Hindawi
Australian Journal of Grape and Wine Research
Volume 2023, Article ID 2117139, 9 pages
https://doi.org/10.1155/2023/2117139

AUSTRALIAN SOCIETY OF VITICULTURE AND OENOLOGY | ASVO

Research Article

In-the-Field Authentication of Grapevine (*Vitis vinifera* L.) cv: Albariño Using Chlorotype Discrimination and a Single SNP Interrogation by LAMP

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Received 9 November 2022; Revised 10 January 2023; Accepted 15 February 2023; Published 28 February 2023

Academic Editor: Serge Delrot

Authentication of grapevine cultivars, *Vitis vinifera* L., is difficult, especially when analytical specimens lack diagnostic ampelographic characters, which prevents the verification of traceability systems aimed at guaranteeing varietal integrity. This issue is problematic when viticultural strategies and regulations associated with high-value wine-producing geographical areas rely on extensive control and monitoring of valuable cultivars. Varietal identification based on grapevine molecular markers is a standardized methodology that requires a specialised laboratory for its application. In contrast, the use of loop-mediated isothermal amplification (LAMP) allows DNA markers to be characterized quickly and easily, without the need for skilled personnel, allowing implementation in-situ or in-the-field. Simultaneous identification of the chlorotype and the interrogation of a single SNP using a portable device have allowed the first discrimination in-the-field of grafted grapevines, without appreciable ampelographic characters, as belonging to the valuable "Albariño" cultivar. This methodology constitutes a valuable tool for cultivar discrimination and can be efficiently implemented in the traceability of valuable grapevine genetic resources.

1. Introduction

The grapevine, *Vitis vinifera* L., contains several worldwide dispersed, highly appreciated, and valuable cultivars but frequently involves a difficult or problematic morphological identification that requires effective methodologies [1–3]. Furthermore, when dealing with reproductive organs lacking most of cultivar diagnostic ampelographic characters, accurate identification is unattainable, and authentication is only supported by documentary traceability. In this context, the viticultural strategies and regulations associated with high-value wine-producing geographical areas demand methodologies for effective cultivar authentication.

The use of nuclear microsatellite markers or simple sequence repeats (SSRs) has been the standardized molecular method of identifying grapevine genotypes in cultivars [4–9]. However, the resolution of allele sizes for these markers requires infrastructure, equipment, trained personnel, and time that do not allow their application outside the laboratory context. In contrast, rapid, sensitive, specific, and simple techniques aimed at in-situ diagnoses, such as loop-mediated isothermal amplification (LAMP) [10, 11]

can be used in conjunction with alternative DNA markers for their efficient application to the traceability of grapevine cultivars. The LAMP reaction only progresses if there is a perfect match between the required oligos and the target sequence, especially at critical positions. By designing alternative oligo versions, specifically targeting each allele, and visualising with which version of the oligos set amplification occurs, it is possible to distinguish both alleles and achieve SNP genotyping [12]. Thus, a colorimetric and fluorescent LAMP assay, based on a single informative SNP, was developed to detect the presence of wheat varieties for the detection of adulterated food products [13].

Grapevine chloroplast DNA is characterized by a lower evolutionary rate than the nuclear genome [14], maternal inheritance [15], and the presence of a reduced number of geographically structured chlorotypes [15, 16]. Therefore, the variation detected in three SSR chloroplasts, responsible for intra- and interspecific length polymorphism in *Vitis*, was useful to demonstrate maternal inheritance in *Vitis* and differences in chlorotype frequencies in cultivars from Spain and Greece. As a result, four chlorotypes were defined based on allelic variants in these three chloroplast loci, named A, B, C, and D, being A the most frequent among Spanish and D among Greek cultivars [15]. Furthermore, the study of the distribution of chlorotype variation across the entire area of the *sylvestris* and *sativa* subspecies allowed the identification of four additional chlorotypes (E to H) [17].

Alternatively, sequencing of noncoding chloroplast regions allowed the definition of four haplotypes designated by the character status at three polymorphic sites (AAA, ATA, ATT, and GTA). In addition, a 54 bp deletion, at the trnC-GCA–petN intergenic spacer, was associated with all haplotypes, except GTA [18, 19]. Interestingly, a relationship is observed among alternative chlorotypes definitions. Thus, the A SSR-based chlorotype is homologous to the sequence-based GTA haplotype, being the most abundant in cultivars from Spain.

The availability of wide genome data allowed the definition of nuclear SNP arrays and SNP data sets designed for various genomic-based studies and, particularly, for cultivar identification. A 48 SNP set [20] was proposed as a genotyping standard for grapevine cultivars, and combined with additional SNP data sets, allowing the analysis of genetic relationships among cultivars [21].

