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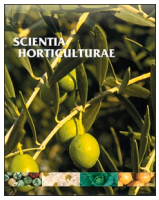

Research Paper

Development of an efficient molecular-marker assisted selection strategy for berry color in grapevine

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ABSTRACT

Marker-assisted selection (MAS) represents one of the most effective strategies to reduce costs and time in plant breeding programs. MAS benefits from the identification of markers in strong linkage disequilibrium (LD) with causative genes and from the development of fast, scalable, and reproducible screening techniques. In grape, berry color is a valuable trait, highly related to the sensory characteristics of fresh grape and wine quality.

The present work aims to develop a MAS strategy to select skin color by High-Resolution Melting (HRM), a 'close-tube' method capable of detecting polymorphisms within PCR-amplified DNA fragments. To identify molecular markers associated with the berry skin color phenotypic trait, the total genome of 3 white-berry and 3 red-berry accessions were sequenced using Illumina technology and aligned to the reference genome PN40024 v4. The sequencing results revealed the presence of three highly polymorphic regions on chromosome 2 between 15 Kbp and 17 Kbp for red berry genotypes. HRM analyses were carried out on a total of 95 genotypes, 70 of which comprised a berry color segregating population: ['Italia' (white berry) x cv 'Crimson Seedless' (red berry)] and 25 genotypes belonging to a commercial grapevine germplasm collection consisting of 11 red and 14 white berry accessions. The HRM profiles of the three regions tested strongly correlated with the phenotypic trait of color.

The results of this work represent an effective, fast, and reliable genotyping strategy that enables the discrimination of genotypes based on the presence or absence of berry pigmentation.

1. Introduction

The development of molecular markers associated with loci controlling important phenotypic traits is crucial to improve the efficiency and cost-effectiveness of breeding programs (Moose and Mumm, 2008). The detection of molecular marker(s) that are predictive for a phenotype of agronomical interest (e.g. fruit quality or resistance to biotic or abiotic stress), enables an early screening and selection of the accessions characterized by a favourable/unfavorable genetic configuration. Compared to the traditional breeding method, marker-assisted selection (MAS) permits a faster selection of the accessions that will be phenotypically assessed and, in parallel, guarantees a reduction in the costs and space needed for the maintenance of the seedlings. MAS is particularly effective for tree crops (characterized by a long juvenility phase) and for fruit quality traits regulated by few and already depicted loci, especially in hybridization programs for species characterized by high heterozygosity, such as grape (Vargas et al., 2023). Berry color is a pivotal part of both table grapes and wine production since it is an essential driver of consumers' appreciation and wine quality (Poni et al., 2018). The genetic control of the berry color (and therefore the anthocyanin content in the skin of the berries) has been attributed to two transcriptional factors (TF): *VvMybA1* and *VvMybA2* (Kobayashi et al., 2004; Walker et al., 2007). These TFs are directly involved in controlling the expression of UDP-glucose: flavonoid 3-O-glucosyltransferase, a key gene in the anthocyanin biosynthesis pathway (UFGT) in grape (Boss et al., 1996; Kobayashi,et al., 2002) as well as in other crops (Zhao et al., 2012). Genetic analysis has revealed that mutations, insertions, and deletions in the *Myb* gene can lead to the gene being inactivated,

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resulting in white berry phenotypes. For instance, the insertion of the retrotransposon *Gret1* on the *VvMybA1* gene has been linked to the loss of berry skin pigmentation in various grape genotypes. (Azuma et al., 2008; Kobayashi et al., 2004; Walker et al., 2007). Furthermore, a non-synonymous single-nucleotide polymorphism (SNP) and a frame-shift mutation in the *VvMybA2* gene (locus *K980*) were correlated with the red berry color (Kerekes et al., 2015). Fouriner-Level et al. (2009) identified in a full-sib grape population a single Quantitative Trait Locus (QTL) on chromosome 2 harbouring a cluster of four *Myb*--type genes (*VvMybA1, VvMybA2, VvMybA3*, and *VvMybA4*) accounting for the 62 % of the overall variation in anthocyanin fruit content. Nevertheless, the screening techniques proposed to date, as well as the molecular markers identified in these genetic regions, are not always effective and applicable for large-scale MAS plans and/or on different genetic backgrounds (Carrasco et al., 2015; Fournier-Level et al., 2009).

Recently, genotype-phenotype association studies have identified three distinct genetic regions located on chromosome 2 in a genetic window spanning from 15 Kbp to 17 Kbp (Guo et al., 2019; Sun et al., 2020). In light of this, several SSRs (Simple Sequence Repeats) mapped in chromosome 2 and showing high linkage-disequilibrium with the skin color were proposed, namely: VMC5g7, VMC8c2, VMC6b11, VMC7 (Doligez et al., 2006; Riaz et al., 2004). Even though these markers exhibited a strong correlation with the berry color, they have not been validated on genetic backgrounds different from those in which they were initially identified. Furthermore, SSR screening and interpretation is often laborious and difficult to scale up for large selection programs. In this context, identifying molecular markers showing robust performances on different genetic backgrounds would represent a significant improvement for the effectiveness of MAS approach.

In parallel, the availability of a fast, reliable and (relatively) unexpansive screening method is another prerequisite for an economically sustainable application of MAS, especially for large-scale breeding programs.

In this context, the high-resolution melting (HRM) approach can represent a valid solution thanks to its high scalability. HRM is a "closed-tube" method that enables the detection of genetic polymorphisms and mutations within PCR (Polymerase Chain Reaction) amplicons through the analysis of fluorescence levels during the thermal denaturation of the DNA (Distefano et al., 2012; Reed and Wittwer, 2004).

In order to set up a marker assisted selection strategy for berry color in grape, the genome of 3 white and 3 red accessions was sequenced enabling a precise characterization of the polymorphisms (SNPs and INDELs) occurring in genetic regions within the three loci already described as involved in this trait.

Finally, a set of markers was developed to confirm the linkage between the phenotype and genotype throughout HRM analyses both on a full-sib population, segregating for berry color, and a heterogeneous vine germplasm collection.

