Label free DNA-based Optical Biosensor applied to Wine Authenticity

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Introduction

The grapevine varieties used in winemaking have a profound influence in the quality of the final product, consequentially having a direct impact on wine's market price. Highly quoted wines belonging to referenced market segments, such as Denomination of Origin (DO), are the preferential target of fraudulent practices (Pereira et al., 2012). Among these practices, the grapevine varietal mislabelling is one of the most common occurrences. Thus a precise mean for grapevine varietal identification in wine samples is of the outmost relevance in order guarantee commercial fairness. Currently, there are several methods available for this purpose, however most of them rely on the analysis of compounds that are influenced by the winemaking processes, the growing environmental conditions and other uncontrolled factors (Pereira et al., 2017). Still, these variables do not affect the grapevine genotype and therefore DNA-based methodologies are considered to be an accurate and efficient mean to identify and discriminate the grapevine varieties (Pereira et al., 2018).
DNA markers can reveal inter and intra-species/varietal diversity through the identification of specific nucleotide sequence within the genome (Scarano and Rao, 2014). Several DNA markers have been described as suitable for varietal identification among the *Vitis vinifera* L. genome. The International Organisation of Vine and Wine recommended a set of six Simple Sequence Repeats (SSRs) suitable for such purpose (OIV, 2009). Additionally, a set of 48 Single Nucleotide Polymorphism (SNPs) markers have been used to genotype the different grapevine varieties (Cabezas et al., 2011).
The need for quicker, simpler and cost-effective analysis is propelling the development of new technologies, as alternatives to the expensive and time consuming lab-based methodologies applied nowadays (Mehrotra, 2016). Particularly, optical biosensors are arising in this field demonstrating a great potential to be applied to wine authenticity assessment (Gomes et al., 2018; Barrias et al., 2019). The use of Long-period gratings (LPGs) is a viable, cost-efficient technology for optical biosensing devices. Indeed, even though LPGs were initially developed as band-rejection filters, there use in sensing applications, such as, temperature, refractive index, biochemical applications, as been increasing (Grattan and Meggitt, 2013). Biosensors based on LPGs respond to small changes in the optical fiber surrounding medium with a shift of the refractive index. When the fiber's outer surface is functionalized with a specific DNA sequence, when the target strand is complementary to the probe, it will hybridize, all these interactions are detected by the system in a highly sensitive and specific manner (Gonçalves et al., 2015; Moreira et al., 2016).

The aim of this study is to develop a DNA-based optical biosensor that allows the identification of different grapevine varieties in leaf, must and wine samples based on SNP information.

Methods overview

The overall methodology followed in this work is resumed in Figure 1. Briefly, leaf, must and wine samples from three grapevine varieties (Malvasia Fina, Trincadeira and Temperanillo (Aragonez)) were collected from certified vineyards. The wines were prepared using certified grapes under controlled microvinification conditions (Pereira et al., 2017).
Genomic DNA samples were extracted from leaf, must and wine samples following the method by Doyle and Doyle (1987), Pereira et al. (2012) and Pereira et al. (2011), respectively. Three SNP markers, previously identified by our group in the gene *F3H* (flavanone 3-hydroxylase; Gomes et al., 2018) were used to design probes suitable to differentiate the three grapevine varieties, using the DNA extracted from the three matrices. Two optical biosensor assays were performed (Assay 1 and Assay 2), involving a functionalization step, followed by hybridization between the Probe and the complementary DNA (when present in the sample) and a regeneration step for further reutilization of the system. Hybridization led to wavelength shifts recorded by the software, allowing for the detection of the event detection. Each assay was performed in triplicates to assure the system's reproducibility.

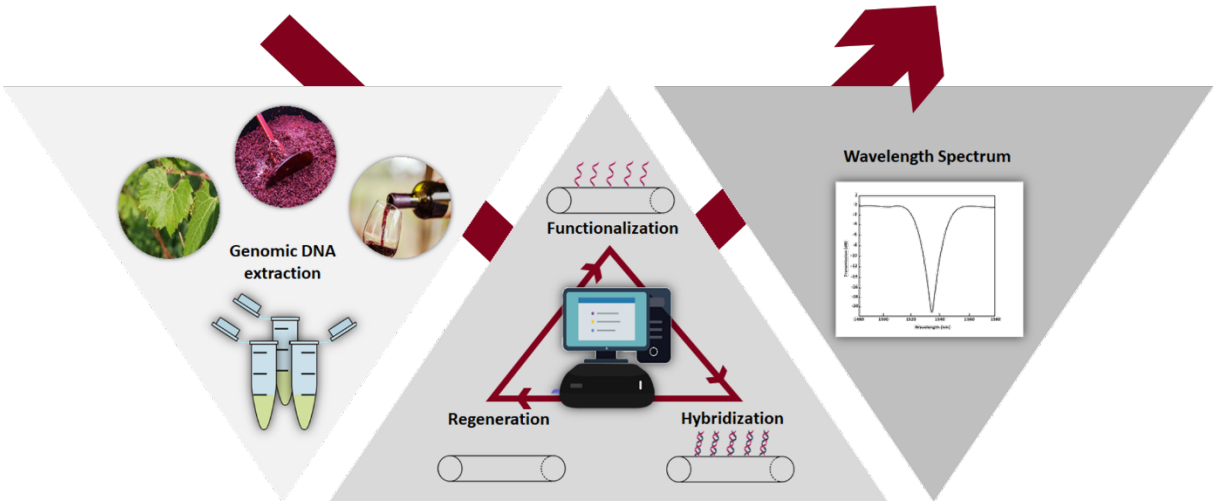

Figure 1. Overall methodology applied in our DNA-based optical biosensor.

