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Genome-Wide Association Study on Berry Traits of Table Grapes

생식용 포도의 과실 특성에 대한 전장 유전체 연관 분석

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ABSTRACT

Exploring genes involved in fruit quality traits and revealing their roles are necessary for the development of high quality cultivars. In this study, berry traits related to quality in table grapes were analyzed by genome-wide association study (GWAS). They included berry and seed weights, tensile strength, soluble solid contents (SSC), titratable acidity (TA), berry flesh firmness, and polymeric tannin contents (PTC) in berry skin. The traits were evaluated for two years, 2018 and 2019. From August until October, uniformly ripe berries were randomly collected from 'Tano Red' and 'Ruby Seedless', and their 269 progeny for measuring the above traits. Phenotype data for the seven traits in the two different years exhibited widely ranged Pearson correlation coefficients from 0.34-0.81, but their *P*-value ranged from 1.126e-07 to 2.2e-16, indicating that the data in the two years were

correlated. Genotyping-by-sequencing method was used for sequence analysis. A total of 148.69 Gb of sequence data including 1.47×10^9 reads, was generated from the 271 grape plants. These reads were aligned to the reference genome of the grape PN40024 12X.v2 genomic sequence. A total of 400,648 single nucleotide polymorphisms (SNPs) were initially obtained by SNP matrix. After trimming, 25,421 SNPs were used for GWAS. After filtering, 2,243 SNPs were selected to construct a linkage map for quantitative trait locus (QTL) analysis. In GWAS, significant SNPs correlated to berry and seed weights, and tensile strength were located in chromosome 18 (chr. 18). The location of the SNPs was around a dominant regulator gene named *SDI* (seed development inhibitor), *VvAGL11*. From the QTL analysis using a linkage map, the QTLs of seed weight were detected only in the linkage group (LG) 18, but those of berry weight were additively detected in LGs 11 and 15. The QTLs of tensile strength common in the two years were only detected in LG 18. In the case of SSC, TA, and berry flesh firmness, there were several SNPs related to each trait for each year. However, no SNPs exceeding the threshold were found common in the two years by both GWAS and QTL analysis. The PTC in berry skin was used as a value for estimating the perceived astringency. Significant SNPs for PTC in berry skin were found in chr. 11 by GWAS and QTL analysis. *VvMybPA2* was identified as a candidate gene for determining astringency of berry skin. In conclusion, significant SNPs were found around the gene *VvAGL11* in the three traits, berry and seed weights, and tensile strength. These traits are significantly affected by the presence of seeds. In the case of the PTC in berry skin, *VvMybPA2*, which is the gene related to proanthocyanidin biosynthesis, was selected as a candidate gene. The identification of the markers closely associated

with these berry traits will be useful for grape molecular breeding.

Keywords**:** genome-wide association study, grape breeding, quantitative trait locus, single nucleotide polymorphism

Student number**:** 2018-26880

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INTRODUCTION

Grape (*Vitis* spp.) is one of the most extensively cultivated fruit plants with table grapes accounting for 36% of the total world production (Office International de la Vigne et du Vin, 2019; Reisch et al., 2012). Breeders in important table grape growing areas have been trying to develop grape cultivars with superior berry traits to those already be on the market. Seedlessness, crisp texture, and suitability for storage are the main goals of table grape breeding (Reisch et al., 2012).

Breeding of perennial fruit species is a long-term activity involving a high investment as compared to annual crops due to two challenges: long juvenile periods and the plant size (Zhebentyayeva et al., 2012). To select one with desired traits from the population by introducing molecular breeding technology can be a great benefit in terms of both saving time and cost. Identifying genes involved in target traits for breeding and developing markers are ongoing in various fruit trees. Most studies have been conducted in the form of various mapping approaches including quantitative trait locus (QTL) mapping. QTL mapping has proved a powerful method to identify regions of the target gene either in F2 populations or recombinant inbred line families (Dirlewanger et al., 2009; Kenis et al., 2008; Korte and Farlow, 2013; Martínez-García et al., 2013; Wu et al., 2014). However, QTL mapping has its limitations when it comes to mapping populations (Korte and Farlow, 2013). A new study method was named genome-wide association study

(GWAS) investigates the association between genotypes and phenotypes, using the entire genome. This method is based on the hypothesis that there are common genetic variants that affect a trait, independent from the effect of pedigree (Visscher et al., 2012). GWAS can serve as a foundation experiment by providing insights into the genetic architecture of the trait, suggesting an informed choice of parents for QTL analysis. The results of GWAS find out the loci of candidates for mutagenesis and transgenics (Korte and Farlow, 2013). Thus, GWAS are often complementary to QTL mapping, when conducted together (Manenti et al., 2009).

GWAS has recently been a common way of studying agriculturally important quantitative traits and natural variations, thanks to the development of next-generation sequencing technologies (Atwell et al., 2010; Guo et al., 2019; Yano et al., 2016). It is also expanding to research related to crops including fruit trees (Cao et al., 2012; Guo et al., 2019; Iwata et al., 2013; Kumar et al., 2013; Lee et al., 2017). There are a few studies on the genetic determination of quantitative traits in fruit trees using GWAS. In apple, GWAS was conducted using 1,200 seedlings of seven full-sib families to reveal significant associations of six fruit traits. Significant associations were found in all six traits, some of which were coincident to known candidate genes (Kumar et al., 2013). In pear, nine agronomic traits were investigated by GWAS, among them harvest time, black spot resistance, and spur number were associated with significant QTLs (Iwata et al., 2013). In peach, GWAS using 104 landrace accessions with 53 simple sequence repeat (SSR) markers

detected associated markers for ten traits related to fruit and phenological period (Cao et al., 2012).

In grape, several QTL mappings and GWAS of berry related traits have been conducted. Berry size and seedlessness are primary targets of breeding programs for table grapes (Cabezas et al., 2006). To this end, there are several approaches for identifying genes to determine seedlessness. The MADS-box gene *VvAGL11* is essential for seed morphogenesis in grapes (Malabarba et al., 2017). The QTL analyses related to seed traits by using seedless grapes as parental resources found QTLs in the region of *SDI* (seed development inhibitor), *VvAGL11* (Cabezas et al., 2006; Doligez et al., 2002; Mejía et al., 2007). Previous studies to identify genes related to soluble solid contents (SSC) and titratable acidity (TA) in grape berries, detected QTLs in various linkage groups (LGs) (Bayo-Canha et al., 2019; Chen et al., 2015; Liu et al., 2007; Viana et al., 2013; Zhao et al., 2015). Several pieces of research regarding berry flesh firmness revealed QTL in LG 18 commonly and other researches detected QTLs in other LGs (Carreño et al., 2014; Correa et al, 2016; Jiang et al., 2020). A GWAS of six table grape berry traits including berry color, berry development period, cluster size, berry weight, and berry flesh texture was conducted by using 179 genotypes comprising a mixture of landraces and cultivars. By the study, QTLs in every trait were founded respectively (Guo et al., 2019).

The goals of this study were to determine the significant genetic regions and find

the candidate genes governing genetic variations in seven berry related traits of table grapes. A pseudo-F2 population was genotyped by genotyping-by-sequencing (GBS) method and phenotypic variations of the seven traits were measured for two years. QTLs detected by GWAS and linkage analysis by genetic map were compared with QTLs in previous studies and candidate genes underlying the QTLs were predicted. In this study, not only the traits previously studied but the tensile strength, which is advantageous shelf-life characteristic and astringency of berry skin were also investigated. A comprehensive understanding of genetic determinism of the berry related traits will facilitate the breeding of new grape cultivars.

MATERIALS AND METHODS

Plant materials and phenotyping

'Tano Red (*Vitis* spp.)' and 'Ruby Seedless (*V. vinifera*)' grapevines, and their 269 progeny grown under field conditions in the experimental orchard of the National Institute of Horticultural and Herbal Science, Rural Development Administration, Wanju (35° 83' N, 127° 03' E), Republic of Korea, were used in this study. The annual precipitations of Wanju area were 1,332.5 and 968.9 mm in 2018 and 2019, respectively, and the corresponding annual average temperatures were 13.9 and 14.2ºC, respectively. Seven berry traits were evaluated. They included berry and seed weights, SSC, TA, berry flesh firmness, tensile strength, and polymeric tannin contents (PTC) in berry skin. From August until October, five or more ripe clusters were harvested from each vine, and then 50-100 berries were randomly selected for determining the above traits.

