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Chronoamperometry Measurement For Rapid Cucumber Mosaic Virus Detection In Plants

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Abstract

Cucumber mosaic virus (CMV) causes major losses to agricultural and horticultural crops around the world. Hence, a rapid assay for the detection of CMV which can be employed in both laboratory and field is essential. A portable electrochemical immunosensor system for the detection of CMV, based on immobilized CMV specific antibodies conjugated with gold nanoparticle was developed for this purpose. The conjugated antibodies were added with polymer and deposited onto carbon screen printed working electrodes. Optimization of the modified surface immunosensor was performed using sandwich immunoassay format (ELISA). The initial ELISA result for the standard curve development showed a limit of detection down to 0.1mg/mL. Subsequently, the immunosensor was tested for cross reactivity with other plant pathogens. The performance of the electrochemical immunosensor revealed that it has a high selectivity in sample matrix with other organism. This immunosensor provides a promising technology for simple and sensitive detection system that is essential in rapid detection of plant pathogens.

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1. Introduction

Cucumber mosaic virus (CMV) is a representative of Cucumovirus, which also includes *Tomato aspermy virus* (TAV) and *Peanut Stunt Virus* (PSV), with newly described *Gayfeather Mild Mottle Virus* (GMMV) as a reputed member (Adams et al. 2009). In contrast to TAV and PSV which have quite a restricted host range, CMV infects a large number of species. CMV, transmitted by several species of aphid, is known to infect more than 1000 species of plants in 365 genera of 85 families (Gallitelli 2000). This common plant virus caused yellow mottling, distorted leaves and stunted growth in a wide range of garden plants, not just cucumbers. CMV was also found to be the most common viruses infecting and damaging chili in Malaysia which results in the losses of yield ranges from 1 0%- 1 5% if infection came in late and may reach up to 60% if plants were infected at early stage. As a result, production of chili is insufficient to meet the local consumption and consequently, an approximately RM30 million worth of chili is imported annually.

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Traditional methods for CMV diagnostics are ELISA and RT-PCR. However, these methods are expensive and require highly trained laboratory staff. Immunosensors provide a promising alternative to currently used detection systems (Hassen et al. 2011). They are analytical devices consisting of antibodies or their fragments coupled to a transducer and they generate an analytical response related to analyte concentration in a sample (Sun et al. 2011).

In spite of its advantages, the ability of immunosensors to detect plant pathogens is still in its infancy in terms of sensor technology. An immunosensor which is high in specificity is necessary to guarantee timely accurate diagnosis of infections. Specificity of an immunosensor can be determined using cross reactivity tests and has been used extensively to determine the degree of similarity between antigens. It is also a commonly evaluated parameter for the validation of immune and protein binding based assays such as ELISA. CMV is known to cross react with tobacco mosaic virus (Regenmortel & Wechmar 1970) and Cowpea Chlorotic Mottle Virus (Smith et al. 2000).

In order to develop an immunosensor with high specificity and detectability, the variability of testing principles is really high considering both sensing surfaces and assay formats. Many attempts exist towards the method of sampling, storage and pre-incubation conditions that could potentially simplify the working protocol. To overcome the challenge of rapid detection of CMV, this work describes the development of a chronoamperometry antibody based immunoassay with nano-gold-polymer- specific antibody modified working carbon electrode for enhanced sensitivity.

2. Material and Methods

2.1 CMV isolate

CMV was propagated in cucumber plants, grown in a greenhouse at 20-25°C. The infected leaves with distinctive symptoms were collected and purified according to the methods as previously described by Mossop et al. (1976). Virus concentration was estimated by measuring the absorbance with a spectrophotometer at 260nm.

2.2 Antibody procedures

The polyclonal antibodies were produced by immunizing Zika white rabbits with an emulsion consisting of purified CMV in Phosphate Buffered Saline and Complete Freund's Adjuvant via subcutaneous injection. The rabbit was bled from the main artery of the ear of the rabbit. Blood samples were taken for antibody purification using a two-step procedure involving ammonium sulphate precipitation and column chromatography using protein A. Subsequently, IgG titers for the antibodies were determined by indirect ELISA method. The bleed which gave the highest titer was selected for conjugation with gold nano-particle. Next, the conjugated antibodies were added with polymer and electro-deposited on screen printed carbon working electrode (SPCE) using a potentiostat.

Meanwhile, the antibody-HRP which will act as the detector on the sensor surface was produced by conjugating antibodies with activated peroxidase. The antibody-HRP was later purified using affinity chromatography and the collected fractions were measured at OD_{280nm}.

2.3 Standard curve development

Purified CMV with concentrations ranging from 0.1 to 1.3 mg/mL was used for the development of CMV standard curve. This was followed with the addition of rabbit polyclonal antibody-HRP conjugate. The current measurements of the antibody-substrate reaction was then conducted using digital electrochemical system comprise of an electron transfer mediator, 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) with H_2O_2 as the substrate system. The change in current for the various CMV concentrations was observed with time at a constant current.

2.4 Cross reactivity study

The experiments were carried out using Tungro viruses (RTBV, RTSV), Papaya Ringspot Virus (PRSV), *Pyricularia oryzae* (Po), *Colletotrichum capsici* (Cc) and *Colletotrichum gloeosporioides* (Cg). The CMV antibody immobilized on SPCE was incubated with the above pathogens followed by washing with phosphate buffered saline with Tween 20 (PBS-T). Next, the SPCE was coated with bovine serum albumin and were washed again with PBS-T. Antibody-HRP was added to the surface of the SPCE prior to electrochemical measurements were performed. At least three

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