2. Material and methods

2.1. Plant material

A total of 95 genotypes encompassing 70 individuals of a segregating population (ItaxCri) of table grapes (constituted in 2018) and 25 vine varieties of a germplasm collection were employed for this study. The ItaxCri population originated from the cross of a white-berry cultivar, ‘Italia’ (as female parent) and a red-berry cultivar, ‘Crimson Seedless’ (as pollen donor) (Puglisi et al., 2022). The seedlings, after molecular validation, were grafted on 140 Ru rootstocks and grown under standard conditions. As for the germplasm collection, this includes 12 autochthonous Sicilian varieties and 13 international varieties chosen for their economic importance and diffusion (Table S1).

2.2. Phenotyping of berry color trait in the segregating population

The grapes were sampled at commercial maturity during the harvesting season of 2022. The color was determined *a priori* employing a descriptive, binary, assessment (Red/White). Subsequently, 15 berries per accession of ItaxCri were randomly selected and analyzed using a Minolta CR410 colorimeter (Minolta Camera Co., Osaka, Japan). The color coordinates adopted were L* (black to white), a* (green to red) and b* (blue to yellow) according to the CIELab color space coordinates (Rolle et al., 2011).

As for the germplasm collection, berry color was annotated as "Red" for pigmented genotypes and “White” for those non pigmented.

2.3. DNA extraction

The DNA of each accession was extracted from 100 mg of young leaves using the DNeasy Plant mini-Kit (Qiagen, Hilden, Germany), following the instructions provided by the manufacturer. The quantity and quality of the DNA were analyzed using a spectrophotometer (Nanodrop 2000, ThermoScientific, Massachusetts, United States). The DNA samples were diluted in 40µl of DNase/RNase-free water (Sigma-Aldrich, St. Louis, Missouri, United States) to a final concentration of 30 ng/µL.

2.4. DNA sequencing and polymorphism detection

The Illumina MiniSeq 150 pair-ends technology was employed to sequence the DNA of three white-berry grape cultivars (‘Italia’, ‘Grillo’, and ‘Carricante’) and three red-berry grape cultivars (‘Crimson Seedless’, ’Nerello Mascalese’ and ’Nero d’ Avola’). The average read depth was 40X (~13.5 Gb). Library preparation followed standard Illumina protocols. The sequencing analyses were submitted to Novogene company (Beijing, China). The short reads were aligned to the PN40024 v4 reference genome (Velt et al., 2023) using the Burrows-Wheeler aligner (Li and Durbin, 2009). Once the aligned genomes were obtained, the selected genomic regions were investigated using Integrative Genomics Viewer software v 2.16.0 (Robinson et al., 2011).

2.5. High-resolution melting assay design for berry color markers validation

2.5.1. HRM primer design

Primers were designed in a genomic window of 400 bp retrieved from the reference genome of *Vitis vinifera* spp *vinifera* PN40024 v4 (Velt et al., 2023) based on three polymorphic region positions described by Guo et al. and Sun et al. (Guo et al., 2019; Sun et al., 2020). The selection of the three regions investigated in the present work built on the results of the works mentioned before, both suggesting the presence of a strong linkage between a genomic region located on chromosome two and spanning between 15 Kbp and 17 Kbp and berry color. In particular, Guo et al. identified a SNP marker within the *vVMybA*3 transcription factor at position 15,641,010 bp, corresponding to the first selected and investigated region (SEQ 1). The same researchers also reported an associated marker at position 15,983,296 bp corresponding to the second selected region (SEQ 2) investigated in this study. Both of these regions are also part of one of the QTLs identified by Sun et al. on a genomic window spanning from 13,368,581 and 16,199,608 bp. The third region (SEQ 3) was selected based on a SNP identified by Guo et al. at position 17,439, 426 bp.

For each genomic region, the primers were designed using Primer3 software version 4.1.0 (https://primer3.ut.ee) and Multiple Primer Analyzer (ThermoScientific, Waltham, Massachusetts, U.S.). Optimal annealing temperatures and master mix were assessed using a gradient PCR with temperatures ranging from 54 to 60 °C based on the primers analysis outputs mentioned above (Mastercycler nexus, Eppendorf, Hamburg, Germany). Amplicon positions and the primer sequences with

the respective optimal annealing temperatures of the analyzed markers are shown in Table 1.

2.5.2. PCR amplification and HRM analyses

PCRs were performed on a 72-rotor using a Rotor-Gene Q 2plex HRM Real-time PCR cycler (Qiagen) in a 20 µl/tube total volume. The total volume of the final reaction mix contains: 60 ng of genomic DNA, 0.25 µM forward and reverse primer, 0.2 mM dNTPs, 2 µl of 10xNH_4 Reaction Buffer, 2 mM $MgCl_2$ solution, 1 U BIOTAQ DNA Polymerase (Meridian Bioscience) and 1.5 µM of SYTO9 Green fluorescent nucleic acid stain (Invitrogen). The PCR amplification and subsequent HRM rounds were performed according to the following steps: (i) initial denaturation at 95 °C for 5 min; (ii) 40 amplification cycles, 95 °C for 15 s, 55–56–57 °C for 30 s (according to the annealing temperature of the primers) and 72 °C for 25 s; (iii) final extension at 72 °C for 2 min; (iv) 95 °C for 1 min; (v) 40 °C for 1 min; (vi) the final HRM step ramping from 70 °C to 88 °C rising by 0.25 °C each step. During the incremental melting step, fluorescence data were continuously acquired. The data were analyzed using Rotor-Gene Q 2.1.0.9 software (Qiagen). The different melting curve plots were generated after normalization and the temperature shift determination.

2.6. Statistical analyses

One-Way analysis of variance (ANOVA) of the segregating population's averaged and normalized colorimetric data was performed. Chi-square independence tests ($\chi 2$) was instead employied to evaluate the association between phenotype and molecular data at $\alpha = 0.05$ significance level for each marker. All the statistical analyses were conducted with the R software (v 4.2.3, R Core Team, 2021) employing the 'stat' package.

