

## Determination of Markers Associated with Important Agronomic Traits of Watermelon (*Citrullus lanatus* L.)

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### 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 was developed 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 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:** Association mapping, ISSR, iPBS, SSR, Sugar parameters.

### 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 programs. 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

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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 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 and contribute to future genetic and breeding programs 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 extraction was performed with the DNA isolation method developed by Doyle and Doyle (1990). The total volume for the PCR reaction was prepared as 15  $\mu$ L: 7.15  $\mu$ L distilled water, 1.5  $\mu$ L 10 $\times$ DNA polymerase buffer, 2.5 mM dNTPs, 5 mM primer, 1U Taq Polymerase, and 20 ng DNA. The prepared PCR mix was analyzed 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 Associated 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 associated mapping study, 26 character-related traits were analyzed 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  $\Delta K$  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 analyzing 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 GenAIEx 6.5 program. The amount of Polymorphic Information (PIC) was determined using Microsoft Excel. For association mapping, QQ 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

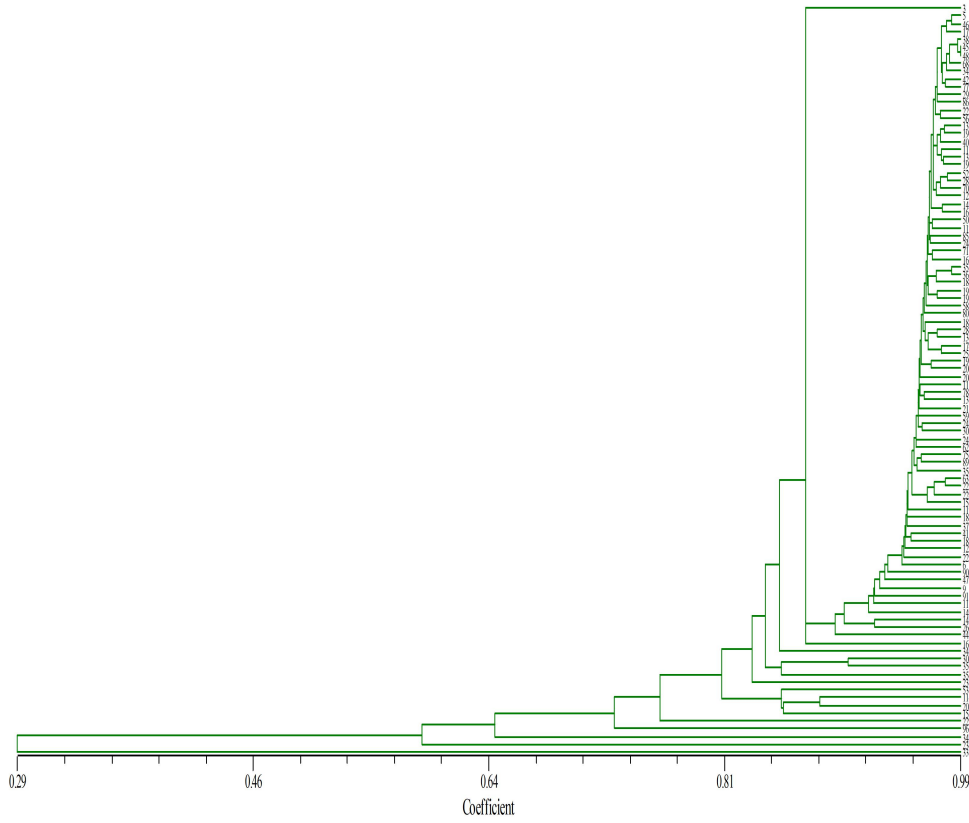
Analysis of molecular characterization were performed with a total of 110 primers, 36 iPBS, 12 ISSR, and 62 SSR in 96 genotypes. The total number of bands obtained was 1,397,

and the number of bands per primer was 12.7. A total of 1,364 of the 1,397 bands obtained were polymorphic, the polymorphism rate was determined to be 97.6% and the band sizes varied between 45-2,100 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 from 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).