Berry and seed weights

Ten intermediate size berries and seeds from the ten berries were weighed and averaged per berry.

SSC and TA

About 50 g of randomly selected berries wrapped with cotton gauze were

squeezed out using a stainless juicer. Using the juice, SSC was measured with a digital refractometer (PAL-1, Atago, Tokyo, Japan). TA was determined by titrating 5 mL of the juice diluted in 35 mL of distilled water with 0.1 N NaOH to an endpoint pH of 8.5, and converted the amount of NaOH consumed until the discoloring point to the amount of tartaric acid. Data for each progeny in each year were the averages of three replications.

Berry flesh firmness and tensile strength

Berry flesh firmness was measured using a TA-PLUS texture analyzer (Lloyd Instr. Ltd., West Sussex, UK) fitted with a 5-mm probe. The equatorial side of each berry was peeled slightly and compressed by 5 mm at a speed of 100 mm/min. The maximum force was recorded and expressed as Newton (N).

Tensile strength was measured using a self-made device. A clip with a string was fixed at the top, a stem with berry was fixed to the clip, and the berries were intercepted by a stainless-steel plate with a hole in the middle. The position of the berry was fixed by the plate and the clip was pulled vertically to pull the stem upwards, and the tension required to separate the berry from the stem was measured. Both data of berry flesh firmness and tensile strength were the averages of five replications.

PTC in berry skin

Three replications were employed and about ten uniform berries were randomly collected from each replicate. The berry skin was separated, washed, lyophilized, and powdered. For the extraction of phenolic compounds, 10 mL of extraction solvent consisting of water, methanol, and acetone (36:16:48, v/v/v) was mixed with 0.3 g of skin powder in a conical tube, followed by sonication at room temperature for 60 min. The extraction medium containing extracted phenolic compounds was separated by centrifugation at 4,500 rpm for 30 min and filtered using a syringe filter (0.2 μm in pore diameter), and the filtrate was stored in a refrigerator until analysis.

PTC in the extract was estimated using bovine serum albumin (BSA) precipitation assay (Harbertson et al., 2015). For the precipitation of polymeric tannin, 0.5 mL of extract was added to 1 mL of 1 mg/mL BSA in washing buffer (170 mM NaCl in 200 mM acetic acid, pH 4.9) in a 2-mL microcentrifuge tube and incubated at room temperature for 10 min. After centrifugation at 10,000 rpm for 2 min and discarding the supernatant, 1 mL of the washing buffer was added, vortexed, and centrifuged again. After discarding the supernatant again, 875 μL of resuspension buffer (5% triethanolamine in 8.3 M urea aqueous solution, pH 7.0) was added, vortexed, and incubated at room temperature for 10 min to resuspend the protein/tannin sediment. To 96-well microplate, 175 μL of each sample solution was transferred and 25 μL of $FeCl_3$ solution (10 mM $FeCl_3$ in 10 mM HCl) was added, covered with a lid, incubated with shaking for 2 min, and halted for 8 min

on a microplate reader (Multiscan GO, Thermo Fisher Scientific, Waltham, MA, USA). The absorbance of the reaction mixture (Abs1) was recorded at 510 nm against the blank solution consisting of resuspension buffer and the $FeCl_3$ solution. In the same way, the absorbance of the reaction mixture (Abs2) consisting of the resuspended sample solution and 10 mM HCl was recorded as well. The absorbance value Abs1 subtracted from Abs2 was used to estimate PTC according to a tannic acid calibration curve. The amount of PTC was expressed as μg/mL tannic acid equivalent.

DNA extraction, sequencing, and single nucleotide polymorphisms (SNP) calling

Immediately expanded leaves were randomly collected separately from the 271 grapevines. Genomic DNA was extracted from the leaves using a DNeasy Plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Sequence analysis was performed by using the GBS method. The purity of the extracted DNA was increased using a DNA purification kit. DNA was cleaved using *Ape*KI restriction enzyme, and then a barcode, a sequencing adapter, was attached to the cleaved region. These library fragments were amplified by performing a multiplex polymerase chain reaction. Multiplex sequencing was performed to generate SNP using a HiSeq 2500 (Illumina, San Diego, CA, USA).

SNP matrix design

Demultiplexing was performed using the barcode sequence, and the adapter sequence was removed. DynamicTrim and LengthSort programs of SolexaQA (v.1.13) package were used for trimming according to quality. The pretreated clean reads were mapped into the reference genome (PN40024 12X.v2) using the BWA (0.6.1-r104) program. The raw SNPs were detected and consensus sequences were extracted from the BAM format file created by the mapping procedure using the SAMtools (0.1.16) program. The raw SNP position was obtained by comparing each sample with the reference genome and then used as a candidate for constructing a list of the unions. At this time, a blank area (non-SNP loci) was filled from the sample's consensus sequence to design the SNP matrix.

Association study

From the 400,648 SNPs obtained through the SNP matrix, 25,421 SNPs were selected based on a minimum allele frequency above 5% and a missing data below 30% (Table 1). Phenotype data of the seven berry traits collected twice in the years 2018 and 2019 were used as continuous variables. GWAS was performed using TASSEL 5.0 (Bradbury et al., 2007).

Linkage mapping

The linkage map was constructed with JoinMap 4.0 software (Van Ooijen, 2006) using the regression mapping algorithm. A population type was cross pollinator

Table 1. SNP marker selection for GWAS by filtering.

Trimming stage	Filter criteria	No. of SNPs
1	Total SNP matrix	400,648
2	Minor allele frequency (MAF) > 5%	138,986
3	Missing data < 30%	149,535
4	MAF > 5% and missing data < 30%	25,954
5	Chromosomes 1 to 19	25,421

Five hundred and fifty-three SNPs that cannot be aligned to chr. 1 to 19 were excluded.

(CP), which indicates a cross between two heterozygous diploid parents, with unknown linkage phases (Van Ooijen, 2006). Among five segregation types (lm×ll, nn×np, hk×hk, ef×eg, and ab×cd) of CP populations, three of the segregation types (lm×ll, nn×np, and hk×hk) were genotyped in this study. The 'lm×ll' describes markers with first parent being heterozygous and second parent being homozygous, the 'nn×np' describes markers with first parent being homozygous and second parent being heterozygous, and the 'hk×hk' describes markers with both parents being heterozygous (Ban and Choi, 2018). From the 25,421 SNPs, 8,557 SNPs were divided into three segregation types (Table 2). After removing the redundant SNP markers, 2,418 SNPs remained for linkage map construction. Finally, 2,243 SNPs were used to construct the genetic map. Kosambi mapping function was applied to convert recombination rates into genetic distances (Kosambi, 2016). LGs were estimated by applying the independence logarithm of odds (LOD) threshold ranges from 2 to 22, and constructed with a linkage LOD of at least 5.0.

QTL analysis

QTL mapping was performed on the population using the consensus map and phenotypic data. The method was maximum likelihood via an expectation-maximization algorithm by R software package, version 3.6.3 (http://www.r-project.org). LOD thresholds were estimated by 1,000 permutation tests with significance at $P < 0.05$. Finally, candidate genes were detected in 200 kb-region

Table 2. Allelic segregation types used to construct the genetic linkage map.

Segregation type	No. of SNPs	No. of selected SNPs
nn×np	2,740	771
lm×ll	3,575	880
hk×hk	2,242	767
Total	8,557	2,418

In every segregation type, markers for linkage map construction were selected per 300 kb chromosome region.

around each QTL using the reference genome (PN40024 12X.v2) and description information of the National Center of Biotechnology Information (NCBI). The genomic locations of all the predicted genes were retrieved from the ensemble plant BioMart server (http://plants.ensembl.org/biomart/martview).

Statistical analysis

Statistical analysis was performed in the R software package. Pearson correlation analysis was performed between traits within years and between years for each trait.

RESULTS AND DISCUSSION

Phenotype data of berry traits

Phenotype distributions of the seven berry traits are shown in the graphs of Fig. 1. Most traits showed normal distribution except seed weight and PTC in the berry skin in 2018. Pearson correlation coefficients between the two years widely ranged from 0.31 to 0.81, but their *P*-values ranged from 1.126e-07 to 2.2e-16, indicating that the data in the two years were correlated (Table 3). Among them, the annual variations were higher in TA and SSC than those in the other traits between the two years. These annual variations may be due to environmental factors or differences in harvest time.