3. Results

3.1. Berry color phenotyping

An ANOVA was conducted on the three parameters of berry color in the ItaxCri population. The results revealed a significant phenotypic difference (p-value < 0.01) for the three color parameters between the individuals showing red and white berry (Fig. 1). For L* (relative luminance) values, the red berry and white berry accessions were characerized by an average value of 27 and 41.7 respectively (Fig. 1A). The values of a* (which negative values indicate green and positive values indicate magenta) showed that the red berry accessions had an average of 6.7 and the white berry accessions had an average of −5 (Fig. 1B). Finally, the colorimetric values of b* (which negative values indicate blue and positive values indicate yellow) showed that the red berry accessions had an average of 3, while the white berry accessions had an average of 12.5 (Fig. 1C).

3.3. DNA sequencing

To better understand the genetic diversity within the loci proposed to be associated with the skin color, three cultivars with red berries ('Crimson Seedless', 'Nerello Mascalese' and 'Nero d'Avola') and three with white berries ('Italia', 'Grillo' and 'Carricante') were resequenced.

Based on the genotype-phenotype association studies conducted by Guo et al. (2019) and Sun et al. (2020), three different genetic regions were selected and investigated. In their work, Guo et al. identified five loci associated with skin color, while Sun et al. discovered ten QTLs associated with berry skin anthocyanin content and composition.

The sequencing data of the three regions revealed no difference between the white berry genotypes and the reference genome. For the first sequence (SEQ 1) (Fig. 2A), the three red berry accessions sequenced showed a SNP at position chr2:15,641,000 bp in heterozygous condition (C/T). Additional SNPs were also detected at position chr2:15,641,089 for 'Crimson Seedless', chr2:15,641,074 bp for 'Nerello Mascalese' and ch2:15,641,018 bp for 'Nero d'Avola' all in heterozygous condition (C/T) compared to the reference genome.

As for the second sequenced region investigated (SEQ 2, Fig. 2B), 'Crimson Seedless' and 'Nerello Mascalese' showed a deletion of 25 bp at chr2:15,983,169 bp and two SNP in heterozygous condition (chr2:15,983,276:T/C and chr2:15,983,293:C/T). 'Crimson Seedless' showed four more SNPs (Fig. 2B). Furthermore, 'Nero d'Avola' showed a 2 bp deletion at position chr2:15,983,176 bp.

Finally, for the third region sequenced (SEQ 3, Fig. 2C), a 12 bp insertion in heterozygous condition was detected at position chr2:17,439,371 bp with an identical motif (TTATATTTTAAA) for both 'Nerello Mascalese' and 'Nero d'Avola'. In addition, three SNPs in heterozygous conditions were detected (Fig. 2C). For this region 'Crimson Seedless' sequencing data displayed a low reads coverage and was not displayed.

3.4. High-resolution melting analysis for marker validation

The analysis of the three genomic regions linked with skin color enabled the design of three primer pairs that were subsequently employed to screen both ItaxCri and the collection of vines. The 95 genotypes were analysed employing an HRM approach, and clear distinctive curves were determined and associated to the skin color (though with different efficiency) for all three amplified regions. For each genomic region deciphered, an HRM marker was implemented, namely: (i) CB_1 for SEQ 1, (ii) CB_2 for SEQ 2 and (iii) CB_3 for SEQ 3. For the CB_1 marker (Fig. 3A and B), the white berry genotypes showed a homozygous peak at 80.8 ± 0.5 °C, while the red berry genotypes showed a melting curve highlighting a heterozygous genotype with two inflexion points at 78 °C and 81 °C (variance of ± 0.5 °C).

As for the CB_2 marker (Fig. 3C and D), the white berry genotypes were characterized again by a homozygous peak at 72 ± 0.5 °C, while the red berry genotypes showed a homozygous peak at 73.5 ± 0.5 °C (Fig. 3D), revealing two clear and distinct normalized curves (Fig. 3C).

The analysis of CB_3 showed a homozygous peak at 79 ± 0.5 °C for both white and red berry genotypes, even though the red berry genotypes presented an additional peak at 75.5 ± 0.5 °C, with a lower fluorescence/temperature ratio (1.5 dF/dT).

In terms of discriminatory efficiency, all three markers proved to be strongly associated with the color phenotype with a *p-value* of the Chi-squared Independence test < 0.05 (Table 2). The CB_1 marker proved to be the most effective for what concerned the analysis of ItaxCri with a discriminatory efficiency of 100 % while, among the germplasm collection, the 'Syrah' cultivar was not efficiently discriminated (Table 3, Fig. S2). The marker CB_2 was characterized by a

Table 1
Molecular markers used for high-resolution melting HRM For each marker, the genetic coordinates (chromosome and physical position), the primer sequences and the temperature of annealing (Ta) were reported.

Marker Name	Position (bp)	Primer Forward	Primer Reverse	Ta (°C)
CB_1	chr2:15,640,923–15,641,124	CAAAGCCCCATCCATGCG	TCATCTTTCCCTATTCCCGTG	57
CB_2	chr2:15,983,160–15,983,392	TCCCTCACCTTTTTACACAACT	ACCCTTCATCCTTTTTTTAGCCT	56
CB_3	chr2:17,439,364–17,439,436	CGTAATCATACTGTTGTGAGCCA	TTGACCCATTTTTTAAAGGCACA	55