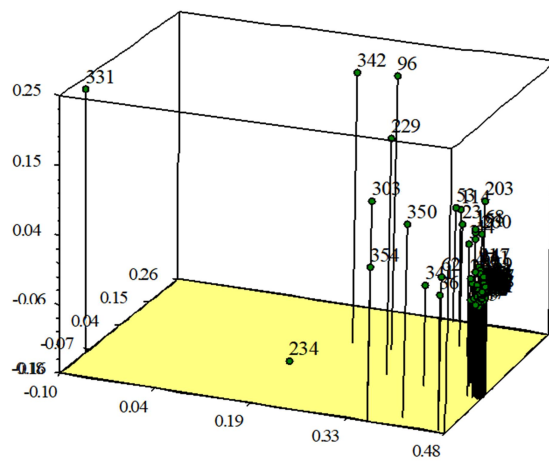
In the principal component analysis obtained using 96 genotypes, the cumulative sum of the first three eigenvalues for the two- and three-dimensional graphs was determined as 89.1. In the three-dimensional PCA graph, the 85 genotypes took place together and formed the first cluster. Genotypes 331, 234, 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).

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).

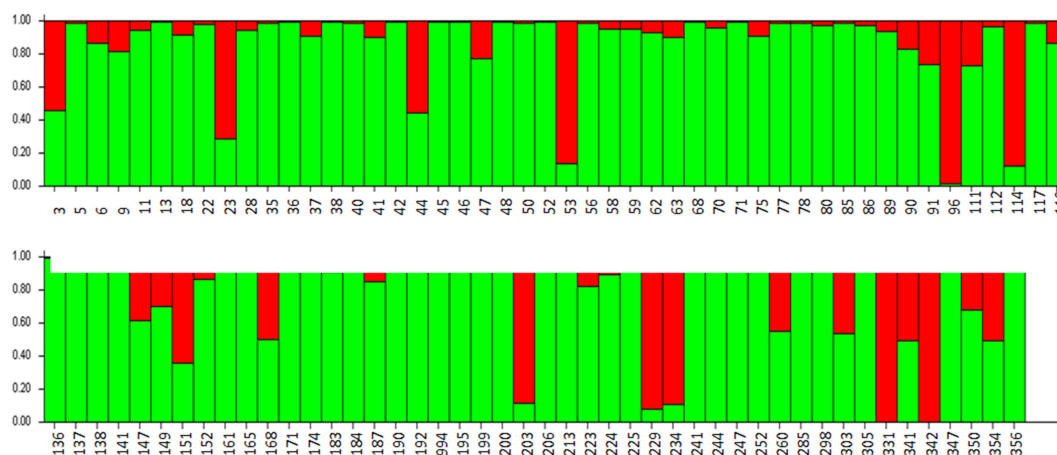
The number of bands obtained per primer (12.7) was higher than Alsohim and



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



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



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

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 higher than those of Alshim 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 ( $H_e$ ) 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