As a result of examining the correlation coefficients between seven berry traits, there were the most positive correlations between seed and berry weights (Fig. 2). This correlation likely results from the fact that gibberellins produced by seeds promote berry growth during berry developmental stages (Francisco et al., 2000). Seed and berry weights positively correlated with tensile strength (Fig. 2). There is no direct evidence related to this correlation, but the maximum tensile strength is normally associated with the separation of the vascular bundles of the seeds inside the berry (Gomes and Ferraz, 2011). It suggests that the physical properties of the vascular tissue in berries vary depending on the presence of seeds or berry size. However, there were negative correlations between berry flesh firmness and berry

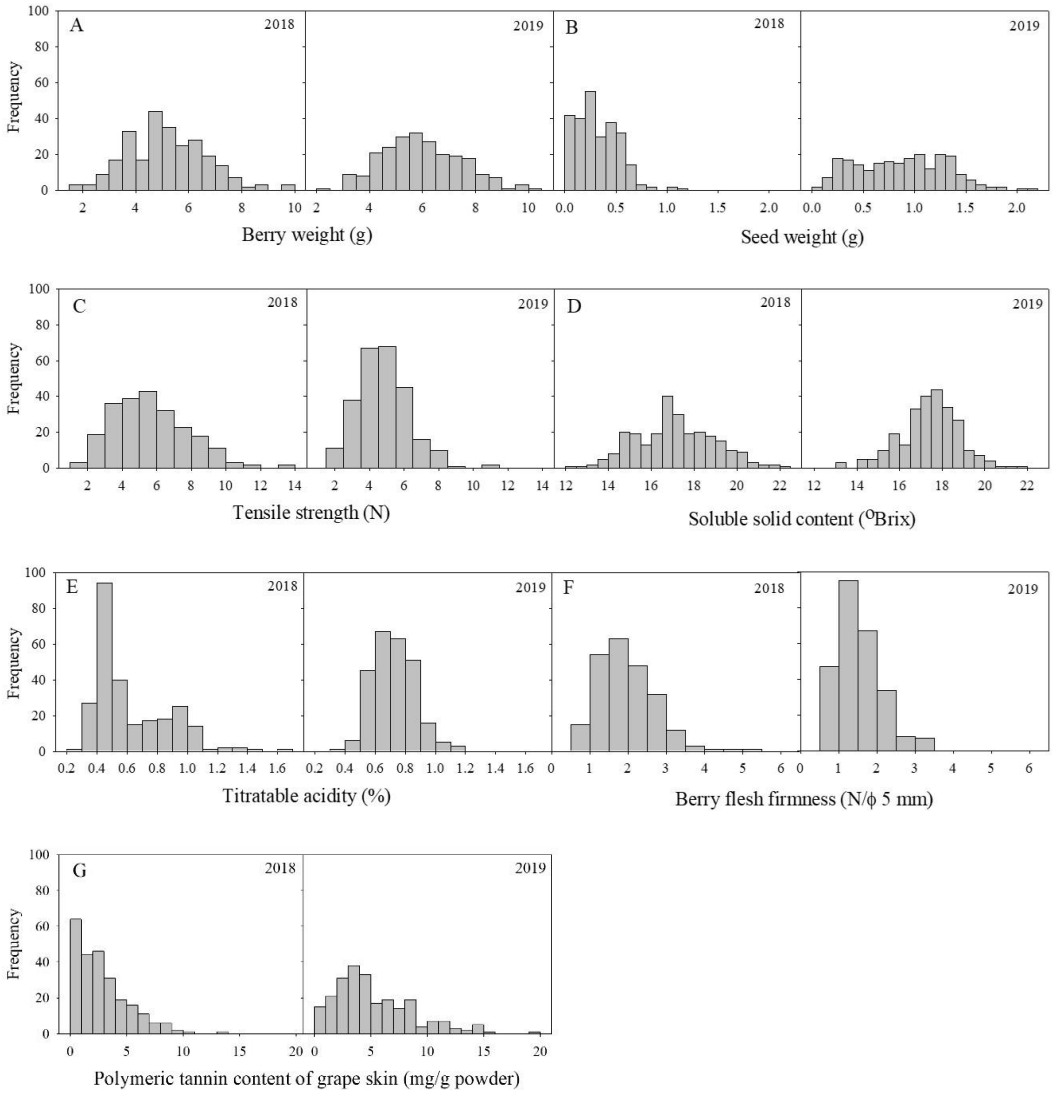

Fig. 1. Distribution of (A) berry weight, (B) seed weight, (C) tensile strength, (D) soluble solid contents, (E) titratable acidity, (F) berry flesh firmness, and (G) polymeric tannin contents in berry skin of table grapes.

Table 3. Phenotypic variation and correlation between two years for berry traits of table grapes.

Trait	Year	Min	Max	Mean	Pearson correlation coefficient
Berry weight (g)	2018	1.74	9.7	5.16±1.48	0.81**
	2019	2.22	10.2	5.95±1.50	
Seed weight (g)	2018	0	1.2	0.32±0.21	0.65**
	2019	0.07	2.15	0.88±0.44	
Tensile strength (N)	2018	1.18	13.2	5.66±2.15	0.47**
	2019	1.78	10.7	4.79±1.48	
SSC (^{o}Brix)	2018	12.3	22	17.1±1.83	0.37**
	2019	13.0	23	17.4±1.42	
TA (%)	2018	0.3	1.6	0.62±0.25	0.34**
	2019	0.25	1.17	0.67±0.14	
Flesh firmness (N)	2018	0.62	5.24	1.96±0.74	0.57**
	2019	0.43	3.35	1.50±0.57	
PTC (mg/g)	2018	0	13.5	2.83±2.34	0.42**
	2019	0.06	19.7	5.23±3.5	

** Significant at $P < 0.01$. SSC, soluble solid contents; TA, titratable acidity; PTC, polymeric tannin contents in berry skin.

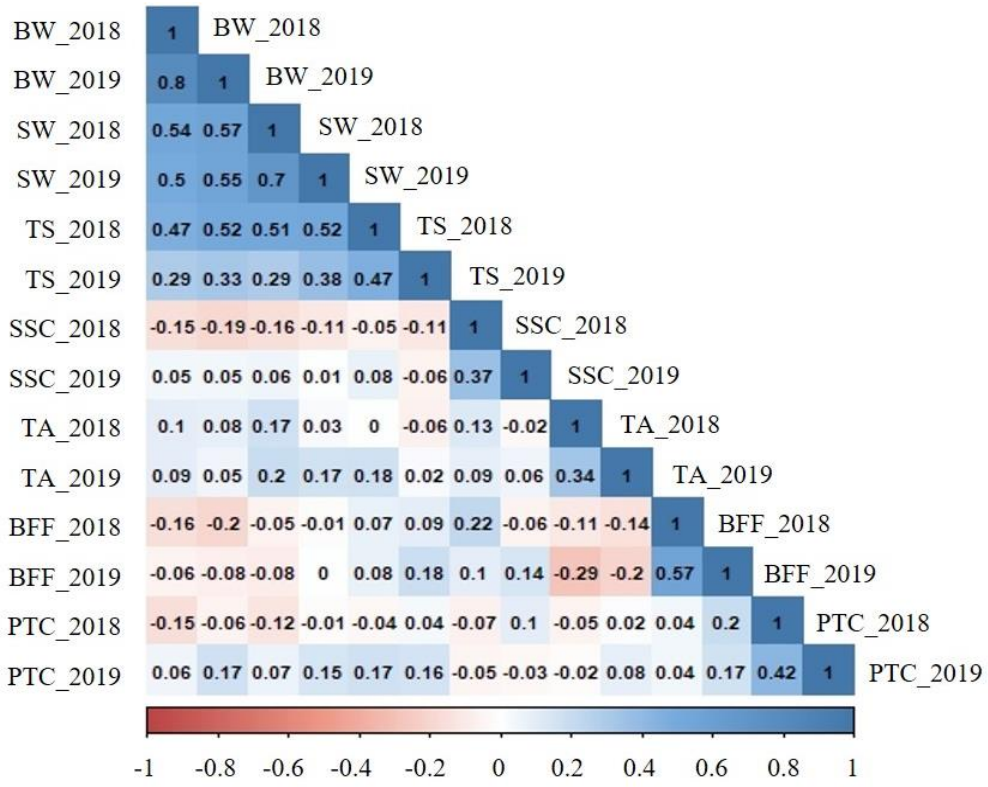

Fig. 2. Correlations among berry traits of table grapes in 2018 and 2019. The color distribution represents the correlation. BW, berry weight; SW, seed weight; TS, tensile strength; SSC, soluble solid contents; TA, titratable acidity; BFF, berry flesh firmness; PTC, polymeric tannin contents in berry skin.

weight, and between berry flesh firmness and TA. Such significant correlations were not observed in the other berry traits. Since the specific factors of each year can be offset by using the average value of the two years, the association study was conducted for each year.