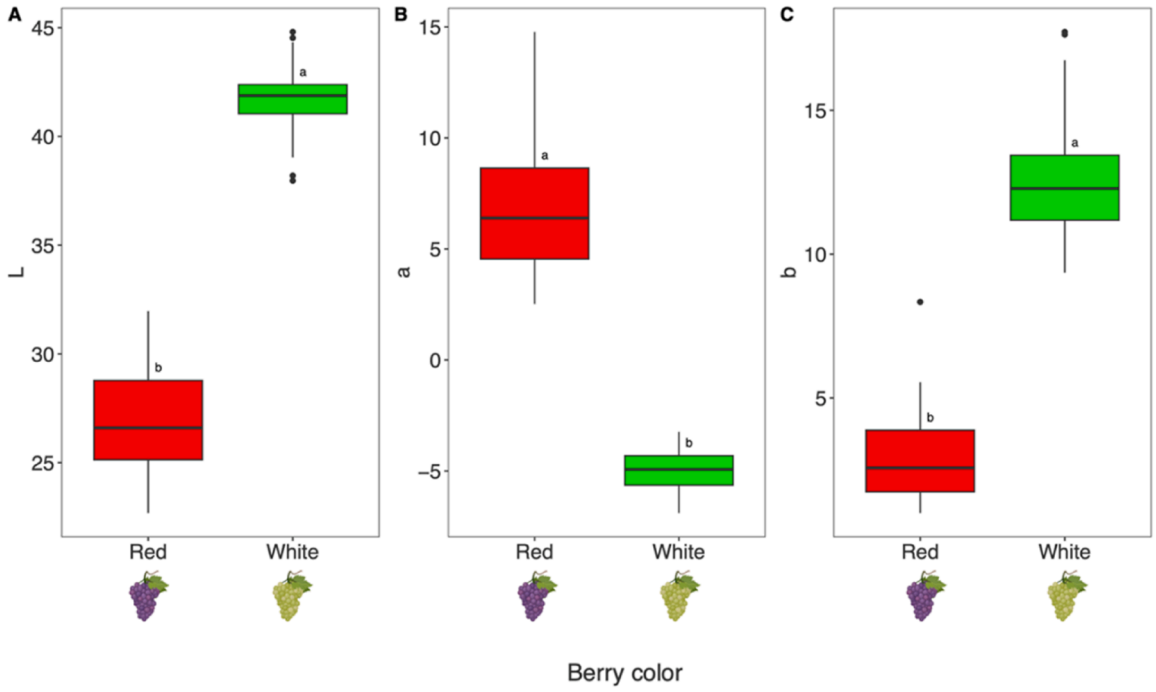

Fig. 1. Phenotypic distribution of the colorimetric data measured on the segregating population Italia x Crimson. In red are represented the red-berry grape accessions, and in green, the white-berry grape accessions. el A showed the boxplot of the L* value,; while B and C referred to the a* and b* parameters respectively. An ANOVA-One Way analysis was carried out combined with a Tukey's honest significant difference (HSD) test to generate compact letters (p-value $\leq$ 0.01, for each colorimetric parameter).

discriminatory efficiency of 98.6 % and 80 % in ItaxCri and the germplasm collection, respectively. CB_2 whosed inconsistent results for 'Pink Muscat', the 3 clones of 'Nero d'Avola', and 'Grenache' (Table 3, Fig. S2). The marker CB_3 resulted in a discriminatory efficiency of 95.8 % for ItaxCri; even though the discriminatory efficiency was the lowest among the markers tested in the segregating population, CB_3 was the only marker characterized by a totally efficient discrimination of the 25 samples composing the germplasm collection (Table 3).

4. Discussion

During the domestication and selection process, grapes underwent profound transformations, leading to the wide phenotypic diversity characterizing the actual varietal panorama. Such variability was particularly noticeable for what concerns the berry color, a trait highly responsive to selective pressures (Fournier-Level et al., 2010).

Extensive research uncovered several polymorphic variants of *Myb* genes affecting the skin berry color trait (Ferreira et al., 2016, 2018; Giannetto et al., 2008; Migliaro et al., 2014). In particular, genetic studies highlighted the occurrence of three mechanisms affecting the regular expression of *VvMybA1* and/or *VvMybA2* genes: (i) the insertion of the *Gret1* retrotransposon in the promoter or 5′ untranslated region (5′-UTR) of *VvMybA1*, resulting in its inactivation (Kobayashi et al., 2004, 2005); (ii) the occurrence of frame-shift mutations in the coding region of *VvMybA2* (Walker et al., 2007); (iii) the deletion of the genomic region harbouring both genes (Walker et al., 2006; Yakushiji et al., 2006). Moreover, different haplotypes have been identified based on the genetic background of the studied grapevine populations. The configuration of *Myb* haplotypes at the berry color locus stands as a pivotal genetic factor influencing anthocyanin composition and their accumulation in berries (Azuma, 2018).

In this scenario, testing the performance of the available genetic markers in different genetic backgrounds is a fundamental prerequisite for an efficient application of MAS (Carrasco et al., 2015; Fournier-Level et al., 2010; Kerekes et al., 2015; Pelsy et al., 2015; Péros et al., 2015). Several studies have shown that the efficiency of molecular markers in grapevine is influenced by the genetic background on which they were identified. Such findings highlight the importance of taking into consideration the genetic background when selecting molecular markers for research or breeding purposes and the importance of validating them in different genotypes (Bennici et al., 2019).

Another key feature of MAS employment is the availability of a cost-effective and easy-to-interpret assay for genotyping. In the present work, three loci linked to berry color were validated on a wide germplasm encompassing both a segregating population and a germplasm collection (Guo et al., 2019; Sun et al., 2020). These regions were deep analyzed by the whole-genome sequencing of six cultivars (three red berries and three white berries); as expected, no differences were detected among the three white varieties and the PN40024 reference genome (also characterized by white berry) (Velt et al., 2023). In the SEQ 1 region, two heterozygous SNPs were identified for red berry genotypes that were causing the characteristic melting curve in the HRM assay (Fig. 3A and B). This agreed with what was reported by Guo et al. (2019), reporting the presence of a SNP located at position chr2:15,641,010 bp correlated with berry color. This SNP is likely found at position chr2:15,641,000 in this study. In this region, the sequencing data of the three red-berry genotypes analyzed revealed the occurrence of additional SNP (s) that played a role in accentuating the differences between the red-berry and the white-berry melting curves.

