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CURRENT OPPORTUNITIES FOR LAB-ON-A-CHIP BASED NANO-DIAGNOSIS FOR SUGARCANE DISEASE

Raja muthuramalingam T., Nithya K., Rasappa Viswanathan*
Division of Crop Protection, ICAR-Sugarcane Breeding Institute, Coimbatore 641007.
Corresponding author: rasaviswanathan@yahoo.co.in

Sugarcane is one of the most important crops in the world, growing in tropical and subtropical conditions. The crop is economically important due to its industrial potential in terms of various products and byproducts. Since sugarcane is a long-term crop, diseases are the primary concern responsible for decreased yield with epidemic occurrences and some of the diseases predominantly spread through seedcanes. Almost all the diseases severely impact sugarcane yield, quality, and ratooning potential [1-3]. Like many plant diseases, sugarcane diseases also challenging to manage in the field. The timely diagnosis is a proven fact that served to minimize losses and other impacts in different crops. However, healthy seed canes and tissue culture raised plants are prominent solutions for the severe seed cane-transmitted diseases. Only a proper diagnostic can facilitate the availability of healthy seed canes and tissue cultured raised seeds. The standard diagnostics facilities are not much feasible for sugarcane farmers throughout the country. Apart from exorbitant laboratory-based diagnostic instruments, very few on-field techniques (Lateral flow assay and LAMP) hardly survived due to questionable sensitivity and specificity. Nevertheless, there is a massive demand for farmer-friendly simple diagnostics methods in the entire world.

Lab-on-Chip is a trending method that addresses the commoner concern over many advantages like very economical, fast and user-friendly then unbelievable sensitivity [4-6]. It is made of a small or micro-level diagnostic platform where it needs a minimal amount of samples to detect [6]. Elsewhere the techniques performed in simple paper-based microfluidics (also called Lab-on-Chip) platform which mostly semi-quantitative

and biodegradable when it comes to environmental concern. Lateral flow assay is one of the best examples of a paper-based (treated cellulose) microfluidics system. Meanwhile, most of the paper-based diagnostic methods are working with the colourimetric principle, since it is the only popular one to detect biomolecules by naked eye. Similarly the colourimetric detection, many kinds of paper based lab-on-chips are fabricated according to the requirement of the detection method and molecules. One of a kind we developed for the diagnosis of *Sugarcane mosaic virus* (SCMV) and *Sugarcane streak mosaic virus* (SCSMV) with gold nanoparticles (AuNPs) as a reporter/signaling agent. The detection methodology is similar to the ELISA but the experimentation is much better like detection time, reagent cost, sensitivity and storage validity. The gold nanoparticle (AuNPs) based colorimetric assay can be very efficient and sensitive especially for biomolecule identification. It is also clearly evident that recent rapid diagnostics assay for Covid-19 contained AuNPs as reporter molecules [7]. Like that, the gold nanoparticle-based colorimetric assay has been extensively used for the rapid detection of many pathogens. Gold nanoparticles exhibit extraordinary properties such as high surface to volume ratio, optical behavior, surface Plasmon resonance (SPR), fluorescence emission, photo-thermal effects, etc [8]. Gold nanoparticles could be synthesized using a reduction reagent, which can donate electrons to the Au ions to convert into nanosized particles. Sodium citrate and Sodium borohydride are the familiar reduction agent that used for gold nanoparticles synthesis. For example, 1mM of gold chloride (HAuCl4) solution at boiling condition added with 20-50mM of reducing agent turned the solution into the attractive deep red-coloured colloidal solution, which indicates the synthesis of gold nanoparticles. Different ratio of reagent concentration results in nanoparticles of varying sizes, shape and surface properties [9]. Nanoparticles sizes between 20 and100nm in spherical shape mostly utilized in biosensing applications. Optimization needed for the homogenous synthesis of particles that should confirm UV-Vis absorbance spectroscopy and transmission election microscopy. Mostly, gold nanoparticles are stable at room temperature for an extended period; when stored at 4ºC and dark, the stability period may increase up to one year. After synthesizing AuNPs, polyclonal antiserum (probes) specific to both sugarcane viruses conjugated with AuNPs by covalent/electrostatic bonding method. Chemicals like NHS/EDC frequently used to build strong bonding between antibody and nanoparticles. In this way, any detection probes of DNA, RNA, aptamers and proteins can be conjugated with nanoparticles [8]. Though, gold nanoparticles generally have a surface affinity to biomolecules which can be conjugated through simple incubation called passivation. Moreover, some specific chemical compounds containing amine ($-NH_2$), carboxyl (-COOH), and thiol (-SH) groups that synthesize AuNPs offer a bioconjugation site without any further surface chemistry modification. It also helps to improve the sensitivity and stability of the nanogold conjugated probe.

In the experiment, size designated, pre-treated, then pre-coated (capture probe) cellulose paper used as a diagnostic platform. Infected plant samples incubated with a pre-coated spot for an hour, then washed and blocked with 2%BSA in PBS. Further dipping the paper strip into colloidal nanogold-probe solution may allow the hybridization with viral antigens. After 5-10 min incubation at RT, presence of a red colour signal on the sample spot revealed the presence of viral particles. It's a simple qualitative test that requires a maximum of 25 min to complete. The assay can be further improved to semi-quantitative through the read of colour intensity produced on the spot. Also, we achieved high sensitive detection of low titer of virus particles followed by the silver enhancement method. Under silver enhancement solution (combination of silver chloride, hydroquinone, sucrose in citrate buffer), the signal level increased 3-fold, which helps in the low concentration of virus detection. It is an autocatalytic process where silver ions further reduced over the gold nanoparticle core, which increased the particle size ten times bigger than its original size [10]. In this way, we obtained the detection sensitivity almost equivalent to the PCR assay. The known virus concentration is likely to generate a standard intensity chart through captured image analysis [11]. Like silver enhancement, we plan to execute another sensitivity enhancement method by using the same gold nanoparticles as "Nanozymes". It is a recent invention by material scientists where nanoparticles reacted as enzymes on substrates (chromogenic substance) result in bright coloured signals [12].

The lab-on-chip method with colourimetric detection principle become an attractive venue for plant disease diagnosis. Metal nanoparticles like gold and silver nanoparticles are extensively used in recent paper-based diagnostics [8]. The antibody probe related detection system is much simplest way of detecting as compared to nucleic acid probe. But there is less availability of antisera for the emerging pathogens, and also the complication in the production of antisera made them a secondary choice to the researchers. Researchers are also finding an alternative to antibody probes such as nucleic acid aptamer, either DNA or RNA, which can detect viruses in samples underwent without any purification process. For example, the gold nanoparticle integrated RPA assays with RNA probe reported were able to detect viruses in unpurified samples [13]. The RPA assay also accommodated in the simple paper-based diagnostic platform. With this simple technical approach, any novel diagnostic methodology can be adapted or created to prevent severe impact by plant pathogens. Henceforth, there are many opportunities and research work are being continued further to reach the road of Lab-on-a-chip-based diagnosis for sugarcane diseases. Such an efficient diagnostics that works in the field condition would support supply of healthy planting materials to the farmers and sustain sugarcane productivity.

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