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Review paper

DNA AUTHENTICATION TECHNOLOGIES FOR PRODUCT QUALITY MONITORING IN THE WINE INDUSTRY

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ABSTRACT
Identification of wine product authenticity is a topical question in the Russian Federation. A solution to this problem can be DNA authentication of wines, which is a technological process of product authenticity control using genetic identification of the main plant ingredient — wine grape varieties. This type of wine verification is carried out by analyzing residual amounts of Vitis vinifera L. nucleic acids extracted from cell debris of final products by molecular genetic methods. The aim of this work is the analysis of the existing methods for extraction of nucleic acids from grapes, wine raw materials and commercial wines, as well as description of the molecular genetic approaches to technical genetic identification of grape varieties and authentication of wines made from them. The obtained data suggest suitability of DNA authentication of wine products as a supplement to earlier approved analytical methods (documentary, visual, sensory, physico-chemical).

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1. Introduction

One of the priorities in Russia over the last decade has been provision of the population with high-quality and safe food products. The alcohol industry is of great importance for economy of the Russian Federation [1]. With that, the wine industry accounts for a significant volume of manufactured products.

In Russia, wine quality is determined by several normed physico-chemical indices [2]. As the experience shows, these indices cannot guarantee the objective conclusion about wine authenticity. Due to the widespread presence of falsified products on the market, the problem of new method development became a topical issue in product quality and safety assessment. Consequently, the key task is extension of the assessment criteria area with more modern methodological base, in particular, the DNA authentication technologies.

DNA authentication of wines is a technological process of product authenticity control by genetic identification of the main plant ingredient — wine grape varieties. This type of wine verification is carried out by analyzing residual amounts of *Vitis vinifera* L. nucleic acids extracted from cell debris of final products by molecular genetic methods. [3].

2. Main part

Analysis of the literature on residual DNA extraction from wine cell debris indicates the following key methods: Pereira [4], Savazzini & Martinelli [5], and Nakamura [6], as well as their modifications [7]. The first two methods mentioned above have the similar extraction stage: precipitation of wine plant debris. This stage is performed using precipitators such as sodium chloride, 2-propanol and sodium acetate with the following centrifugation [4,5]. The method for residual DNA extraction described by L. Pereira et al. [4] is most effective due to high yield of extracted residual nucleic acids (Figure 1).

Methods for DNA authentication of wine raw materials and commercial wines are based on using several genetic markers of nuclear, mitochondrial and chloroplast DNA (Table 1) [5,6].

One of the methods for DNA authentication of wine raw materials is the use of highly polymorphic DNA microsatellite loci. Initially this method was intended for genetic identification of grape varieties [8,9,10,11,12]. Table 2 presents the basic set for identification and certification of grape varieties and hybrids [4,5,6, 13,14,15,16,17,18,19,20].

The *SSR* fragments were amplified by multiplex PCR, which enabled combining several analyzed loci. It is conventional to use this amplification algorithm when working with DNA obtained from grape plant parts (fruit, leaf, stem, root); however, it is not efficient when analyzing the extracted residual nucleic acid from wine [5,6,13,14,15].

Another type of *SSR* markers targeted to chloroplast DNA (*spSSR*) [21,22,23,24,25] has several advantages compared to the analysis of nuclear DNA (*nSSR*) due to the higher copy number of a target per cell, higher resistance to the exonuclease action and lower susceptibility to degradation because of its content in organelles with the double membrane [5, 7].

Analysis of microsatellite loci of chloroplast DNA remains to be an alternative approach to varietal genetic identification of *Vitis vinifera* L. as this type of *SSR* markers has the low discriminatory ability.

Table 1

Markers used for wine DNA authentication

Methods for DNA authentication of wine raw materials and wine products		
SSR- markers of nuclear, mitochondrial and chloroplast DNA of *Vitis vinifera* L	*STS*- markers of nuclear, mitochondrial and chloroplast DNA of *Vitis vinifera* L	*STS*- markers of nuclear, mitochondrial and chloroplast DNA of *Vitis vinifera* L

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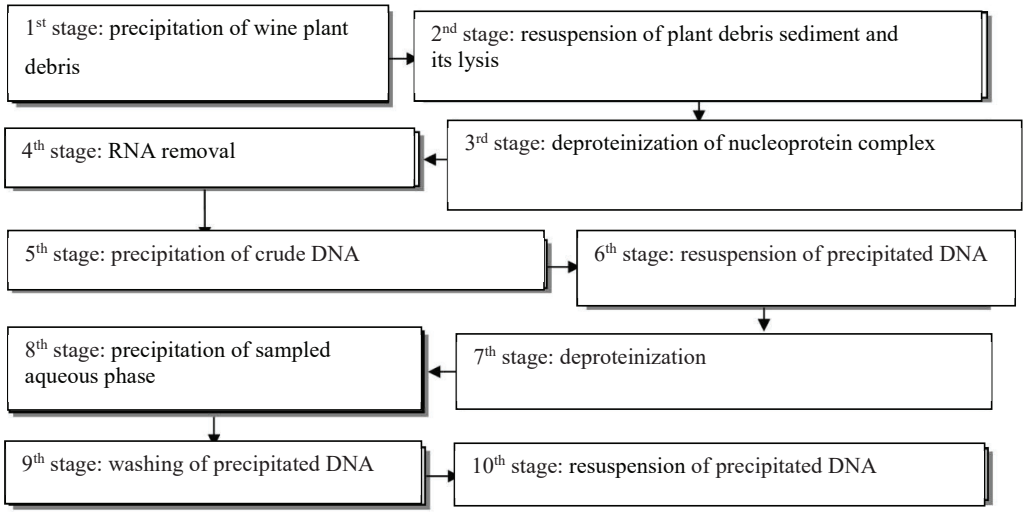

