*., **19**: 578. https://doi.org/10.1186/s12870-019-2122-2

Earl, D.A. and Vonholdt, B.M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the evanno method. *Cons. Genet. Res*., **4**, 359-361. doi: 10.1007/s12686-011-9548-7

Ebadi, M., Soltani, F., Mostofi, Y., Alabboud, M. 2022. Analysis of genetic diversity among watermelon [*Citrullus lunatus* Thunb (Matsum.) and Nakai] accessions by phenotypic and molecular markers. *J. Agric. Sci. Technol*., 24(2): 429-440.

Elias, M.S. 2016. Distinguish among some selective watermelons by using ISSR technology. *Iraqi J. Agric. Sci*., **47**(5):1235-1245. https://doi.org/10.36103/ijas.v47i5.501

Endelman, J. B. 2011. Ridge regression and other kernels for genomic selection with R package rrBLUP. *Plant Genome*, **4**(3): 250-255. https://doi.org/10.3835/plantgenome2011.08.0024

FAOSTAT. 2021. The statistical database (FAOSTAT). FAO, Rome, Italy.

Flint-Garcia, S.A., Thornsberry, J.M. and Buckler, E.S. 2003. Structure of linkage disequilibrium in plants. *Annu. Rev. Plant Biol*., **54**: 357-374.

Fraser, P.D. and Bramley, P.M. 2004. The biosynthesis and nutritional uses of carotenoids. *Prog. Lipid Res*., **43**(3): 228-265. https://doi.org/10.1016/j.plipres.2003.10.002

Khoei, S.G., Mandoulakani, B.A. and Bernousi, I. 2014. Evaluation of watermelon retrotransposon elements in melon. *Am.-Eurasian J. Agric. Environ. Sci*., **14**(6): 516-520. https://doi.org/10.5829/idosi.aejaes.2014.14.06.12343

Khoei, S.G., Mandoulakani B.A. and Bernousi I. 2015. Genetic diversity in Iranian melon populations and hybrids assessed by IRAP and REMAP markers. *J. Agric. Sci. Technol.*, **17**(5): 1267-1277.

Kwon, Y.S., Oh, Y.H., Yi, S.I., Kim, H.Y., An, J.M., Yang, S.Y., Ok, SH. and Shin, J.S. 2010. Informative SSR markers for commercial variety discrimination in watermelon (*Citrullus lanatus*). *Genes Genom*., **32**:115-122. https://doi.org/10.1007/s13258-008-0674-x

Li, B., Lu X., Dou, J., Aslam, A., Gao, L., Zhao, S., He, N. and Liu, W. 2018. Construction of a high-density genetic map and mapping of fruit traits in watermelon (*Citrullus lanatus* L.) based on whole-genome resequencing. *Int. J. Mol. Sci*., **19**(10): 3268. https://doi.org/10.3390/ijms19103268

Mardi, M., Naghavi, M. R., Pirseyedi, S. M., Kazemi Alamooti, M., Rashidi Monfared, S., Ahkami, A. H., Omidbakhsh, M. A., Alavi, N. S., Salehi Shanjani, P. and Katsiotis, A. 2011. Comparative assessment of SSAP, AFLP and SSR markers for evaluation of genetic diversity of durum wheat (*Triticum turgidum* L. var. *durum*). *J. Agric. Sci. Technol.*, **13**: 905-920.

Mashilo, J., Shimelis, H., Odindo, A. and Amelework, B. 2016. Simple sequence repeat markers reveal genetic diversity within and among landrace collections of citron and dessert watermelon from South Africa. *J. Am. Soc. Hortic. Sci*., **141**(6): 598-608. https://doi.org/10.21273/JASHS03870-16

Mashilo, J., Shimelis, H., Odindoa, A.O. and Amelework, B. 2017. Genetic diversity and differentiation in citron watermelon [*Citrullus lanatus* var. *citroides*] landraces assessed by simple sequence repeat markers. *Sci. Hortic*., **214**: 99-106. https://doi.org/10.1016/j.scienta.2016.11.015

Morilipinar, E.O., Dalda-Sekerci, A., Coskun, O.F. and Gulsen, O. 2022. Genetic analysis of local pumpkin populations. *Int. J. Agric. Nat. Sci*., **14**(3): 264-272.

Mujaju, C. and Nybom, H. 2011. Local-level assessment of watermelon genetic diversity in a village in Masvingo Province, Zimbabwe: Structure and dynamics of landraces on farm. *Afr. J. Agric. Res*., **6**(27): 5822-5834. https://doi.org/10.5897/AJAR11.100

Mujaju, C., Sehic, J. and Nybom, H. 2013. Assessment of EST-SSR markers for evaluating genetic diversity in watermelon accessions from Zimbabwe. *Am. J. Plant Sci*., **4**: 1448-1456.