As for the SEQ 2 sequence, there is also agreement between the HRM curve profiles and the sequencing data. Indeed, the HRM curves of the red-berry genotypes showed a lower dF/dT ratio, probably due to the presence of a 25 bp deletion as reviewed by Reed et al. (2007). The SNPs detected at position chr2:15,983,293 and at position chr2:15,983,276 were probably also related to that identified by Guo et al. at position chr2:15,983,296 bp. Our results highlighted the occurrence of four additional SNPs in 'Crimson Seedless', indicating a significant degree of polymorphism in this region. For this marker, the cultivar 'Nero d'Avola' did not show a HRM melting curve congruent with the phenotype (red berry vine). This is probably due to the lack of SNPs in

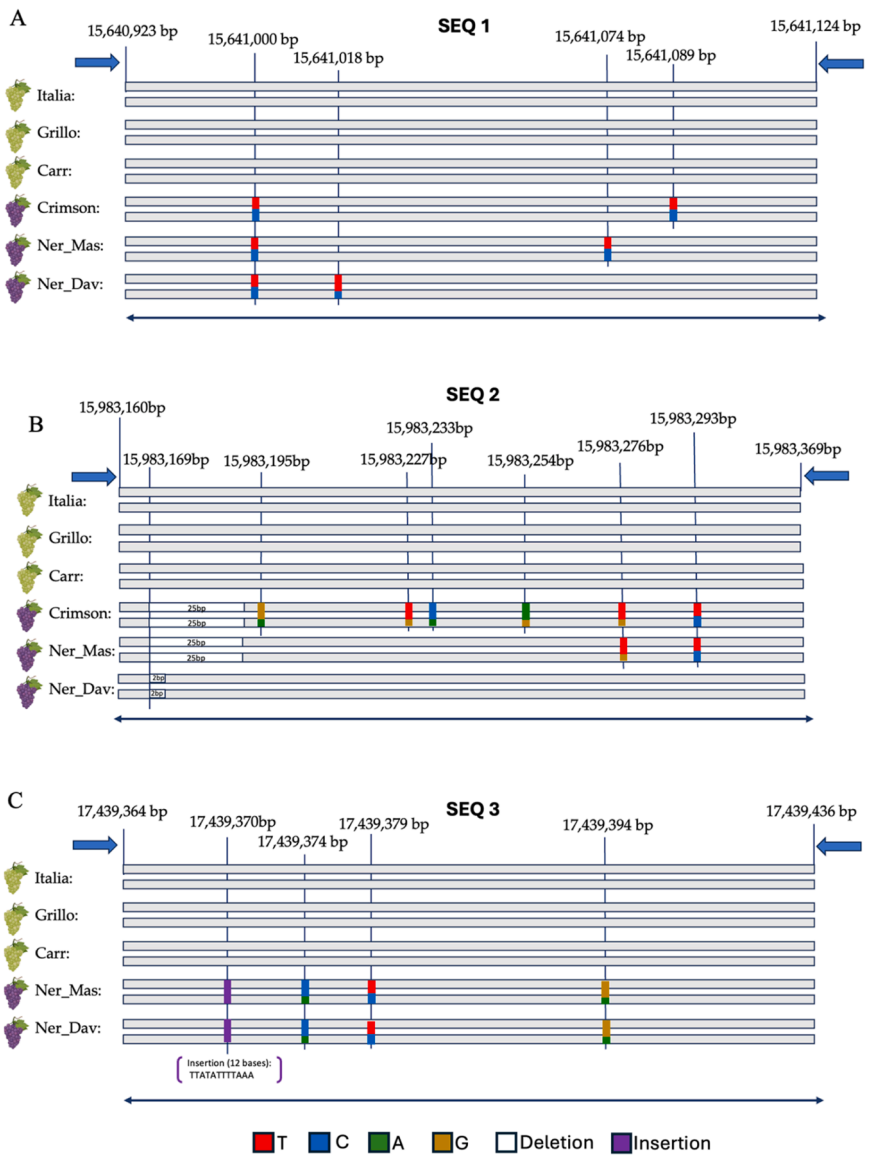

Fig. 2. Sequencing results of chromosome 2 regions associated to berry color trait for SEQ 1 (A), SEQ 2 (B) and SEQ 3 (C). The blue arrows represent the anchor regions of the primers designed for the HRM assay. For each polymorphism and for each region the position in bp is reported..

the amplified region and the presence of a small 2 bp deletion not detected by the HRM analysis. Furthermore, the amplified regions of the CB_1 and CB_2 markers were located within the two QTLs intervals detected by Sun et al. (2020) in the region comprised within chr2:13, 368,581 and chr2:16,199,608 bp and related to the anthocyanin content in berries skin.

As for the CB_3 marker, although Guo et al. (2019) identified a SNP linked to skin color at position chr2:17,439,426 bp, the HRM results showed a heterozygous profile of the two samples with red berry, likely due to the occurrence of a 12 bp insertion, and/or the presence of three SNPs with a heterozygous genetic configuration (Fig. 3E and F). Summing up, all markers tested were predictive for skin color (Chi-squared Independence test < 0.05); nevertheless, inconsistencies were detected in all three cases, highlighting the need for a preliminary evaluation of the genetic background under evaluation when MAS protocols are applied. In this context, further studies are necessary to investigate the genetic determinants and develop additional markers showing a full predictivity for skin color and also able to capture the different color nuances characterizing grape panorama.

5. Conclusion

In light of the crucial role that berry color plays in determining grape and wine quality, MAS techniques, especially when complemented with fast and unexpansive screening methods such as HRM assay, represent a noteworthy advancement in grapevine breeding. The following work proposes an effective, fast and reliable genotyping strategy that easily allows the discrimination of genotypes based on the presence/absence of berry pigmentation, highlighting the potentiality and limitations of the available molecular markers for berry color, especially when different genetic backgrounds are analysed. These findings can serve as a valuable resource for grapevine breeders in their quest to develop superior grape cultivars with desirable traits.

Author contributions

All authors have read and agreed to the published version of the manuscript.