The 18K SNPs array, developed by the GrapeReseq European project [22], has been used for several grapevine studies [23–25]. In one of them, 945 European accessions of *V. vinifera* were genotyped, and that was the basis for selecting a simple set of 14 SNPs, filtered from the initial 18K SNPs array, that provided enough resolution for their identification [26]. The *Vitis* International Variety Catalogue (https://www.vivc.de) currently includes SNP profile for many varieties including (1) the 48 plus 24 SNP markers developed by Cabezas et al. [20] and (2) 14 plus 26 SNP loci proposed by Laucou et al. [26]. Considering these markers, LAMP can also be applied to interrogating SNPs in situ, quickly and efficiently [27].

The EU regulations impose obligations to plant, replant, or graft those wine grape varieties that are authorized for each territory [28]. In particular, 31 varieties have been authorized in the autonomous region of Galicia (North--West Spain) [29]. The EVEGA (Galician Viticulture and Enology Station) germplasm bank maintains a grapevine collection to preserve, study, and promote these valuable grapevine genetic resources from the diverse protected designations of origin located in this region [5, 30]. Consequently, those authorized and valuable cultivars must be traceable through the cultivar multiplication, distribution, and commercialization chain, in any developmental stage or vegetative status, preferably in situ. To achieve this, traceability available data on chlorotype classification and genotyping provide basic information for the initial step of identification of the cultivars hosted at EVEGA [15], including "Albariño," the most economic and enologically important grapevine cultivar. Thus, nuclear and chloroplast genotyping data for the EVEGA accessions are available for this purpose from different sources [20, 21].

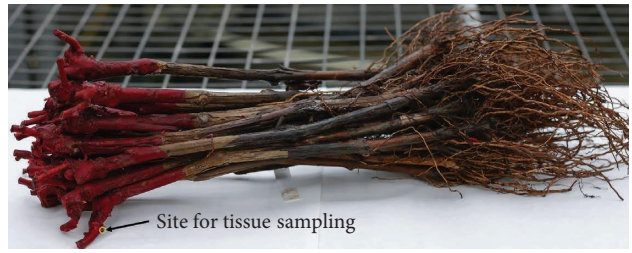

Figure 1: Certified grafted grapevines belonging to the "Albariño" cultivar and used in this study. Small tissue samples are taken from a bud present in the grafted scionwood.

This study proposes a rapid, simple, and accurate methodology for in-situ cultivar authentication based on LAMP amplification. LAMP-based detection systems harbouring chloroplast polymorphisms and SNP loci that contain diagnostic information were designed and evaluated for their potential for quick and simple in-field authentication of grafted shoot tissue samples.

2. Materials and Methods

A set of six relevant cultivars from EVEGA collection, "Mencía" (prime name in VIVC database "Mencia," VIVC number 7623), "Treixadura" ("Trajadura," 12629), "Castañal" ("Castanal," 23051), "Zamarrica" ("Cainho da Terra," 26692), "Godello" ("Gouveio," 12953) and "Loureira"("Loureiro Blanco," 6912), in addition to the main target, "Albariño" ("Alvarinho," 15689), was selected for this study. DNAs were isolated with DNA Easy Mini Plant Kit (Qiagen) from leaf samples from the EVEGA germplasm bank to test and evaluate LAMP-based detection protocols. The sampled cultivars carry A ("Mencía," "Castañal," "Godello," and "Loureira") or D ("Treixadura" and "Zamarrica") chlorotypes.

Total DNA from testing grapevines ($N = 6$) for direct use in LAMP reactions was prepared from a dormant bud of a certified grafted scion wood (Figure 1). A cross section of the bud ($<2\,\text{mm}^2$ $x < 0.5\,\text{mm}$) was immersed in 50 μL of digestion buffer (10 mM KCl and 0.5% casein), incubated at

95°C for 5 min in a thermocycler/dry bath, and kept on ice for an additional 5 min [31]. The lysate was clarified, and debris was pelleted by a microfuge spin. A 2 μL sample of the lysate was 1/10 diluted with PCR-grade water before adding it to the reaction. For in-the-field implementation, two methods were tested: one as described, but incubation was carried out at 70°C, and without the centrifugation step ("Li17" method). Alternatively, the bud section was digested in AP1 buffer (50 μL) (Qiagen) for 10 min at 65°C. A clear solution was then transferred to a new tube and incubated with 1 volume of Mag-Bind® Total Pure NGS (Omega Bio-Tek) at ambient temperature for 5 min. Nucleic acid purification was then performed following the standard beads protocol (Omega-Bio-Tek, Norcross, USA) ("Qbeads" method). DNA quantities were fluorometrically estimated with Qubit (Thermofisher Scientific, Waltham, MA, USA).

The LAMP oligos were designed using Primer Explorer (https://primerexplorer.jp/e/; Eiken Chemical Co., Ltd., JAPAN) and manually revised, and edited. A target sequence was used as a reference or control, from a conserved region of the LOC100244283 gene located in chromosome 7, associated with resistance to mildew infection [32]. Internal FIP and BIP oligos were designed to hybridize with the specific allele sequence or SNP, preferably involving their 5′ end. The chlorotype-specific oligos were designed from chloroplast DNA, involving those sequences displaying diagnostic polymorphisms for the chlorotypes A and D [18, 19]. The designed oligo sets were evaluated for amplification efficiency and chlorotype specificity. Furthermore, LAMP oligo combinations were designed and tested for the interrogation of the diagnostic SNP 191_100, belonging to the 48 SNP set and informative for the authentication of cv. "Albariño" [20]: Albariño is the only Iberian cultivar which shows a TT homozygous genotype for this SNP, among more than 350 existing in the ICVV-DNA database (Ibáñez personal communication).