SNP calling and alignment

A total of 148.69 Gb of sequence including 1.47×10^9 reads, was generated from the 271 grape plants including parental lines. These reads were aligned to the grape PN40024 12X.v2 genomic sequence. A total of 400,648 SNPs were initially obtained for these genotypes. After trimming, 25,421 high-quality SNPs remained for further analysis. These high-quality SNPs covered all 19 chromosomes and were almost evenly distributed across the whole genome (Fig. 3). The largest number of SNPs was found on chr. 18 (2,243 SNPs), followed by chr. 13 (1,767 SNPs), whereas the smallest number of SNPs was found on chr. 7 (795 SNPs). The SNPs on each chromosome were distributed consistently with the physical length of the corresponding chromosome. The average marker density was 19.12 kb/SNP. The highest SNP marker density was 14.97 kb/SNP in chr. 5, whereas the lowest marker density was 24.78 kb/SNP in chr. 4.

Linkage map and QTL analysis

A linkage map was built using 2,243 SNP markers distributed on 19 LGs (Fig.

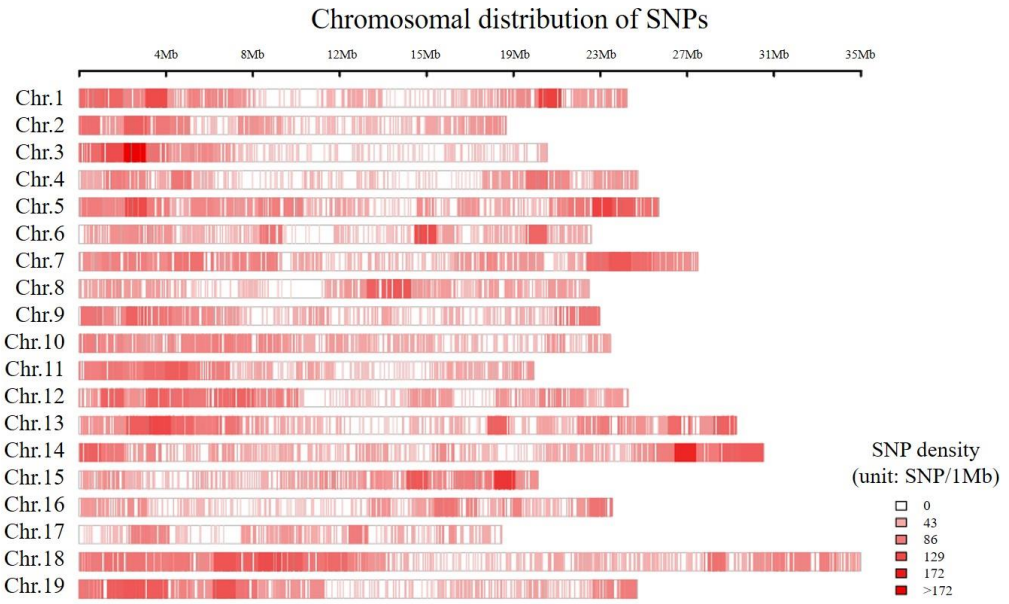

Fig. 3. SNP distributions on 19 chromosomes of table grapes. The 25,421 SNPs were evenly distributed throughout all chromosomes.

4). This map had a total length of 2,068 centiMorgans (cM) and the average marker distance of 0.99 cM (Table 4). In the high-density map of a previous study with the whole-genome resequencing data, the average marker distance was 0.88 cM (Jiang et al., 2020). In the present study with the genomic data from GBS methods, a high-density linkage map could be constructed comparing to other genetic maps (Bayo-Canha et al., 2019; Viana et al., 2013).

SNPs related to berry and seed weights

The Manhattan plot showed a distinct peak of significant SNPs related to berry weight on chr. 18 (Fig. 5A, B). The threshold [$-\log(P) = 5.7$] was calculated and corrected by using the Bonferroni method ($\alpha = 0.05$, n = 25,421). There were 55 significant SNPs related to berry weight common in 2018 and 2019, above the threshold. Except for two SNPs in the other chromosomes, 53 SNPs were located in 27.6-34.4 Mbp on chr. 18 (Table 5).

In the case of seed weight, 102 loci above the threshold were associated with seed weight and common in the two years (Fig. 5C, D). Among them, except for eight loci, 94 loci were located in 24.9-34.4 Mbp on chr. 18 (Table 5). This wide range included the locus of gene *VvAGL11* (26.8 Mbp on chr.18).

Parthenocarpy and stenospermocarpy are genetically mediated seedlessness in grape berries (Bouquet and Danglot, 1996; Cabezas et al., 2006). In stenospermocarpy, although pollination occurs, the seeds fail to develop due to the early degeneration

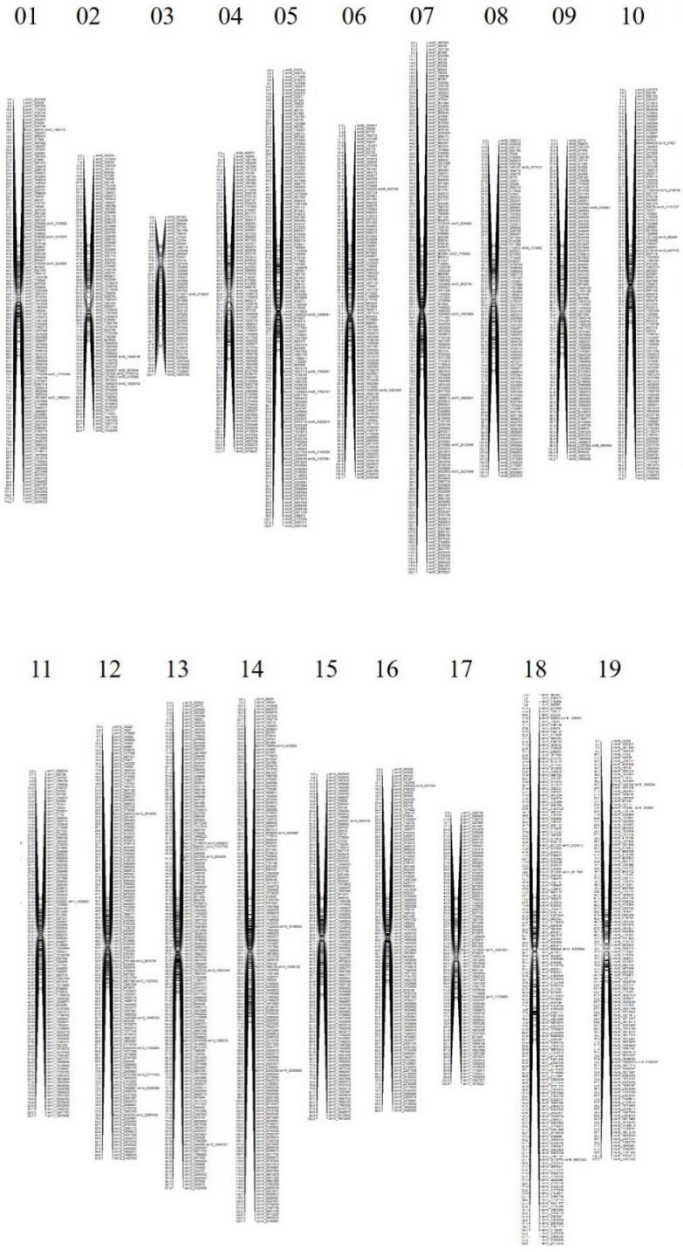

Fig. 4. Consensus genetic map of table grape population constructed using 2,243 SNP markers distributed on 19 LGs.