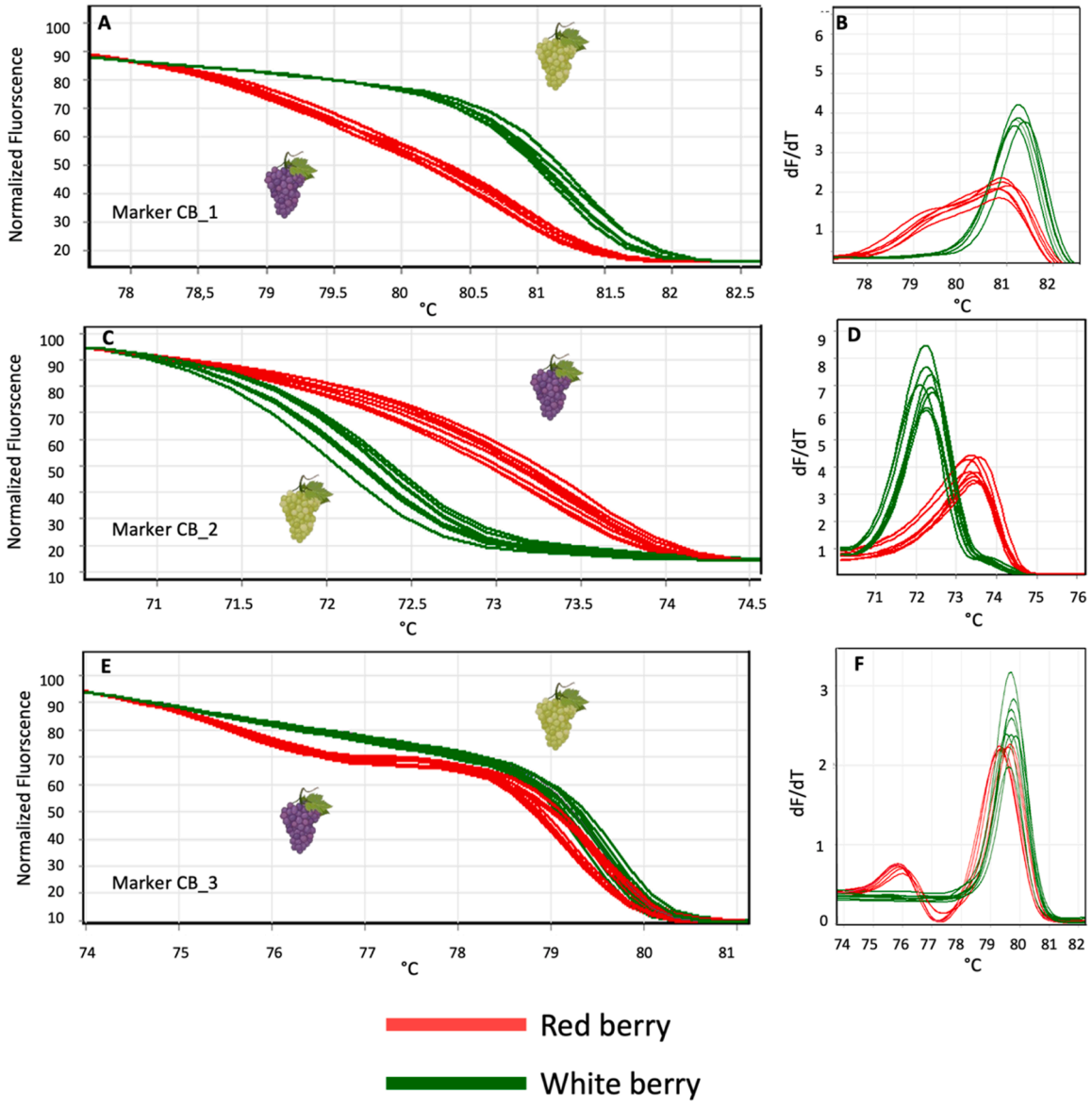

Fig. 3. HRM analysis plots of red (red lines) and white berry (green lines) genotypes analyzed with CB_1 (A, B), CB_2 (C, D), CB_3 (E, F) markers. Left-hand panels represent the normalized graphs; right-hand panels represent the melting curves.

Table 2
Discriminatory efficiency, $\chi 2$ and *p-values* of the color trait predictability for the three markers analyzed in the genotypes of both the segregating table grape population ($n = 70$) and of the germplasm collection ($n = 25$).

Marker name	[Ita x Cri] segregating population			Germplasm Collection		
	Discriminatory efficiency	χ^2	*p-value*	Discriminatory efficiency	χ^2	*p-value*
CB_1	100 %	66	4.4e-16	96 %	17.8	2e-5
CB_2	98.6 %	58.7	1.8e-14	80 %	7.2	0.007
CB_3	95.8 %	45.3	1.6e-11	100 %	21.15	4.3e-06

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CRediT authorship contribution statement

Leonardo Paul Luca: Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Mario Di Guardo:** Writing – review & editing, Validation, Data curation. **Stefania Bennici:** Investigation. **Filippo Ferlito:** Investigation. **Elisabetta Nicolosi:** Writing – review & editing, Funding acquisition. **Stefano La Malfa:** Writing – review & editing, Funding acquisition. **Alessandra Gentile:** Writing – review & editing, Supervision, Funding acquisition. **Gaetano Distefano:** Writing – review & editing, Methodology, Conceptualization.

Table 3
HRM analysis results of the three regions associated with berry color trait on the cultivars belonging to the germplasm collection. The genotypes are classified as D (Discriminated) when the molecular analysis and the observed phenotype match and N.D. (Not Discriminated) when the molecular marker analysis and the observed phenotype don't match.

Grape cultivar	Berry color	CB_1	CB_2	CB_3
Crimson Seedless	Red	D.	D.	D.
Grenache	Red	D.	N.D.	D.
Syrah	Red	N.D.	D.	D.
Cabernet Sauvignon	Red	D.	D.	D.
Petit Verdot	Red	D.	D.	D.
Pink Muscat	Red	D.	N.D.	D.
Nerello Mascalese	Red	D.	D.	D.
Nero d'Avola clone 1	Red	D.	N.D.	D.
Nero d'Avola clone 2	Red	D.	N.D.	D.
Nero d'Avola clone 3	Red	D.	N.D.	D.
Frappato	Red	D.	D.	D.
Italia	White	D.	D.	D.
White Muscat	White	D.	D.	D.
Chardonnay clone 1	White	D.	D.	D.
Chardonnay clone 2	White	D.	D.	D.
Petit Manseng	White	D.	D.	D.
Gewurztraminer	White	D.	D.	D.
Catarratto clone 1	White	D.	D.	D.
Catarratto clone 2	White	D.	D.	D.
Grillo	White	D.	D.	D.
Carricante clone 1	White	D.	D.	D.
Carricante clone 2	White	D.	D.	D.
Carricante clone 3	White	D.	D.	D.
Luisa	White	D.	D.	D.
Inzolia	White	D.	D.	D.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113522.