Two different methods were used for the detection of amplification. A colorimetric LAMP assay was performed in a 20 μL reaction volume using WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB, Ipswich, MA, USA). Oligo concentrations were adjusted to 0.2 μM for F3 and B3, 1.6 μM for FIP and BIP, and 0.4 μM for Loop oligos. 1 μL aliquots of DNA solutions were added to the reaction. Reactions were incubated at 65°C for 55 min in a thermocycler/dry bath, and then, colour was visualized and inspected at the time-to-read (55 min). A colour change (Fuchsia to yellow) originates from the decrease of pH caused by the DNA amplification in a reaction with minimal buffering capacity and the presence of a dye (phenol red) sensitive to pH change [33].

In addition, a fluorescent LAMP assay was carried out using the WarmStart® LAMP Kit (DNA & RNA) 2X Master Mix (DNA & RNA) (NEB) and including LAMP Fluorescent Dye x1 (NEB), being the oligo concentrations and DNA added volume same as above. The reactions, covered by 12 μL of mineral oil, were also performed at 65°C for 45 min-1 hour, and fluorescence was observed in real time and recorded on a DNAiTECH device (DNAiTECH, Marlborough, New Zealand).

3. Results and Discussion

Certified grafted grapevines are the main format for the distribution of quality grapevines controlled by a traceability system aimed at guaranteeing the cultivars identity, according to the regulations established by the Administration and the wine regulatory councils in Galicia (NW Spain). The certified grafted vines examined lacked any morphological identifying character, which can be a source of error or unintended substitution.

The sampling of a dormant bud causes damage to the graft, making it unusable in the case of single-bud graftings (Figure 1). However, the significance of the obtained information outweighs the limited potential damage to a batch of grapevines and does not hinder this authentication analysis based on in-situ amplification detection using LAMP oligos specific to both chlorotypes and SNP 190_100 genotype.

3.1. The Grapevine Chlorotype Discrimination. A relevant chlorotype-specific polymorphism was found in the non-coding region between the trnC-CGA tRNA and the petN gene [19] and confirmed by de-novo chloroplast sequencing (GenBank: CM041971.1 and CM041973.1) in two "Albariño" clones (Quinteiro et al., unpub.). This 54 bp indel polymorphism located at position 30,133-30,186 of the chloroplast sequence NC_007957 was selected as a target for the chlorotype-specific system. The loop (Vvin-ctA + 54.1) and part of the BIP (Vvin-ctA + 54.1.BIP) oligos were designed to anneal within this 54 bp length sequence present exclusively in chlorotype A (GTA), absent for all other chlorotypes, and thus conferring a chlorotype A specificity. A FIP and a BIP oligo (ctD-54.4.FIP/BIP) were designed to anneal to the 54 bp deletion position observed for the D and other non-A (ctD*) chlorotype sequences resulting, in the set of cultivars used, in a chlorotype D* specificity (Figure 2, Table 1).

The reference (REF), chlorotype A (ctA), and chlorotype D* (ctD*) oligo systems were successfully evaluated using high-quality DNA isolated and purified by standard protocols (DNeasy Tissue Mini Plant, Qiagen) (Figure 3(a)). For REF and ctA oligo systems, the expected colour shift to yellow was observed for 3 replicates at 45 mins containing 12.5 ng of template DNA in a colorimetric LAMP. A quantity of 1.25 ng provoked a colour shift in the ctA system after 60 mins of incubation. The ctD* system showed lower sensitivity at low DNA concentrations (Figure 4).

The chlorotype-specific systems also perform well using the fluorescence-based methodology. In this case, the ctA system presents a delay in signal emission, compared to the ctD* signal, probably due to its longer length. In both cases, the time-to-read can be set to less than 30 mins if the same template quantity as above (12.5 ng) is used (Figure 3(b)).

The similar performance of ct systems and REF reflects the high efficiency of the REF oligo system, which anneals to a nuclear sequence with a concentration lower than that of the chloroplastic one (Figure 5). The localisation for the REF oligo system, within the LOC100244283 gene, was based on ideal thermodynamic criteria. In contrast, the localisation of

Table 1: Oligonucleotide sequences for LAMP interrogation of chlorotypes for cultivar authentication in *Vitis vinifera* L. FIP and BIP oligos included F1c-F2 and B1c-B2 sequences, respectively.