Mujaju, C., Zborowska, A., Werlemark, G., Garkava-Gustavssson, L., Andersen, S.B. and Nybom, H. 2011. Genetic diversity among and within watermelon (*Citrullus lanatus*) landraces in southern Africa. *J. Hortic. Sci. Biotechnol*., **86**:353-358. https://doi.org/10.1080/14620316.2011.11512773

Nasri, S., Abdollahi Mandoulakani, B., Darvishzadeh, R. and Bernousi, I. 2013. Retrotransposon insertional polymorphism in Iranian bread wheat cultivars and breeding lines revealed by IRAP and REMAP markers. *Biochem. Genet*., **51**: 927-943. https://doi.org/10.1007/s10528-013-9618-5

Ocal, N., Akbulut, M., Gülşen, O., Yetişir, H., Solmaz, I. and Sari, N. 2014. Genetic diversity, population structure and linkage disequilibrium among watermelons based on peroxidase gene markers. *Sci. Hortic*., **176**:151-161. https://doi.org/10.1016/j.scienta.2014.07.001

Prothro, J., Sandlin, K., Gill, R., Bachlava, E., White, V., Knapp, S.J. and McGregor, C. 2012. Mapping of the Egusi seed trait locus (eg) and quantitative trait Loci associated with seed oil percentage in watermelon. *J. Am. Soc. Hortic. Sci*., **137**(5): 311-315. https://doi.org/10.21273/JASHS.137.5.311

Reddy, U.K., Nimmakayala, P., Levi, A., Abburi, V.L., Saminathan, T., Tomason, Y.R.,Vajja, G., Reddy, R., Abburi, L., Wehner, T.C., Ronin, Y., Karol, A. 2014. High-resolution genetic map for understanding the effect of genome-widerecombination rate on nucleotide diversity in watermelon. *G3-Genes Genom. Genet*.,, **4**: 2219-2230. https://doi.org/10.1534/g3.114.012815

Ren, Y., McGregor, C., Zhang, Y., Gong, G., Zhang, H., Guo, S., Sun, H.,Cai, W., Zhang, J. and Xu, Y. 2014. An integrated genetic map based on four mapping populations and quantitative trait loci associated with economically important traits in watermelon (*Citrullus lanatus*). *BMC Plant Biol*., **14**(1): 33. https://doi.org/10.1186/1471-2229-14-33

Sandlin, K., Prothro, J., Heesacker, A., Khalilian, N., Okashah, R., Xiang, W., Bachlava, E., Caldwell, D.G., Taylor, C.A., Seymour, D.K., White, V., Chan, E., Tolla, G., White, C., Safran, D., Graham, E., Knapp, S. and McGregor, C. 2012. Comparative mapping in watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai]. *Theor. Appl. Genet*., **125**(8): 1603-1618. https://doi.org/10.1007/s00122-012-1938-z

Singh, D., Singh, R., Sandhu, J. S. and Chunneja, P. 2017. Morphological and genetic diversity analysis of *Citrullus* landraces from India and their genetic inter relationship with continental watermelons. *Sci. Hortic*., **218**: 240-248. https://doi.org/10.1016/j.scienta.2017.02.013

Soghani, Z.N., Rahimi, M., Nasab, M.A. and Maleki, M. 2018. Grouping and genetic diversity of different watermelon ecotypes based on agro-morphological traits and ISSR marker. *Iheringia - Ser. Bot*., **73**(1): 53-59. https://doi.org/10.21826/2446-8231201873107

474 Sudha, R., Samsudeen, K., Rajesh, M.K. and Niral, V. 2022. Molecular marker assisted
475 confirmation of hybrids in coconut (*Cocos nucifera* L.). *Indian J. Genet. Plant Breed*.,
476 **82**(3): 369-372. https://doi.org/10.31742/ISGPB.82.3.15
477 Verma, M. and Arya, L. 2008. Development of EST-SSRs in watermelon (*Citrullus lanatus*
478 var. *lanatus*) and their transferability to *Cucumis* spp. *J. Hortic. Sci. Biotechnol*., **83**(6):
479 732-736. https://doi.org/10.1080/14620316.2008.11512452
480 Yagcioglu, M., Gulsen, O., Yetisir, H., Solmaz, I. and Sari, N. 2016. Preliminary studies of
481 genom-wide association mapping for some selected morphological characters of
482 watermelons. *Sci. Hortic*., **210**: 277-284. https://doi.org/10.1016/j.scienta.2016.08.001
483 Zhang, J., Yang, J.J., Zhang, L.K., Luo, J., Zhao, H., Zhang, J.N. and Wen, C.L. 2020. A new
484 SNP genotyping technology target SNP-seq and its application in genetic analysis of
485 cucumber varieties. *Sci. Rep*., **10**: 5623. https://doi.org/10.1038/s41598-020-62518-6