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Onsite Single-Step Device for Early Detection of Infections and Drought Stress in Vineyards

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Summary:

Agriculture is an important part of the economy of most countries. In general, it is important to monitor and prevent biotic (infections) and abiotic stresses (droughts or excessive watering) that can significantly affect crop yield. For this reason, there is a need to develop a fast and low-cost platform that can be used in the field to monitor plant response to various types of stresses. For portability, microfluidics was the platform of choice for targeting two biomarkers: abscisic acid, related to plant ripening and to plant response to drought stress; and salicylic acid, related to the plant response to infection.

Keywords: microfluidics, biosensor, antibody, aptamer, capillarity

Introduction

Biotic (infections) and abiotic stresses (droughts and floods) greatly affect the yield in food and feed production. The incidence of these stress factors has become increasingly more frequent and damaging because of climate change. To prevent the loss of crops, it would be beneficial to develop a portable, low cost, user-friendly platform, that can be used in the field to perform tests for the early detection of changes in the vine, thus allowing for prompt and appropriate response. Currently, routine sampling of crops must be sent to central analytical laboratories which is time-consuming and costly.

A device based on microfluidics technology that can provide the needed portability and potential low cost due to the small volume of reagents. Also, because of the smaller distances for diffusion, the reaction times can be faster making it appropriate for regular on-site testing. Under stress conditions, the concentration of several phytohormone in the plant changes. In this paper, we demonstrate a microfluidic device that can monitor two such phytohormones: abscisic acid (ABA) for tolerance mechanisms against drought stress, and salicylic acid (SA) for infections. [1]

Methods and Results

ABA is detected using a competitive immunoassay (Fig.1(A)). The sample under analysis is mixed with a conjugated molecule composed of ABA and bovine serum albumin (BSA), labelled with Alexa430 (ABA-BSA-Alexa430).

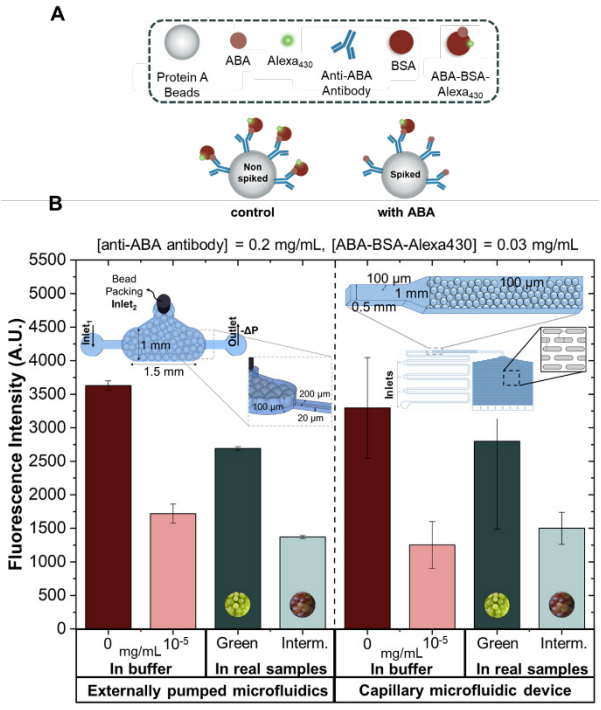

Fig. 1. Competitive Immunoassay for ABA detection: (A) Schematic representation of the assay for a spiked and non-spiked sample (B) Fluorescence response curve for different target ABA concentrations and for different stages of ripening, for the two different systems. The left side uses externally pumped microfluidics, and the right side uses a capillary microfluidic device. The error bars represent the ± standard deviation.

In the presence of ABA, the free analyte in solution will compete with the ABA-BSA-Alexa430 for the binding sites of the anti-ABA antibody, bound by the constant zone of the antibody to protein A microbeads, and therefore, the higher the concentration of free analyte, the lower the fluorescence signal. This method was developed using a microfluidic device, fabricated with polydimethylsiloxane (PDMS) using soft lithography techniques, using external syringe pumps. [1] Self-pumping microfluidic devices that rely on capillary forces to drive the fluids were also developed to increase ease of use and the portability. [2] Fig.1(B) shows the ABA detection both in buffer and in real grape samples picked during the *veraison*. In this stage of ripening, there is a surge in ABA which can be detected as a decrease in the fluorescence signal. As can be seen in Fig 1(B), the two different devices, one that uses an external pumping system, on the left, and the capillary device, on the right, are both capable of detecting ABA, and the results for both devices are identical.

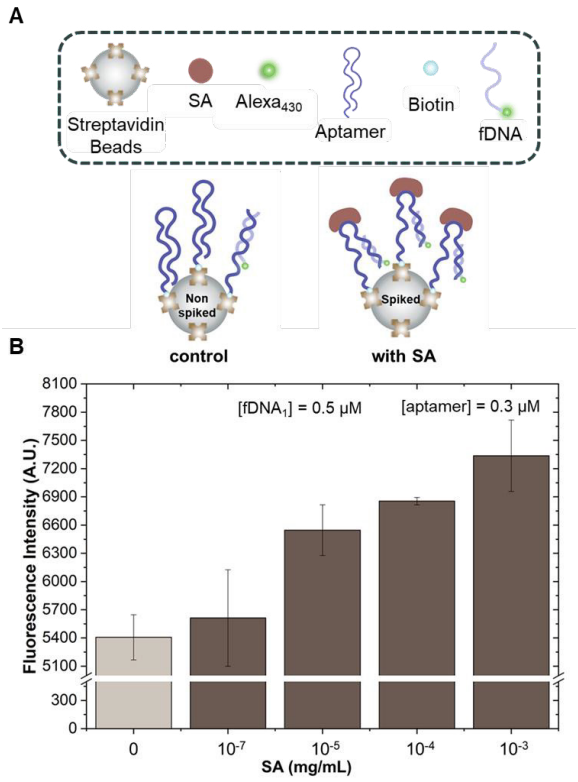

Fig. 2. Assay for SA detection in binding buffer: (A) Schematic representation of the aptamer assay for a spiked and non-spiked sample (B) Fluorescence response curve for different target SA concentrations. The error bars represent the ± standard deviation.

Additionally, an assay for SA detection using biotinylated SA aptamers immobilized on streptavidin beads as a capture agent was also developed (Fig.2(A)). A fluorescently labeled DNA strand complementary to the SA aptamer, binds to the aptamer leading to an increase in signal. In the presence of SA, the binding affinity increases leading to an increase in the fluorescence signal. Preliminary results also show the detection of SA in a microfluidic chip (Fig.2(B)). Using the SA detection method, it is possible to detect concentrations within the range of interest for infections, which is in the 10^{-6}-10^{-4} mg/mL range.

Due to the complexity of the cell matrix of the grapevines and grapes, sample pre-treatment is required before analysis. [1] Since the sample treatment should be performed in the field, the currently required centrifugation step must be replaced prior to in-field usage.

In our presentation, we will describe the microfabrication of the standard and capillary microfluidic chips, the protocols for the assays for detection of the phytohormones ABA and SA, the sample preparation protocols, and the validation of the assays with real grape samples.

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