Oligo name	Sequence 5′⟶3′
Reference LAMP oligo system	
Vvin-ref-loc7.1.F3	GGATTTTATAGCAAATGCCAGT
Vvin-ref-loc7.1.B3	AGTTCCACATGAGAAGGTG
Vvin-ref-loc7.1.FIP	CTGCTGTGGGAATCTTTTAAGCAC-TGATACTTTCTTCACGCACT
Vvin-ref-loc7.1.BIP	CTTTCATCGCATATTACACTCTCCA-TTTGTTCGAGCTGCCATC
Vvin-ref-loc7.1.LF	CCTCCCCAATCTCACCCTATC
Vvin-ref-loc7.1.LB	AATCCACGAGATCCTTCACTGA
Chlorotype A specific LAMP oligo system	
Vvin-ctA + 54.1.F3	CGGTCTTGAATTATATTGGATAGCT
Vvin-ctA + 54.1.B3	TCCCATTTTATCTAATCCTTATTGTC
Vvin-ctA + 54.1.FIP	GCACTCAGAAACTCTCATGAATCTGT-GGGGGAATCTAATTAGTAGTTGT
Vvin-ctA + 54.1.BIP	AATTCTTTCTTTTCGGTAATCCCTAGTC-CCCGATTGTTTCACAGAGA
Vvin-ctA + 54.1.LF	ATACAAAGTATCTTCTGCCCTTTCC
Vvin-ctA + 54.1.LB	AACGATAGACTGTCTCCCGATT
Chlorotype D-specific LAMP oligo system	
Vvin-ctD – 54.4.F3	TTAGTAGTTGTGGAAAGGGCA
Vvin-ctD – 54.4.B3	GGGAATGTTACTAATGAACATGTAAG
Vvin-ctD – 54.4.FIP	CCGAAAGAAAGAATTTCTTTTTGCACTT-ACTTTGTATACAGATTCATGA GAGTT
Vvin-ctD – 54.4.BIP	AATTCTTTCTTTCGGGAGACAATAAGGA-GTTCCCAGTATTAATGAAAGG CA
Vvin-ctD – 54.4.LF	ATTGATTGAATGCTCGAGCACTCAG
Vvin-ctD – 54.4.LB	GGGAAATAGAGGTATGTGATAGTAGATAGTGAT

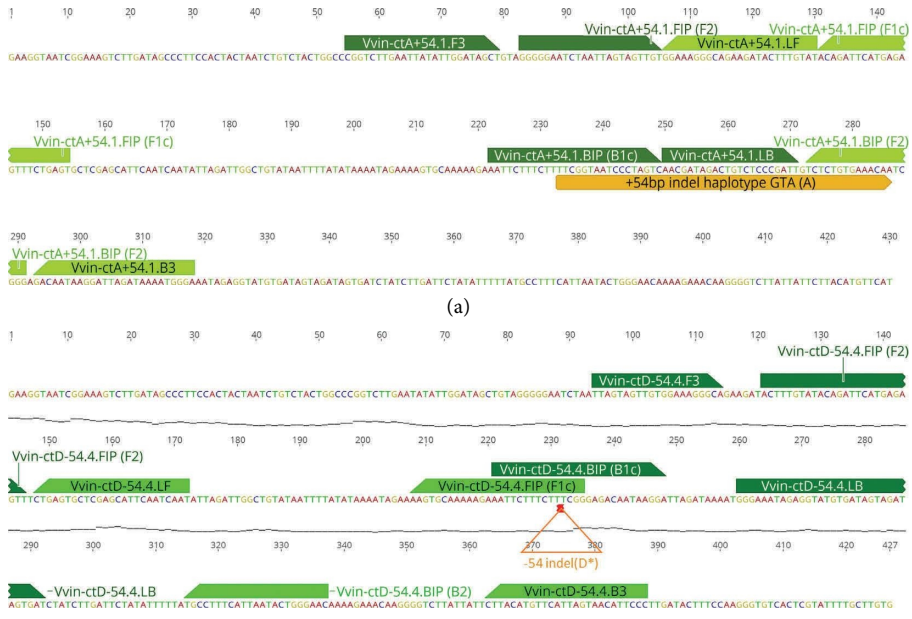

Figure 2: Location of the LAMP oligos and the 54 bp length chlorotype-diagnostic indel within the type A chlorotype (a) and type D* (b) chloroplast target sequence.

the oligos for the chloroplast-localised systems was positioned to confer annealing specificity to the chlorotype sequence, without showing fully ideal design parameters. As a result, the REF system is suitable as a reference system that validates the DNA obtained as a template for the amplification of both nuclear and chloroplast sequences.