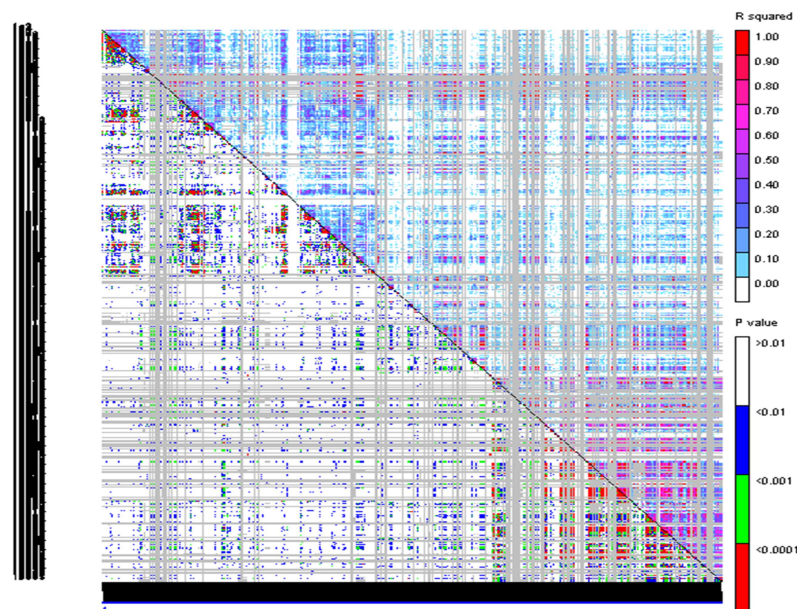


markers had LD values at certain degrees. Out of 103,927 marker pairs, 28,795 showed LD at the 0.05 level (27.7%), 17,782 at the 0.01 level (17.1%), and 7,915 at the 0.001 level (7.6%). The mean LD value ( $D'$ ) among the loci showing a statistically significant LD was 0.54. LD is usually evaluated 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".

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 analyzing 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). Based on these markers, the rate of explanation of the ovarian height of the model 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 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). Based on these markers, the rate of explanation of the



**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.

hairiness value in the ovary of the model 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 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 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 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 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 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, and 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 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 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 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 was 68.6% (Table 1).

There were two independent variables at  $P < 0.05$  level (SSR-CMTm144.550 and SSR-CMTp158.1050) in the model obtained with seed number values. The rate of explanation of the number of seeds in the model was 11.3%. In the model obtained with seed width values, there were two independent variables 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 was 34.1%. In the model obtained with seed thickness values, there

**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

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 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 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 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 was 61.1%. In the model obtained according to the MLM (K+Q) model with the sucrose character, there were five independent variables 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 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 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 was 46.1% (Table 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, diameter, height, skin thickness, 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 analyzed 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 QTLs for sugar parameters 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 they used populations with different genetic diversity. Populations obtained by crossover have much more limited genetic diversity than natural populations. This reduces the number of associated markers identified.

These findings suggest that there is a 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. With the data obtained, it is possible to determine the effect of genetic variation on the results of the associated mapping study to determine the genes associated with these characters, to contribute to future genetic and breeding programs, and to be used in Marker-Assisted Selection (MAS) studies.

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### تعیین نشانگر (مارکر) های مرتبط با صفت های زراعی مهم هندوانه (*Citrullus lanatus* L.)

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#### چکیده

تجزیه و تحلیل ارتباط با استفاده از اطلاعات فنوتیپی و نشانگرهای مولکولی ممکن است اطلاعات ارزشمندی را برای اصلاح مولکولی و انتخاب به کمک نشانگر فراهم کند. هدف از این پژوهش تعیین نشانگرهای مرتبط با پارامترهای قند و صفت های مهم زراعی هندوانه و برآورد سطح تنوع ژنتیکی بود. ۹۶ لاین هندوانه با ترکیب داده های نشانگر SSR (تکرار توالی ساده)، ISSR (تکرار توالی بین ساده) و iPBS (محل اتصال بین پرایمینگ) ژنوتیپ شدند. این ژنوتیپ ها همچنین از نظر ساختار جمعیت، عدم تعادل پیوندی (LD) (linkage disequilibrium) و نقشه برداری ارتباطی (AM=association mapping) پارامترهای قند و سایر صفت های مهم زراعی مورد ارزیابی قرار گرفت. در تجزیه و تحلیل، ۵۸۳ نشانگر تا حدی دارای مقادیر LD بودند. یک مدل خطی کلی توسعه داده شد با استفاده از ماتریس Q که ساختار جمعیت را در نقشه برداری ارتباطی نشان می دهد، یک مدل خطی پیچیده با استفاده از ماتریس خویشاوندی، و یک مدل خطی پیچیده با

استفاده از هر دو مدل خطی ماتریس Q و K. نرخ توضیح (explanation rates) مدل رگرسیون برای ۲۶ صفت از ۱۱.۳٪ تا ۸۱.۳٪ متغیر بود. بالاترین میزان تبیین مدل رگرسیونی برای سفتی میوه (۸۱/۳٪) و ارتفاع میوه (۷۸/۲٪) اندازه گیری شد. ممکن است تعیین ژن های مرتبط با صفتهای مورد مطالعه امکان پذیر باشد، تا به مطالعات ژنتیکی و اصلاح نژادی آینده کمک کرده و در مطالعات انتخاب به کمک نشانگر (MAS) استفاده شود.