Table 4. Distribution of SNP markers on the genetic linkage map.

Linkage group	No. of mapped markers	Linkage group length (cM)	Average marker distance (cM)
1	127	114.2	0.90
2	88	98.4	1.12
3	49	104.1	2.12
4	90	117.7	1.31
5	143	108.5	0.76
6	108	116.1	1.08
7	166	108.5	0.78
8	103	116.1	1.26
9	98	128.7	1.15
10	122	130.2	0.65
11	105	112.2	0.89
12	138	79.3	0.67
13	152	93.7	0.64
14	162	91.9	0.8
15	105	97.8	0.95
16	104	129.1	0.83
17	170	148.1	0.87

Table 4. Continued.

Linkage group	No. of mapped markers	Linkage group length (cM)	Average marker distance (cM)
18	170	148.1	0.87
19	129	107.7	0.84
Total	2,243	2068	0.99

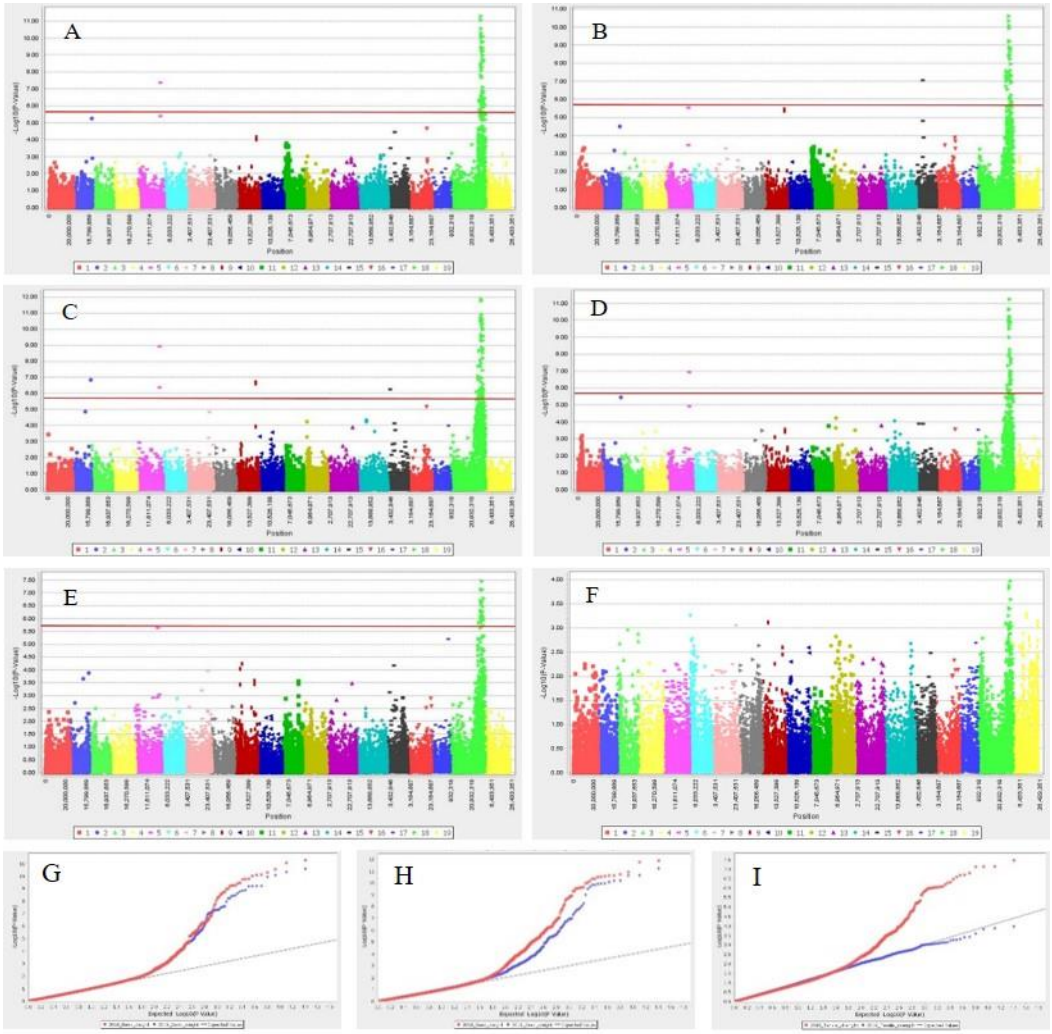

Fig. 5. Manhattan plots (A-F) and Q-Q plots (G-I) of SNP markers from mixed linear models for berry weight (A, B, G), seed weight (C, D, H), and tensile strength (E, F, I) of table grapes. In Manhattan plots $-\log_{10}(P)$ on y-axis, chromosomal position on x-axis. Red horizontal lines depict the significance threshold [$-\log_{10}(P) = 5.7$]. In Q-Q plots, red dots represent the data of 2018 and blue dots represent the data of 2019. (A) Manhattan plot of berry weight in 2018, (B) Manhattan plot of berry weight in 2019,

(C) Manhattan plot of seed weight in 2018, (D) Manhattan plot of seed weight in 2019, (E) Manhattan plot of tensile strength in 2018, (F) Manhattan plot of tensile strength in 2019, (G) Q-Q plots of berry weight, (H) Q-Q plots of seed weight, and (I) Q-Q plots of tensile strength.

Table 5. Summary of the SNPs related to berry and seed weights, and tensile strength of table grapes commonly detected in 2018 and 2019 by GWAS.

Trait	Year	No. of significant SNPs	Chr.	SNP location (Mbp)	Maximum $-\log_{10}(P)$
Berry weight	2018, 2019	1	2	18.2	7.5
		1	5	22.1	9.9
		53	18	27.6-34.4	15.9
Seed weight	2018, 2019	1	2	18.2	13.6
		2	5	22.1	19.5
		2	9	19.3	12.9
		1	12	3.5	6.7
		2	15	2.5-6.7	10.6
		94	18	24.9-34.4	24.2

of the endosperm and abnormal development of integuments (Pratt, 1971). Unlike parthenocarpy, stenospermocarpy can produce berries of a size compatible with commercial requirements (Ledbetter and Burgos, 1994). Thus, numerous genetic studies about stenospermocarpic seedlessness have been conducted for adapting to seedless table grape breeding (Mejia et al., 2011; Zhang et al., 2017).

The most accepted model for genetic inheritance of stenospermocarpic seedlessness in grapevines is based on the expression of three independent recessive genes under the control of a dominant regulator gene named *VvAGL11* (Bouquet and Danglot, 1996; Doligez et al., 2002; Lahogue et al., 1998). This model has partly been confirmed by several studies that all reported a major QTL for seedlessness with *VvAGL11* on LG 18 (Cabezas et al., 2006; Costantini et al., 2008; Mejia et al., 2007; Zhang et al., 2017). Numerous other minor QTLs were found in different LGs, but they were not reproducible across different seasons and were not present in all crosses (Cabezas et al., 2006; Costantini et al., 2008; Doligez et al., 2013; Mejia et al., 2007; Zhang et al., 2017). Thus, the molecular characterization of the *SDI* locus is a key step toward understanding the molecular mechanisms underlying seedlessness (Mejia et al., 2011). In the present study, the phenotypic data of berry and seed weights were highly correlated (Fig. 2). Furthermore, the GWAS results with berry weight data were similar to those with seed weight data (Fig. 5A, B, C, D). The significant SNPs of both traits were detected at chr. 18. All previously published QTL studies in a stenospermocarpic seedlessness background

found a major QTL for both berry and seed weights on LG 18 (Cabezas et al., 2006; Costantini et al., 2008; Doligez et al., 2002; Mejía et al., 2007; Zhang et al., 2017). On the other hand, there is research to find other QTLs for berry weight which do not colocalize with QTLs of seed traits (Doligez et al., 2013).

The classical source of seedlessness in grape breeding programs is 'Sultanina', also called 'Thompson Seedless' (Doligez et al., 2002). By that resource, *SDI* gene is inherited to almost every seedless cultivar. In the present study, the *SDI* gene from pollen parent 'Ruby Seedless' made an effect on the result of GWAS. The significant SNPs related to berry and seed weights were found at a common site in the chromosome, which was known as the main gene controlling the presence of seeds. Some SNPs related to seed weight were located in the locus of *SDI* gene (chr. 18: 26.8 Mbp), but those of berry weight were near to the *SDI* gene but not exactly matched.