Results

The results are represented in Figure 2 and have been divided in two assays. In Assay 1, the biosensor was able to detect the complementary sequence present in the Trincadeira (TA) DNA obtained from all the three sample matrices (Figure 2), with statistically significant wavelength shifts. When Malvasia Fina (MF) must and wine DNA samples were tested, the wavelength shifts obtained were slightly lower, but also statistically significant (Figure 2). These results are explained by the fact that MF is heterozygotic in the three SNP positions of the *F3H* sequence under study, and therefore it can hybridize with the probe, although in a less significant way, since it only has half the copy number in relation to the homozygotic grapevine varieties.
Concerning Assay 2, the biosensor was able to detect the complementary sequence present in the TA DNA in the must and wine DNA samples (Figure 2). The wavelength shift obtained after the insertion of Temperanillo (Aragonez - TR) must was also significant, which can be due to the fact that TR differs from TA only in one of the SNPs studied. Both partial hybridization or deposition of DNA in the biosensor optical fiber's surface can occur and explain the results obtained. On the other hand the results obtained from the TR and TA leaf DNA are not conclusive, since the wavelength shifts associated with both samples were not significant. Since more positive results were obtained with DNA extracted from processed

samples with a much higher complexity (must and wine), the observed results for the leaf DNA of both varieties can possibly be explained by an unsuccessful denaturation step, which is always applied before the DNA insertion in the biosensor, so ssDNA can be available to hybridize with the Probe.

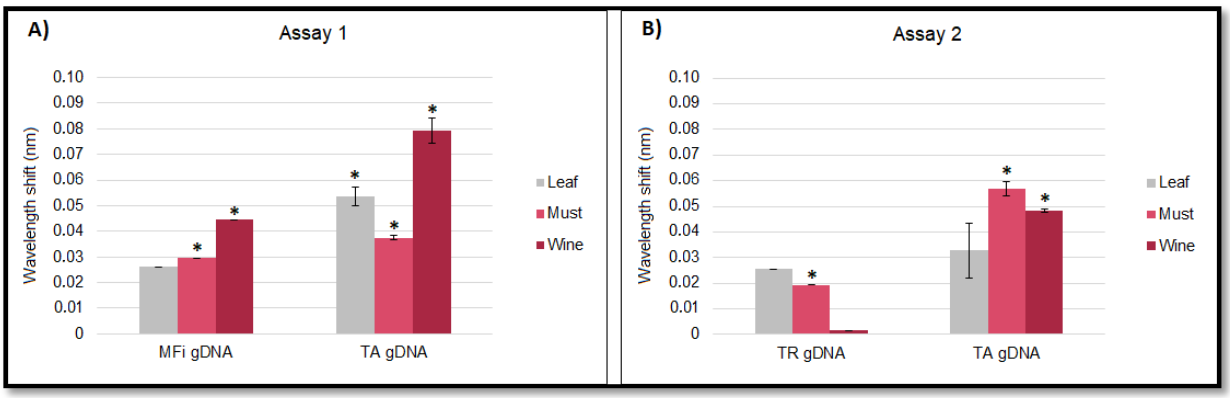

Figure 2. Representation of the biosensor response in the presence of A) Malvasia Fina – MF - gDNA (heterozygous in the three studied SNPs) and Trincadeira - TA DNA (complementary) in leaf, must and wine gDNA samples; B) Temperanillo (Aragonez) - TR gDNA (one mismatch), and TA DNA (complementary), in leaf, must and wine gDNA samples. Statistical analysis was performed regarding data from three replicates.
**Values statistically significant to the non-complementary target signal for a p-value < 0.05.*

Conclusion and Future Perspectives

The development of technology that allows to correctly fingerprinting grapevine varieties throughout the entire wine-chain is a hot research topic in the wine authenticity field. Our DNA-based optical biosensor showed a great potential to be applied for such purpose, since all three grapevine varieties were distinguished in all sample types, including DNA from processed samples with high complexity, such as the wine sample. Moreover, our method does not require costly processing, since it is not necessary to use purify the DNA or to label it in any way. Indeed this simpler optical fiber-based approach is more cost effective, when compared to the current options available in the market and with further testing and optimization it will be possible to turn this technology in a portable system for grapevine fingerprinting. Nonetheless it is necessary to pay a special attention to the probe design when dealing with heterozygous locus, in order to assure that the biosensor is able to precisely discriminate the grapevine varieties. The biosensor setup requires further testing to increase the desired effectiveness in the grapevine varietal discrimination, and to explore the quantification possibility.

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Abstract

The wine market is very competitive and subjected to fraudulent practices. Therefore, there is a need to develop methods to certify wine in order to assure that consumers' and producers' interests are indeed protected. One of the most common type of fraudulent practices is associated to grapevine varietal misidentification. Although several technological approaches have already been attempted, this is still a major issue in the wine industry. DNA-based technologies are still the most reliable, however, wine DNA is very hard to recover and most of the times it is very hard to use in PCR-based assays. Therefore, there is a need to seek for less costly alternative platforms that can be broadly used. In this work we developed a simple and low-cost method for DNA detection and quantification, based on the ssDNA (DNA-probe) immobilization in an optical fiber long period grating (LPG) and subsequent hybridization. The DNA is not labelled, and the hybridization is monitored *in situ* by following in real-time the optical sensor response. This strategy was applied using DNA extracted from leaf, must and wine samples using several *Vitis vinifera* L. varieties. The

adopted methodology is able to detect a difference of only one base pair between DNA sequences. The quality of extracted wine DNA does not seem to have a significant influence in the hybridization process, and therefore in the detection efficiency. This platform constitutes an innovative, simple and low-cost platform that can be broadly applied to grapevine varietal identification in wine samples.