3.2. Testing In-Situ Simple DNA Isolation from a Vitis Dormant Bud Tissue Sample. A key limiting step in point-of-care or in-the-field DNA analysis is to provide amplifiable DNA, even more so when the tissue sampled is scarce, rich in inhibitory compounds, and not an ideal source of DNA. However, the lysate obtained by the "Li17" method from the

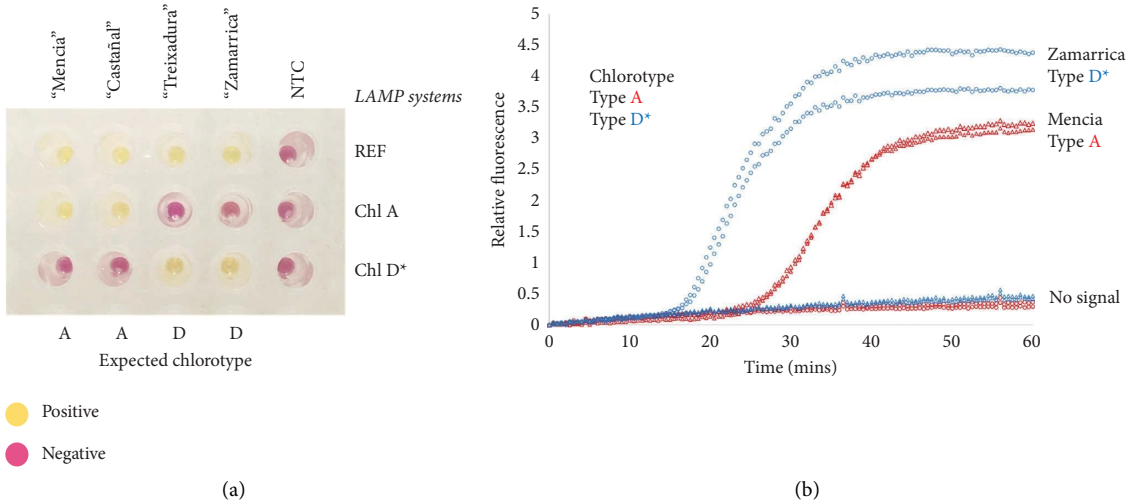

Figure 3: (a) Chlorotype-specific colorimetric LAMP amplification using the reference (REF), chlorotype A (ctA), and chlorotype D* (ctD*) oligo systems and template DNA from four different cultivars bearing A or D chlorotypes. Negative reactions show a fuchsia colour, while positive reactions are yellow. (b) Chlorotype-specific fluorimetric LAMP amplification using the chlorotype A (ctA) and chlorotype D* (ctD*) oligo systems and template DNA from two different cultivars bearing A and D chlorotypes.

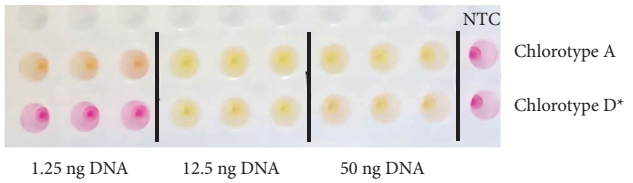

Figure 4: Differential amplification using the ctA and ctD* LAMP systems at different concentrations of DNA template isolated from "Mencia" and "Zamarrica" cultivars, respectively.

grapevine bud section amplified correctly in all tissue samples tested (Figure 5(a)). In this case, using one microlitre of an 1/10 dilution of the lysate obtained, the time-to-read was between 50–60 min for the reference nuclear system (REF). The colour change of the REF colorimetric reaction provides a suitable control test for the presence of amplifiable DNA and the accuracy of the rapid tissue lysate protocol performed. The absence of a colour change in the REF colour reaction test would indicate the unfeasibility of the DNA sample for testing.

Identification of the chlorotype of an unknown sample directly from a dormant tissue lysate resulted in the detection of chlorotype A (Figure 5(b)). This analysis was carried out using tubes preloaded with chlorotype A and D* specific LAMP components (3 replicates) and a tube containing the digestion buffer, kept refrigerated for 2 weeks. Lysate (1 μL of a 1/10 dilution) loaded into LAMP reaction tubes, and incubated at 65°C for 50 min in a 12 V thermoblock generated the colour change in those tubes that carry the ctA system (Figure 5(b)).

When testing DNA obtained from the "Qbeads" method, the reading time was significantly lower, with reading time values generally between 15 and 30 min. The purification achieved with the use of magnetic beads must be the reason for the higher efficiency of the "Qbeads" method. Thus, similar fluorimetric signals were obtained when using 1 μL of the 20 μL of the DNA solution obtained using the Qbeads method or 10 ng of template DNA purified with a conventional kit (Qiagen) (Figure 6). Therefore, we recommend the use of the "Qbeads" method, whenever possible and when rapid analytical conclusions are required. Both DNA isolation protocols allow for the implementation of the analysis under various circumstances, particularly in the field.