SNPs related to tensile strength

By the comparison of correlation among the seven berry traits, tensile strength was highly correlated with seed and berry weights in 2018 (Fig. 2). In 2019, however, the pattern of tensile strength of individuals was changed. A number of significant SNPs related to tensile strength were also observed in the locations on chr. 18: 27.3-33.4 Mbp in 2018 (Fig. 5E, F). These results suggest that the tensile strength is associated with the presence of seeds.

Tensile strength is the fruit detachment force (FDF) between stem and berry. FDF is composed of the linking force between berry 'brush' and berry flesh, and tensile strength of the abscission zone between the pedicel and berry (Deng et al., 2005). In the present study, the linking force between brush and berry flesh appeared to be the main factor determining tensile strength in both years, because the location of significant SNPs was near the region of *VvAGL11*. However, significant SNPs above the threshold were not found in 2019.

QTL analysis of seed and berry weights, and tensile strength

By QTL analysis using the linkage map, the QTLs of berry weight were detected in LGs 11 and 15 along with in LG 18 (Fig 6A, B, Table 6). It's different from the result of GWAS, but both results mainly detected the SNPs in chr. 18 (Table 5, 6). In the previous studies, the QTLs of berry weight were found in LGs 15, 18 (Cabezas et al., 2006; Mejía et al., 2007), and 11 (Doligez et al., 2013). In each study, the maps newly constructed by the populations make it difficult to compare the exact location of the QTLs (Cabezas et al., 2006; Doligez et al., 2013; Mejía et al., 2007). However, the QTLs found at the location similar to the previous studies indicate that seeds are not the only factor affecting the berry weight. The QTLs of seed weight were detected only in LG 18 (Fig 6C, D, Table 6). However, more SNPs related to seed weight in the other chromosomes were detected in

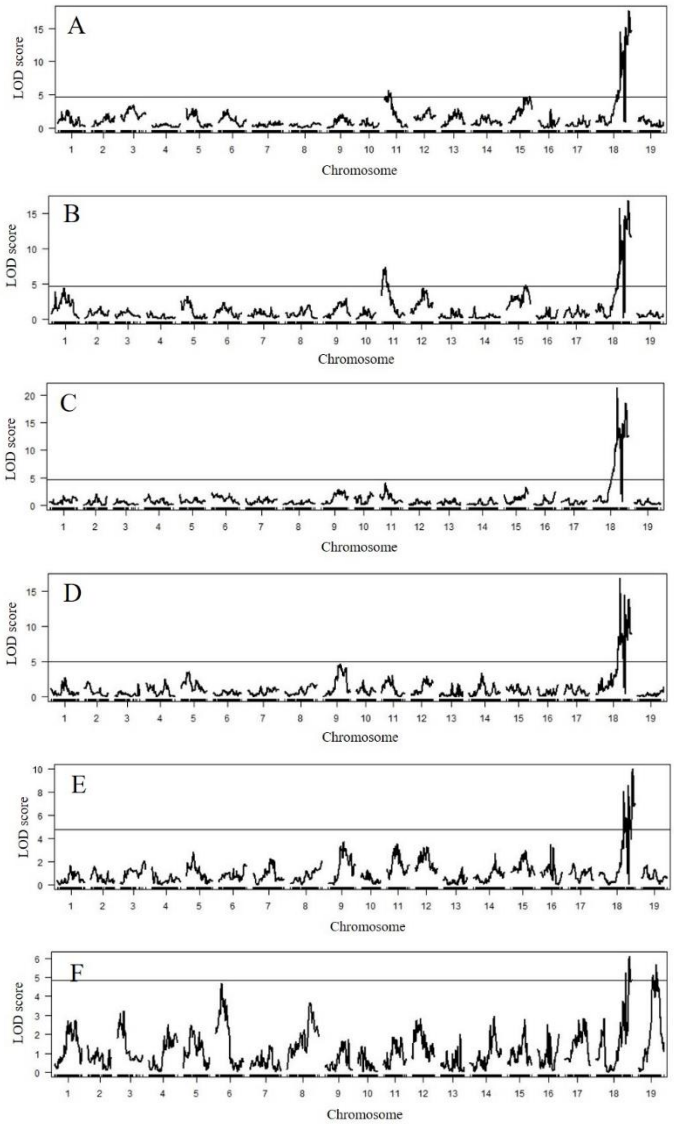

Fig. 6. LOD score plots of SNP markers from QTL analysis using linkage map for berry and seed weights, and tensile strength of table grapes. LOD on y-axis, chromosomal position on x-axis. (A) QTLs of berry weight in 2018, (B) QTLs berry weight in 2019, (C) QTLs of seed weight in 2018, (D) QTLs of seed weight in 2019, (E) QTLs of tensile strength in 2018, and (F) QTLs of tensile strength in 2019.

Table 6. Significant QTLs for seed and berry weights and tensile strength of table grapes.

Trait	Year	LOD threshold	Linkage group	No. of markers	No. of genes in QTL flanked region
Berry weight	2018	4.64	11	20	90
			15	2	33
			18	58	175
	2019	4.69	11	29	235
			15	2	33
			18	53	153
Seed weight	2018	4.71	18	64	183
	2019	4.95	18	53	153
Tensile strength	2018	4.79	18	37	71
	2019	4.85	18	9	54
			19	17	189

GWAS (Table 5).

By QTL analysis, the QTLs of tensile strength were detected in LGs 18 in 2018 and 2019 commonly (Fig 6E, F, Table 6). In 2019, more QTLs of tensile strength were detected in LG 19 (Fig 6F, Table 6). Although the studies related to hormones affecting tensile strength are ongoing, genetic mechanisms on tensile strength remain to be elucidated. Around QTLs, no significant candidate genes were related to hormones, but there were some genes that influence the delivery of carbohydrates and other functions.

SNPs related to SSC and TA

No significant SNPs related to SSC common in 2018 and 2019 were detected in GWAS (Fig. 7A, B). Similar instability of QTLs across the years has widely been reported in grapes (Costantini et al., 2008; Fanizza et al., 2005; Grzeskowiak et al., 2013). Only one SNP (chr. 16: 16.5 Mbp) related to SSC investigated in 2019 was above threshold (Fig. 7). The region near 200 kb both sides from the locus includes the gene, *stilbene synthase*. Stilbenes are a small family of phenylpropanoids. In addition to their participation in defense mechanisms in plants, stilbenes display important pharmacological properties (Parage et al., 2012). Although the broad functions of stilbene, the correlation between sugar and stilbene is not clear until now.