References

Azuma, A., 2018. Genetic and environmental impacts on the biosynthesis of anthocyanins in grapes. Hort. J. 87 (1), 1–17. https://doi.org/10.2503/hortj.OKD-IR02.

Azuma, A., Kobayashi, S., Mitani, N., Shiraishi, M., Yamada, M., Ueno, T., Kono, A., Yakushiji, H., Koshita, Y., 2008. Genomic and genetic analysis of Myb-related genes that regulate anthocyanin biosynthesis in grape berry skin. Theor. Appl. Genet. 117 (6), 1009–1019. https://doi.org/10.1007/s00122-008-0840-1.

Bennici, S., Di Guardo, M., Distefano, G., La Malfa, S., Puglisi, D., Arcidiacono, F., Ferlito, F., Deng, Z., Gentile, A., Nicolosi, E., 2019. Influence of the genetic background on the performance of molecular markers linked to seedlessness in table grapes. Sci. Hortic. 252, 316–323. https://doi.org/10.1016/j.scienta.2019.03.060.

Boss, P.K., Davies, C., Robinson, S.P., 1996. Analysis of the expression of anthocyanin pathway genes in developing vitis vinifera L. cv shiraz grape berries and the implications for pathway regulation. Plant Physiol. 111 (4), 1059–1066. https://doi.org/10.1104/pp.111.4.1059.

Carrasco, D., De Lorenzis, G., Maghradze, D., Revilla, E., Bellido, A., Failla, O., Arroyo-García, R., 2015. Allelic variation in the VvMYBA1 and VvMYBA2 domestication genes in natural grapevine populations (Vitis vinifera subsp. sylvestris). Plant Systemat. Evolut. 301 (6), 1613–1624. https://doi.org/10.1007/s00606-014-1181-y.

Distefano, G., Caruso, M., La Malfa, S., Gentile, A., Wu, S.-B., 2012. High resolution melting analysis is a more sensitive and effective alternative to gel-based platforms in analysis of SSR – an example in citrus. PLoS One 7 (8), e44202. https://doi.org/10.1371/journal.pone.0044202.

Doligez, A., Adam-Blondon, A.F., Cipriani, G., Di Gaspero, G., Laucou, V., Merdinoglu, D., Meredith, C.P., Riaz, S., Roux, C., This, P., 2006. An integrated SSR map of grapevine based on five mapping populations. Theor. Appl. Genet. 113 (3), 369–382. https://doi.org/10.1007/s00122-006-0295-1.

Ferreira, V., Fernandes, F., Pinto-Carnide, O., Valentão, P., Falco, V., Martín, J.P., Ortiz, J.M., Arroyo-García, R., Andrade, P.B., Castro, I., 2016. Identification of Vitis vinifera L. grape berry skin color mutants and polyphenolic profile. Food Chem. 194, 117–127. https://doi.org/10.1016/j.foodchem.2015.07.142.

Ferreira, V., Castro, I., Carrasco, D., Pinto-Carnide, O., Arroyo-García, R., 2018. Molecular characterization of berry skin color reversion on grape somatic variants. J. Berry Res. 8 (3), 147–162. https://doi.org/10.3233/JBR-170289.

Fournier-Level, A., Le Cunff, L., Gomez, C., Doligez, A., Ageorges, A., Roux, C., Bertrand, Y., Souquet, J.-M., Cheynier, V., This, P., 2009. Quantitative genetic bases of anthocyanin variation in grape (*Vitis vinifera* L. ssp. *sativa*) berry: a quantitative trait locus to quantitative trait nucleotide integrated study. Genetics 183 (3), 1127–1139. https://doi.org/10.1534/genetics.109.103929.

Fournier-Level, A., Lacombe, T., Le Cunff, L., Boursiquot, J.-M., This, P., 2010. Evolution of the VvMybA gene family, the major determinant of berry colour in cultivated grapevine (Vitis vinifera L.). Heredity (Edinb) 104 (4), 351–362. https://doi.org/10.1038/hdy.2009.148.

Giannetto, S., Velasco, R., Troggio, M., Malacarne, G., Storchi, P., Cancellier, S., De Nardi, B., Crespan, M., 2008. A PCR-based diagnostic tool for distinguishing grape skin color mutants. Plant Sci. 175 (3), 402–409. https://doi.org/10.1016/j.plantsci.2008.05.010.

Guo, D.-L., Zhao, H.-L., Li, Q., Zhang, G.-H., Jiang, J.-F., Liu, C.-H., Yu, Y.-H., 2019. Genome-wide association study of berry-related traits in grape [Vitis vinifera L.] based on genotyping-by-sequencing markers. Hortic Res 6 (1), 11. https://doi.org/10.1038/s41438-018-0089-z.

Kerekes, A., Lorenzis, G.De, Szőke, A., Kiss, E., Failla, O, 2015. Analysis of VvMybA1 and VvMybA2 genes in grape bud sports. Vitis: J. Grapevine Res. 54, 45–48. https://api.semanticscholar.org/CorpusID:83979821.

Kobayashi, ..S., Ishimaru, ..M., Hiraoka, ..K., et al., 2002. Myb-related genes of the Kyoho grape (Vitis labruscana) regulate anthocyanin biosynthesis. Planta 215, 924–933. https://doi.org/10.1007/s00425-002-0830-5.

Kobayashi, S., Goto-Yamamoto, N., Hirochika, H., 2004. Retrotransposon-induced mutations in grape skin color. Science 304 (5673), 982. https://doi.org/10.1126/science.1095011. –982.