Figure 1. Stages of sample preparation and extraction of Vitis vinifera L. DNA from wine by the Pereira method

Table 2

***SSR* markers of nuclear DNA used for large-scale identification and certification of grape varieties and hybrids partly suitable for DNA authentication of wines**

No	SSR locus	Sequence of oligonucleotide primers	Range of allele lengths, bp
1	VVS2	5′-CAGCCCGTAAATGTATCCATC-3′	123–165
		5′-AAATTCAAAATTCTAATTCAACTGG-3′	
2	VVMD5	5′-CTAGAGCTACGCCAATCCAA-3′	220–268
		5′-TATACCAAAAATCATATTCCTAAA-3′	
3	VVMD7	5′-AGAGTTGCGGAGAACAGGAT-3′	231–267
		5′-CGAACCTTCACACGCTTGAT-3′	
4	VVMD25	5′-TTCCGTTAAAGCAAAAGAAAAAGG-3′	243–275
		5′-TTGGATTTGAAATTTATTGAGGGG-3′	
5	VVMD27	5′-GTACCAGATCTGAATACATCCGTAAGT-3′	173–223
		5′-ACGGGTATAGAGCAAACGGTGT-3′	
6	VVMD28	5′-AACAATTCAATGAAAAGAGAGAGAGAGA-3′	216–285
		5′-TCATCAATTTCGTATCTCTATTTGCTG-3′	
7	VVMD32	5′-TATGATTTTTTAGGGGGGTGAGG-3′	234–272
		5′-GGAAAGATGGGATGACTCGC-3′	
8	VrZAG62	5′-GGTGAAATGGGCACCGAACACACGC-3′	173–219
		5′-CCATGTCTCTCCTCAGCTTCTCAGC-3′	
9	VrZAG79	5′-AGATTGTGGAGGAGGGAACAAACCG-3′	236–270

The use of microsatellite DNA as a source of *STS* is also mentioned in the literature. Sequence Tagged Site (STS) is a short unique sequence, which amplified profiles serve as molecular genetic markers [4, 13]. For example, S. Nakamura et al. (2007) [6] developed the experimental *STS* primer sets for certain *SSR* loci of mitochondrial and chloroplast DNA [10, 21] and tested them in PCR for identification of *Vitis vinifera* L. varieties, as well as DNA authentication of wines produced from them.

As for *SNP* markers [26], they are also suitable for DNA authentication of wines [12]. *SNP* markers have the following advantages:

- differentiation of individual *Vitis vinifera* L. genotypes in single-varietal wines and assemblage wines with the possibility of quantitative assessment of plant ingredients
- efficiency in the analysis of the fragmented DNA of low quality.

Table 3 presents the primer and probe sets for real-time PCR with fluorescent hybridization detection, which are used in genetic identification of the *Sangiovese* variety and DNA authentication of wine produced from it by the single-nucleotide polymorphism (*SNP*) analysis in three analytical positions (98, 222 and 244) [7].

Another variant for application of *SNP* markers is to use the knowledge about single nucleotide polymorphism in several genes of *Vitis vinifera* L. incorporated into the method for high-resolution melting (*HRM*) curve analysis based on the real-time PCR platforms [12, 26, 27].

HRM analysis is an effective genotyping technology [28,29] with combined PCR stages and highly specific and sensitive detection with a possibility to differentiate several genotypes within one analysis, which is also suitable for wine DNA authentication [12, 26].

3. Conclusion

Analysis of methods for extraction of residual nucleic acids from final alcoholic products indicates the topicality and prospects of using DNA authentication as a molecular genetic

method for controlling safety of alcoholic beverages and detecting adulteration. The use of DNA technologies facilitates the most reliable determination of product authenticity in the wine industry. Molecular marker systems are suitable for identification of wine grape (*Vitis vinifera* L.) varieties and can ensure traceability throughout the life cycle of a final product.

Table 3

Real-time PCR primers and probes for three *SNP* positions applied in genetic identification of the *Sangiovese* variety and DNA authentication of wine produced from it

SNP	PPCR Round	Oligonucleotide primers and TaqMan probes	PCR product
98	1st PCR round with external primers	5′-TTCAAAGCGAAGAACCAG-3′ 5′-ACCCTTCAACAAACCAAC-3′	790 bp
	2nd PCR round with nested primers and TaqMan probes	5′-GTTAGTGTAAGGTGATGCC-3′ 5′-TTTCCTAATCCTTGTTGG-3′ 5′-FAM-TAGGATTTATGAAGGGAAG-3′-BHQ1 5′-VIC-TAGGATTTATGAAGGCAAG-3′-BHQ2	136 bp
222	1st PCR round with external primers	5′-AGACTGACTTTTGAAACACC-3′ 5′-TTCCTGGATTGGGTATG-3′	889 bp
	2nd PCR round with nested primers and TaqMan probes	5′-AAGACACCCACCAAGTTC-3′ 5′-CCAGGCAAGTAACACAAG-3′ 5′-FAM-AGCAATGTGGGCTGA-3′-BHQ1 5′-VIC-AGCAATGTGGGCCGA-3′-BHQ2	128 bp
244	1st PCR round with external primers	5′-AAACGCAGGAGAATGTC-3′ 5′-TTCAACCTGATGCCTAAC-3′	721 bp
	2nd PCR round with nested primers and TaqMan probes	5′-AATCCCCATCCCGAAGTG-3′ 5′-CCCAGTTCCATTCCTACACC-3′ 5′-FAM–CCTTTCTGGGTTGAACA-3′-BHQ1 5′-VIC–CCTTTCTGGGTTGCACA-3′-BHQ2	136 bp

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