By the QTL analysis using linkage map, 105 SNPs were detected as QTLs

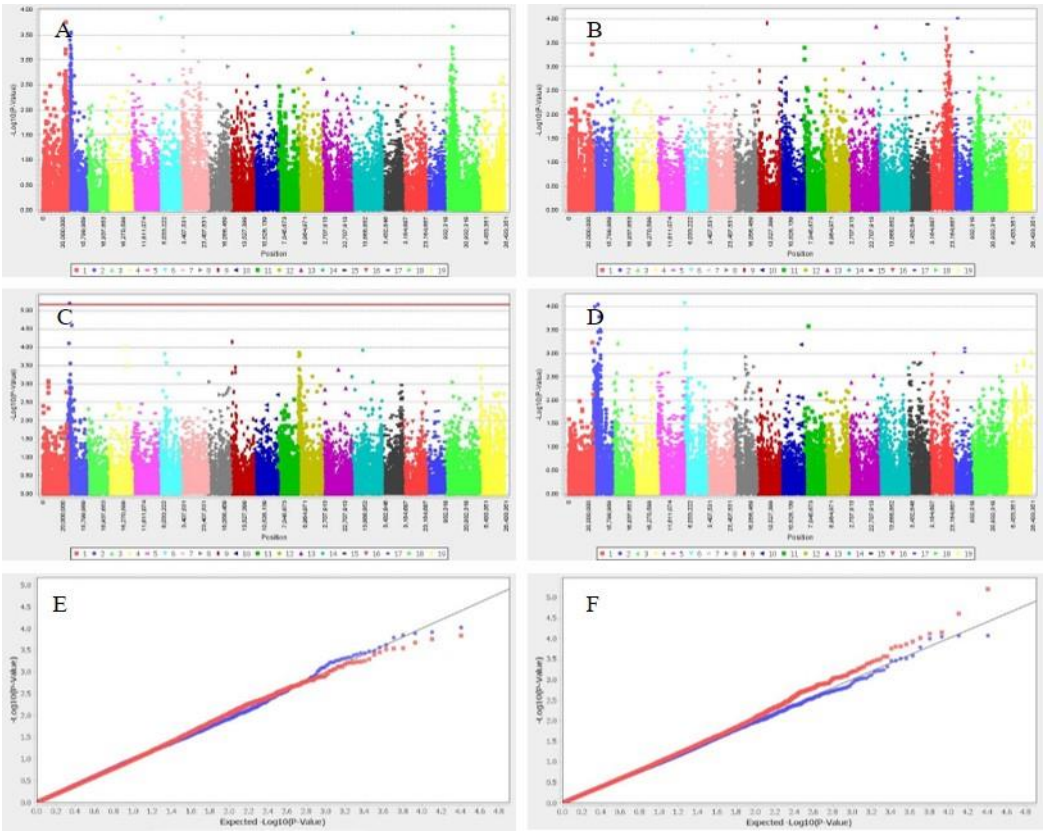

Fig. 7. Manhattan plots (A-D) and Q-Q plots (E-F) of SNP markers from mixed linear models for SSC (A, B, E) and TA (C, D, F) of table grapes. In Manhattan plots, $-\log_{10}(P)$ on y-axis, chromosomal position on x-axis. Red horizontal lines depict the significance threshold [$-\log_{10}(P)$ = 5.7]. In Q-Q plots, red dots represent the data of 2018 and blue dots represent the data of 2019. (A) Manhattan plot of SSC in 2018, (B) Manhattan plot of SSC in 2019, (C) Manhattan plot of TA in 2018, (D) Manhattan plot of TA in 2019, (E) Q-Q plots of SSC, and (F) Q-Q plots of TA.

related to SSC in LGs 2, 7, 16, and 18 (Fig. 8). Although no common QTLs were detected between the two years, there were the candidate genes related to SSC encoding sugar transporter near the markers chr02_3629174 and chr02_3629265, and sucrose synthase in the 200 kb region near the marker, chr07_3674058 (Table 7). According to the previous studies, the concentrations of glucose, fructose, and total soluble sugars were quantitatively inherited. Broad sense heritability varied between 0.6 and 0.7 depending on progeny and year (Liu et al., 2007; Wei et al., 2002). Many QTL analyses have been conducted to identify the genetic factor of SSC. QTLs related to sugars were located within ten LGs (1, 2, 3, 4, 7, 9, 11, 14, 17, and 18). Among them, QTLs in LG 14 were observed for fructose, glucose, and total sugar in common (Chen et al., 2015). In the hybrid mapping population ('87-1' × '9-22'), QTLs related to SSC were detected in LG 3, and the molecular marker was VVIH02 (Zhao et al., 2015). In the other case, the SSC measured by per cluster, QTL in LG 3 was obtained and the nearest marker was VMC1a5 in 5.95 Mbp on chr. 3 (Viana et al., 2013). Although a multitude of investigations, a common consensus has not been found. In the present study, the SNPs related to TA common in the two years were not detected (Table 7). TA of berry is known to be severely influenced by temperature during the growing season (Barnuud et al., 2014a, b). Only two SNPs were significantly related to TA with the data of 2018.

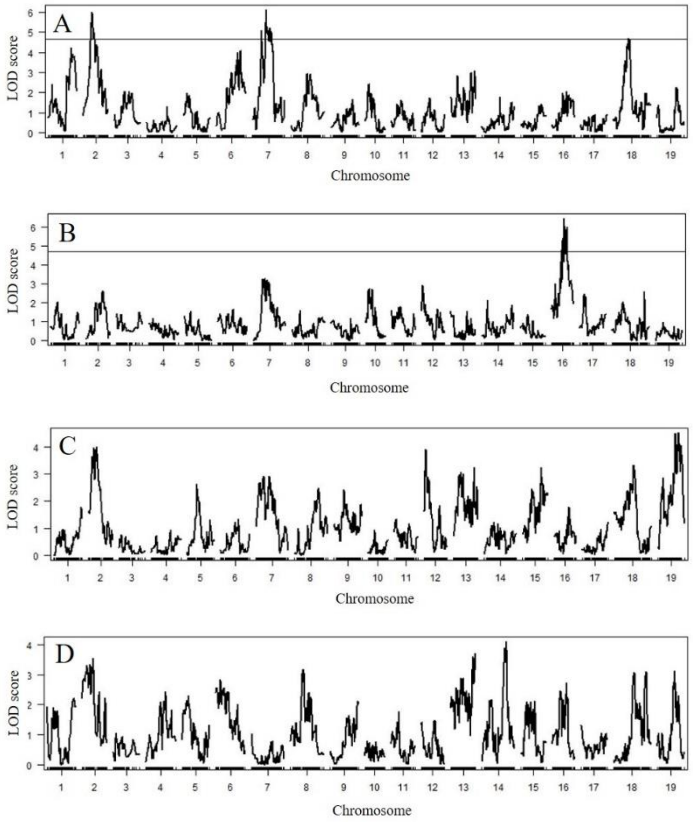

Fig. 8. LOD score plots of SNP markers from QTL analysis using linkage map for SSC and TA of table grapes. LOD on y-axis, chromosomal position on x-axis. (A) QTLs of SSC in 2018, (B) QTLs of SSC in 2019, (C) QTLs of TA in 2018, and (D) QTLs of TA in 2019.

Table 7. Significant QTLs for SSC and TA of table grapes.

Trait	Year	LOD threshold	Linkage group	No. of markers	No. of genes in QTL flanked region
SSC	2018	4.65	2	18	251
			7	63	263
			18	1	25
	2019	4.71	16	23	168
TA	2018	10.24	-	-	-
	2019	4.62	-	-	-

The region near 200 kb both sides from the site included 47 genes. The descriptions of the genes were amine oxidase, 3-hydroxy-3-methylglutaryl coenzyme A synthase, phospholipase D, expansin, and anthocyanidin synthase. In the previous QTL analysis for pH, QTLs were found across LGs 1, 6, 11, 13, and 16. The SSR marker VVMD21 in LG 6 accounted for 10.3% of total pH variation, and for the TA they found QTL across LGs 6, 13, and 19 (Viana et al., 2013). In the other study with complex parentage, SNPs related to acids within LGs 6, 13, and 18 were overlapped with the above results (Chen et al., 2015). QTLs related to wine acidity were detected on chr. 2, and the cofactor was vvib23 (chr. 2: 4.86 Mbp) (Bayo-Canha et al., 2019). In the present study, the results of GWAS showed two SNPs on chr. 2 related to the data of 2018 (Fig. 7C). One of them was about 600 bp departed from the marker vvib23, along with the result of the above research.

In the case of SSC and TA, precise phenotyping is particularly difficult because they are quantitative traits influenced by harvest time. The data of several years should be accumulated to reduce the error.

Genetic control of physical properties of berry

A large number of QTLs were found for fruit flesh firmness on LGs 1, 4, 5, 9, 10, 13, and 18 (Carreño et al., 2014). By other QTL analysis, the determinants for this trait were found to be distributed in LGs 8 and 18 (Correa et al., 2016). A stable

QTL for flesh firmness across seasons has been identified on LG 8. This QTL is mainly given by a male allelic and an additive effect. Together, these two QTLs explained ~27.6% of the phenotypic variance (Correa et al., 2016). In the present study, no significant SNPs above threshold ($-\log_{10}(P) = 5.7$) were detected from GWAS. In the QTL analysis from the linkage map, 76 SNPs above the threshold were detected in LGs 1, 10, 11, and 14 (Table 8). There were 443 genes in the region 200 kb up and downstream of the 76 SNPs, including the predicted genes encoding polygalacturonase precursor near the marker chr14_27565587, senescence-associated carboxylesterases near the marker chr14_28111743, and indole-3-acetic acid near the chr11_3696338.