3.3. Interrogation of a Single Diagnostic SNP for the "Albariño" Cultivar. The distribution of chlorotypes is heterogeneous globally. However, chlorotype A is predominant in the Iberian Peninsula, and specifically, 73% of the autochthonous varieties conserved by EVEGA have it. Thus, the chlorotype diagnostic for a plant or reproductive tissue is not very informative although it can detect incoherent labelling. Therefore, it is necessary to interrogate SNP diagnostic positions that allow for an unambiguous authentication of these varieties, including through the design and evaluation of LAMP macroarrays based on previously reported data [20]. However, reliable authentication of a problem grapevine using a single SNP is considered to be the most efficient case. Here, we demonstrate how the interrogation of the nuclear SNP 191_100 [20] allows the discrimination of the "Albariño" cultivar from the 6 other selected varieties in this work, due to its exclusive TT genotype, confirmed by the whole genome de-novo sequencing (GenBank: GCA_023513765.1 and GCA_023513795.1) of two "Albariño" clones (Quinteiro et al. unpub.). The SNP flanking oligos for LAMP were designed to discriminate between the T and the C alleles, and the latest being absent in "Albariño," while it occurs in the rest of the selected autochthonous varieties (Table 2).

The SNP 191_100 genotype (TT, CT, and CC) was recorded for 195 genotyped cultivars, with a prominent presence of cultivars from the Iberian Peninsula. All of them

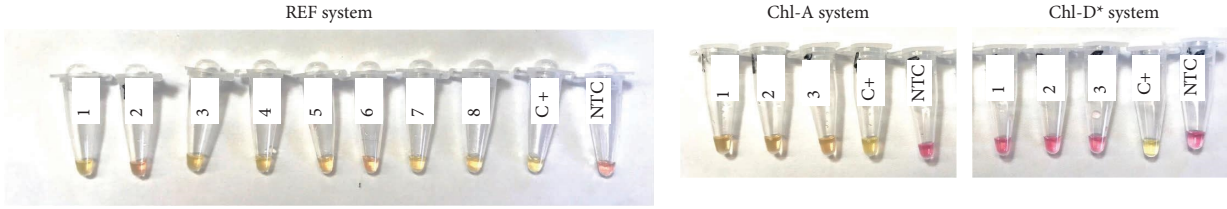

Figure 5: Colorimetric LAMP reactions (12.5 μL) performed on standard PCR tubes in a portable thermoblock using in-field DNA isolations following the Li17 method. (a) The template DNA obtained directly from four lysates (2 replicates per lysate) is tested with the reference REF system. (b) The DNA from one lysate (3 replicates) is tested with the ctA (Chl-A) and ctD* (Chl-D*) systems to identify the carried chlorotype. Reactions included a positive (C+) and negative control (NTC).

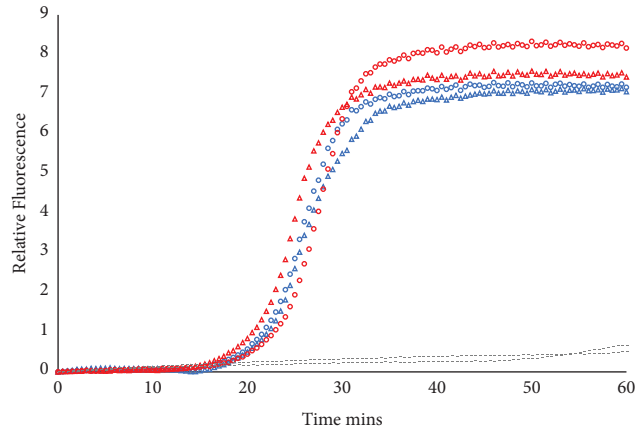

Figure 6: Real-time fluorescent LAMP amplification profiles for oligo LAMP systems targeting the T allele at the nuclear marker SNP 191_100 in the "Albariño" variety (homozygous TT). The time-to-read, around 20 min, was similar using 10 ng of Qiagen purified DNA (blue, 2 replicates) and using 1 μL of DNA extracted in the field with the Qbeads method (red, 2 replicates). Dot lines show the NTC reactions.

Table 2: Design of oligos for LAMP interrogation of the diagnostic SNP 191_100 aimed at the identification of the "Albariño" variety. FIP and BIP oligos included F1c-F2 and B1c-B2 sequences, respectively.

Oligo name	Sequence $5' \longrightarrow 3'$
Vvin-191.2.F3	TTCAAACAGAAAACARAAGGA
Vvin-191.1.B3	TGAAACTGTGACCCCAAT
Vvin-191.C.1.FIP	GGAAAGTTTTTATTTTCCCTTTGGGA-ACTTGTGATGTTCACAGATGA
Vvin-191.C.1.BIP	CAGCCAAACCAAGATGCTTTG-CTTCAAATGGGGCACTACA
Vvin-191.T.1.FIP	AGAAAGTTTTTATTTTCCCTTTGGGA-ACTTGTGATGTTCACAGATGA
Vvin-191.T.2.BIP	TAGCCAAACCAAGATGCTTTGTC-AAATGGGGCGCACTACA
Vvin-191.1.LF	AATCTATAGGGATTAACTACTTTTCAAATCT
Vvin-191.2.LB	GTCCAGCAATTMGGTTCTYGAATG

show a non-TT genotype [20]. However, a TT genotype for the SNP 191_100 has been reported for very few varieties up to now (VIVC database accessed 7 November 2022), mostly from France (vg. Manseng Gros Blanc, Manseng Petit Blanc, Verdot Petit) and Italy (vg. Bianchetto di Saluzzo, Muline) and only "Albariño" from the Iberian Peninsula. This makes the possibility of obtaining false positives in an analysis aimed at authenticating the cultivar "Albariño" in the Galician region unlikely in practice.