Kobayashi, S., Goto-Yamamoto, N., Hirochika, H., 2005. Association of VvmybA1 gene expression with anthocyanin production in grape (Vitis vinifera) skin-color mutants. J. Japanese Societ. Horticult. Sci. 74 (3), 196–203. https://doi.org/10.2503/jjshs.74.196.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25 (14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

Migliaro, D., Crespan, M., Muñoz-Organero, G., Velasco, R., Moser, C., Vezzulli, S., 2014. Structural dynamics at the berry colour locus in *V itis vinifera* L. somatic variants. Aust. J. Grape Wine Res. 20 (3), 485–495. https://doi.org/10.1111/ajgw.12103.

Moose, S.P., Mumm, R.H., 2008. Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. Plant Physiol. 147 (3), 969–977. https://doi.org/10.1104/pp.108.118232.

Pelsy, F., Dumas, V., Bévilacqua, L., Hocquigny, S., Merdinoglu, D., 2015. Chromosome replacement and deletion lead to clonal polymorphism of berry color in grapevine. PLoS Genet. 11 (4), e1005081 https://doi.org/10.1371/journal.pgen.1005081.

Péros, J.-P., Launay, A., Berger, G., Lacombe, T., This, P., 2015. MybA1 gene diversity across the Vitis genus. Genetica 143 (3), 373–384. https://doi.org/10.1007/s10709-015-9836-3.

Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., Ferrara, G., Matarrese, A.M.S., Gallotta, A., Bellincontro, A., Mencarelli, F., Tombesi, S., 2018. Grapevine quality: a multiple choice issue. Sci. Hortic. 234, 445–462. https://doi.org/10.1016/j.scienta.2017.12.035.

Puglisi, D., Las Casas, G., Ferlito, F., Nicolosi, E., Di Guardo, M., Scollo, F., Saitta, G., La Malfa, S., Gentile, A., Distefano, G., 2022. Parents' selection affects embryo rescue, seed regeneration and the heredity of seedless trait in table grape breeding programs. Agriculture 12 (8), 1096. https://doi.org/10.3390/agriculture12081096.

R Core Team, R. F. for S. C. (2021). *R: a Language and Environment for Statistical Computing*.

Reed, G.H., Wittwer, C.T., 2004. Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. Clin. Chem. 50 (10), 1748–1754. https://doi.org/10.1373/clinchem.2003.029751.

Reed, G.H., Kent, J.O., Wittwer, C.T., 2007. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics 8 (6), 597–608. https://doi.org/10.2217/14622416.8.6.597.

Riaz, S., Dangl, G.S., Edwards, K.J., Meredith, C.P., 2004. A microsatellite marker based framework linkage map of Vitis vinifera L. Theor. Appl. Genet. 108 (5), 864–872. https://doi.org/10.1007/s00122-003-1488-5.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., Mesirov, J.P., 2011. Integrative genomics viewer. Nat. Biotechnol. 29 (1), 24–26. https://doi.org/10.1038/nbt.1754.

Rolle, L., Giacosa, S., Gerbi, V., Novello, V., 2011. Comparative study of texture properties, color characteristics, and chemical composition of ten white table-grape varieties. Am. J. Enol. Vitic. 62 (1), 49–56. https://doi.org/10.5344/ajev.2010.10029.

Sun, L., Li, S., Jiang, J., Tang, X., Fan, X., Zhang, Y., Liu, J., Liu, C., 2020. New quantitative trait locus (QTLs) and candidate genes associated with the grape berry color trait identified based on a high-density genetic map. BMC Plant Biol. 20 (1), 302. https://doi.org/10.1186/s12870-020-02517-x.

Vargas, A.M., Fernández-Pastor, M., Castro, F.J., Martínez, M.A., Gómez-Cifuentes, A., Espinosa-Roldán, F., Cabello, F., Muñoz-Organero, G., de Andrés, M.T., 2023. Strategy to minimize phenotyping in the selection of new table grape varieties. In: BIO Web of Conferences, 56, p. 01030. https://doi.org/10.1051/bioconf/20235601030.

Velt, A., Frommer, B., Blanc, S., Holtgräwe, D., Duchêne, É., Dumas, V., Grimplet, J., Hugueney, P., Kim, C., Lahaye, M., Matus, J.T., Navarro-Payá, D., Orduña, L., Tello-Ruiz, M.K., Vitulo, N., Ware, D., Rustenholz, C., 2023. An improved reference of the grapevine genome reasserts the origin of the PN40024 highly homozygous genotype. G3 (Bethesda). https://doi.org/10.1093/g3journal/jkad067.

Walker, A.R., Lee, E., Robinson, S.P., 2006. Two new grape cultivars, bud sports of Cabernet Sauvignon bearing pale-coloured berries, are the result of deletion of two regulatory genes of the berry colour locus. Plant Mol. Biol. 62 (4–5), 623–635. https://doi.org/10.1007/s11103-006-9043-9.

Walker, A.R., Lee, E., Bogs, J., McDavid, D.A.J., Thomas, M.R., Robinson, S.P., 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. Plant J. 49 (5), 772–785. https://doi.org/10.1111/j.1365-313X.2006.02997.x.

Yakushiji, H., Kobayashi, S., Goto-Yamamoto, N., Tae Jeong, S., Sueta, T., Mitani, N., Azuma, A., 2006. A skin color mutation of grapevine, from black-skinned pinot noir to white-skinned pinot blanc, is caused by deletion of the functional *VvmybA1* allele. Biosci. Biotechnol. Biochem. 70 (6), 1506–1508. https://doi.org/10.1271/bbb.50647.

Zhao, Z.C., Hu, G.B., Hu, F.C., Wang, H.C., Yang, Z.Y., Lai, B., 2012. The UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT) gene regulates anthocyanin biosynthesis in litchi (Litchi chinesis Sonn.) during fruit coloration. Mol. Biol. Rep. 39, 6409–6415. https://doi.org/10.1007/s11033-011-1303-3.