Genetic control of berry astringency

Sensory astringency of berries was more highly correlated with PTC in berry skin than with monomeric and dimeric tannin contents (Ma et al., 2014; Peleg et al., 1999). In GWAS, eight SNPs in chr. 11 were significant for the two years (Fig. 9). The eight SNPs were located in 558-1,340 kbp in chr. 11. In the region, 111 genes were detected including genes encoding phenylalanine ammonia-lyase (PAL) and MybPA2. The position of *VvMybPA2* is chr. 11: 1,060-1,067 kbp. *VvMybPA2*, which promotes proanthocyanidin biosynthesis, was mainly expressed in the exocarp of young berries and in the leaves (Terrier et al., 2009). Although there are various genes involved in the tannin biosynthesis, research on the genes involved in the

Table 8. Significant QTLs for berry flesh firmness and PTC in berry skin of table grapes.

Trait	Year	LOD threshold	Linkage group	No. of markers	No. of genes in QTL flanked region
Berry flesh firmness	2018	4.94	-	-	-
	2019	4.84	1	5	24
			10	1	7
			11	57	80
			14	13	236
Polymeric tannin	2018	5.94	11	17	203
content	2019	5.65	11	32	54
			13	31	128

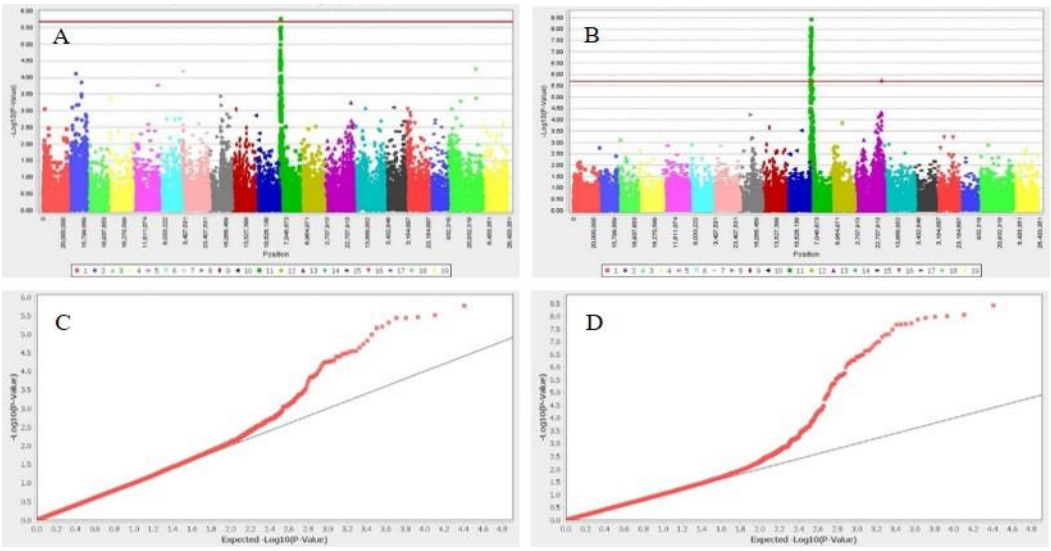

Fig. 9. Manhattan plots (A, B) and Q-Q plots (C, D) of SNP markers from mixed linear models for PTC in berry skin of table grapes. In Manhattan plots, $-\log_{10}(P)$ on y-axis, chromosomal position on x-axis. Red horizontal lines depict the significance threshold [$-\log_{10}(P) = 5.7$]. In Q-Q plots, red dots represent the data of 2018 and blue dots represent the data of 2019. (A) Manhattan plot of PTC in berry skin in 2018, (B) Manhattan plot of PTC in berry skin in 2019, (C) Q-Q plot of PTC in berry skin in 2018, and (D) Q-Q plot of PTC in berry skin in 2019.

condensation process of tannin is lacking. In the present study, by using the polymeric tannin contents as phenotype data, the candidate gene could be related to the condensation process of tannin. In QTL analysis from the linkage map, 63 SNPs related to PTC in berry skin were detected in LGs 11 and 13 (Fig. 10A, B, Table 8). The QTLs were located in 5.7-18.4 cM in LG 11, and 60.3-80.4 cM in LG 13. In the region 200 kb up and downstream of the 63 SNPs, there were the genes encoding sugar transport protein, and disease resistance protein including *VvMybPA2* and *PAL*. To identify genetic factors related to quantitative traits, both GWAS and QTL analysis using a linkage map should be applied. Further investigations are needed to better characterize and screen all the proposed candidate genes.

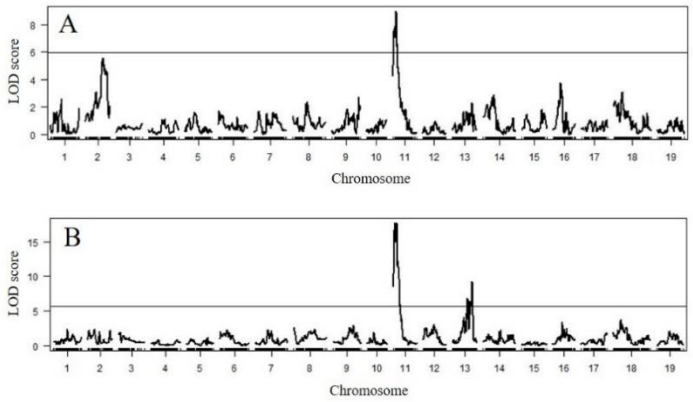

Fig. 10. LOD score plots of SNP markers from QTL analysis using linkage map for PTC in berry skin of table grapes. LOD on y-axis, chromosomal position on x-axis. (A) QTLs of PTC in berry skin in 2018, and (B) QTLs of PTC in berry skin in 2019.

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ABSTRACT IN KOREAN

과수 육종의 주된 목표는 고품질의 과실을 생산하는 품종을 선발하는 것이다. 과실의 품질을 결정하는 대부분의 형질들은 양적 형질이다. 이러한 형질들에 관여하는 다수의 유전자를 탐색하고 그 역할을 밝히는 것은 육종을 효율적으로 수행하기 위해 필요한 사항이다. 본 연구에서는 육종의 목표에 해당하는 7가지 형질에 관여하는 유전자를 탐색하기 위하여 유전체 전장 연관 분석을 수행하였다. '타노레드'와 '루비씨들리스' 그리고 그 둘을 교배하여 만든 269개체의 자손을 이용하여 실험을 진행하였다. 실험에 이용한 형질은 총 7개로 과립 무게, 종자 무게, 인장 강도, 당도, 산 함량, 과육 경도, 과피 내 고분자 타닌 함량이다. 표현형 조사는 2018년과 2019년 두 해 동안 실시하였는데 성숙기에 해당하는 8월부터 10월에 과실 시료를 무작위로 채취하여 위의 형질들을 조사하였다. 두 해 동안 조사한 표현형 데이터의 상관 계수는 0.34-0.81에 해당하여 넓게 분포하였으나, *P*-값은 1.126e-07부터 2.2e-16에 해당하여 각각의 형질은 연차 간 상관 관계가 있는 것으로 나타났다. Genotyping-by-sequencing 방법을 통해 유전체 DNA의 염기 서열 분석을 수행하였다. 총 271개체로부터 얻은 148.69Gb의 유전 정보를 포도 표준 유전체인 PN40024 12X.v2에 정렬하였다. 총 400,648개의 단일 염기 다형성을 얻었으며 이후 조건에 맞춰 25,421개의 단일 염기 다형성을 선발하여 유전체 전장 연관 분석에 이용하였다. 또한 그 중 2,243개의 단일 염기 다형성을 이용하여 연관 지도를 작성하고 양적 형질 유전자좌를 탐색하여 본 연구의 결과를 검토하였다. 과립 무게, 종자 무게, 인장 강도와 관련된

유의한 단일 염기 다형성은 18번 염색체 상에 있는 무핵 연관 주동 유전자로 알려진 *VvAGL11*의 주변에 위치하였으나 당도, 산도, 과육 경도의 경우에는 두 해 모두 공통되는 유의한 결과를 찾을 수 없었다. 포도 과피의 떫은맛을 결정하는 과피 내 고분자 타닌 함량의 경우 연관된 단일 염기 다형성이 11번째 염색체에서 발견되었으며 그 주변에서 발견된 *VvMybPA2*이 후보 유전자로 선발되었다. 따라서 본 연구 결과, 유전체 전장 연관 분석을 통해 생식용 포도 과실의 품질을 결정하는 형질과 관련된 후보 유전자들을 선발할 수 있었으며 해당 분석 방법의 적용 가능성을 확인할 수 있었다.