For the in-the-field implementation of the SNP 191_100 interrogation, we have used protocols that have been proven to be the most efficient. Thus, DNA was extracted from grafted grapevines using the Qbeads method, and amplification was carried out using fluorescent LAMP on a DNAiTECH portable device.

As a result of the specificity of the systems designed for the target sequences of the T and C alleles, amplification in "Albariño" (genotype TT) was only obtained with the system

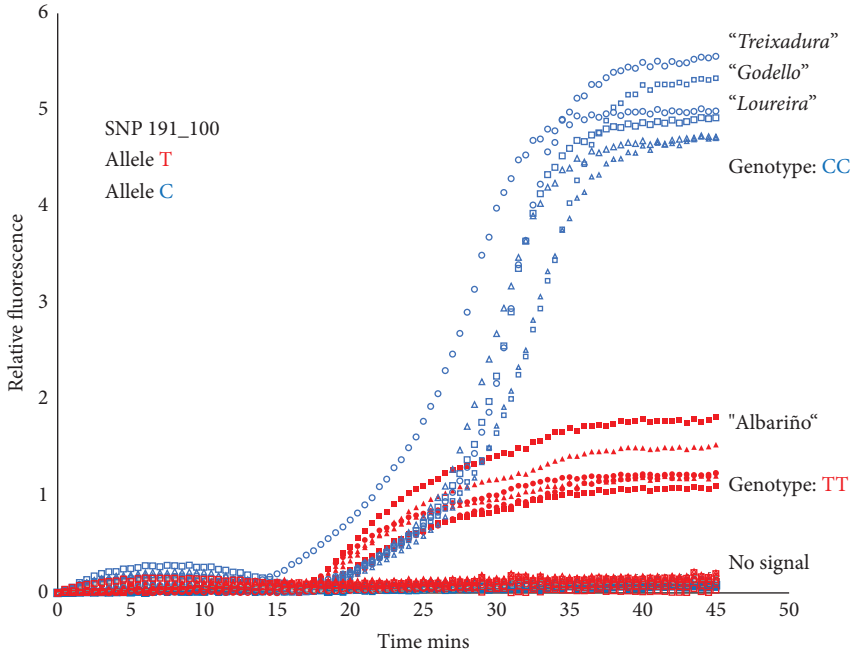

FIGURE 7: The "Albariño"-specific genotype (TT) for SNP 191_100 was interrogated to differentiate "Albariño" from other Galician varieties studied homozygotic for the C allele, such as "Treixadura," "Godello," and "Loureira".

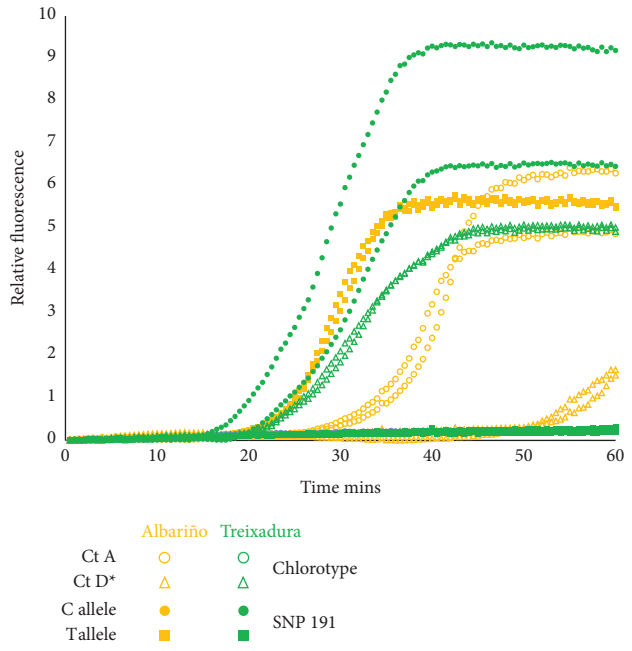

FIGURE 8: LAMP-based test that simultaneously interrogates the presence of a specific chlorotype (A-open circles- or D*-open triangles-) and the genotype at the SNP 191_100 (C allele (filled circles) or T allele (filled squares)) for the "Albariño" (yellow colour) and "Treixadura" (green colour) varieties. Positive reactions are detected between 15 and 35 mins (time-to-read). The negative NTC controls are not visible behind the points with a lack of fluorescence signal. Two replicas of each cultivar were used.

targeting the T allele. In contrast, for "Treixadura," "Godello," or "Loureira" (genotype CC), only the signal for the system targeting the C allele was detected (Figure 7).

3.4. Combination of Chlorotype and Nuclear Polymorphisms Interrogation to Discriminate Albariño. The diagnostic nuclear SNP analysis was also tested accompanied by a simultaneous interrogation of the carried chlorotype. In the example showed, the inference of the chlorotype is also discriminatory as "Albariño" carries chlorotype A while "Treixadura" carries chlorotype D (Figure 8).

In addition, the inclusion of the chlorotype test has an alternative application, functioning as a check for the presence of amplifiable template DNA, thus replacing the REF system. A sample must give a signal for one of the two systems aimed at chlorotyping. In the case of the absence of signal for both chloroplastic systems, the sample should be considered invalid for genotyping. Otherwise, a negative result for the allelic systems would indicate a problem in the oligos annealing to the target sequences. This occurs when not considering polymorphisms in the flanking sequences located at sensitive positions of the oligos. Therefore, we have characterized in the cultivar "Albariño" and other cultivars those polymorphisms in the flanking region not previously described to be considered during the design of the oligos systems.

After an authentication analysis, it is possible to obtain a nonconformity result according to the traceability documentation. In that case, the DNA sample can be subjected in the laboratory to a more complete counteranalysis based on standard SSR [9] or SNP panel methodologies [20].

The methodology proposed here has shown the possibilities of LAMP to authenticate or discriminate a grapevine in a variety of scenarios, including in-situ and field analyses with simple and portable equipment and from a minimal tissue sample. Oligos systems evaluated here and aimed at discriminating the chlorotype and a diagnostic SNP of the "Albariño" cultivar in the set of studied cultivars, constitute just an example to show the methodology for the authentication of valuable grapevine genetic resources. Its implementation may be very useful for quality control in nurseries or for fast preliminary discrimination by regulatory boards controlling a variety identity in Origin Denominations.

This LAMP methodology can be effectively applied in cases where there are described diagnostic SNPs flanked by thermodynamically suitable sequences to accommodate oligos design. A general implementation can be achieved in two different ways. The ideal implementation is the case presented here for Albariño cv. where the variety of interest carries a genotype that is unique among the most probable varieties in the particular context. Similar cases in the frame of varieties authorized in Origin Denominations in Galicia are represented by "Sousón" ("Vinhao," VIVC: 13100, SNP 1229_219, GG) or "Caiño Longo" (VIVC: 5178, Vvi10353, AA). Nevertheless, this is not the expected case in the sets of SNPs available at the VIVC since they were selected to maximize their capacity to distinguish varieties, and so to contain the largest minimum allele frequency.

Alternatively, in the absence of a rare genotype previously described, a panel is selected by combining the minimum number of SNPs required to obtain a reliable authentication. A set of 3 SNPs (2 replicates) can be simultaneously analysed with the portable device used here, while a larger number of SNPs can be evaluated by sequential reactions. The current accessibility to massive genotyping data in a cost-effective and quick way promises a relative universality of this methodology for several *Vitis vinifera* cultivar traceability issues.

4. Conclusions

DNA isolated from a grafted bud sample by the "Qbeads" method, based on the use of magnetic beads, is suitable for efficient LAMP genotyping in the field.

The detection of a positive LAMP reaction can be done with the naked eye after a colorimetric change, or through the detection and analysis of fluorescence emission with a mobile application on an ad-hoc-designed device, which is clearly advantageous.

Discrimination in the field of the grapevine chlorotype is possible by LAMP using oligo systems targeting diagnostic chlorotype polymorphisms. However, the chlorotype provides limited information, and it is necessary to combine it with the use of more informative markers.

A nuclear SNP (SNP 191_100) has been selected that shows a near-unique genotype (TT) for the cultivar "Albariño." The rest of the known genotyped cultivars from the Iberian Peninsula have at least a C allele for this SNP. As a result, the interrogation of this SNP allowed the first DNA authentication of a cultivar in the field.

Data Availability

The oligo and methodological data used to support the findings of this study are included within the article. The SNPs data used to support the findings of this study have been obtained from the references cited and the Vitis International Variety Catalogue (VIVC) (https://www.vivc.de/; database accessed 07/11/22).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Quinteiro Lara and Díaz-Fernández Angela contributed equally to this work.

Acknowledgments

This work was supported by the "Validation of molecular tools in the authentication and characterization of grapevine varieties" action in the project FEADER 12/32/408/310322/11 (European Agricultural Fund for Rural Development (EAFRD)), financed by EAFRD/FEDER (75%), Regional Government of Galicia (Xunta de Galicia) (17.5%), and Ministry of Agriculture Food and Environment (Spain) (7%). Ángela Díaz-Fernández held a predoctoral contract in "Estación Enológica y de Viticultura de Galicia" (EVEGA) provided by the "Agencia Estatal de Investigación (AEI)–Ministerio de Ciencia e Innovación". The authors would like to thank César Iglesias Vázquez for his initiative and support for the realization of this project. Ángela Díaz-Fernández thanks CITI staff for their welcome and help during her various working periods. The authors thank the anonymous reviewer for his insightful comments and suggestions that have improved the manuscript.

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