Journal of Applied Sciences Research, 9(11): 5568-5571, 2013
ISSN 1819-544X
This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLES

Comparative Study Between Elisa And Surface Plasmon Resonance (Spr) For Rice Tungro Disease Detection

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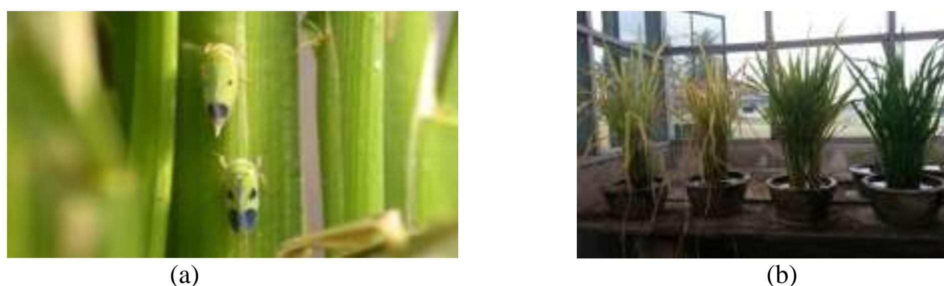
ABSTRACT

Rice tungro disease (tungro) is one of the most damaging diseases of rice in South and Southeast Asia. This disease is caused by dual infections from rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Both are transmitted by green leaf hoppers (GLH).This disease make serious loss of income to farmers when their crops are affected with an approximately 5-10% annual losses of rice yield in Asia was affected by rice tungro disease. This disease contributes to serious loss of income to farmers and also accounts for an approximately 5-10% annual loss of rice yield in Asia.Immunosensors based Surface Plasmon Resonance (SPR) and ELISA that used specific antigen-antibody reaction format have become a promising tool for the quantification of viruses. However both method need to compare which is to be used to determine the antigen with highly sensitive, specific, rapid, and label free detection for the analysis.

Key words: Rice tungro bacilliform virus (RTBV), rice tungro spherical virus (RTSV), Surface Plasmon Resonance (SPR), Enzyme linked immunosorbent assay (ELISA)

Introduction

Rice tungro disease (RTD) is the most severe viral disease. It hasbecome a major constraint in stable rice production in the South and Southeast Asia since mid-1960s where epidemics of the disease were recorded (Herdt, R.W., 1991). This disease is caused by dual infection of two viruses which is rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV) (Hibino, H., 1978). Both viruses are transmitted by *Nephotettixvirescens*and also known as the Green Leafhopper (GLH) as shown in fig 1(a).(Cabauatan, P.Q. and H. Hibino, 1985). Disease detection during the early stage of infection is important for preventing the reduction of yield in crop production and offering better solution to the farmers. An early detection of plant diseases can also help to cure the infected plants or delay the serious complications of the disease. (RTD) was selected asthe most destructive diseases of rice in South and Southeast Asia, where epidemics of the disease have occurred since the mid-1960s. Apart from that, both infected viruses will give symptoms such as stunting and yellow or orange yellow discoloration of the leaves, plant stunting and reduced in yield as shown on fig 1(b) (Azzam, O. and T.C.B. Chancellor, 2002).



(a) (b)

Fig. 1: (a) Vector of RTD is *Nephotettixvirescen* which acts as a transmitter of RTBV and RTSV (b) Rice Tungro Disease Symptoms in paddy.

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Detection of tungro disease at the field is generally carried out by visual observations of typical symptoms. However, the two types of virus particles are sometimes present independently of each other in some plants, causing widely different symptoms. Therefore, it is imperative to develop more specific detection techniques. Specific polyclonal antibody raised against the two viruses separately, can be used in a variety of immunodiagnostic techniques in order to detect the presence of RTBV or RTSV in infected leaf samples. Enzyme linked immunosorbent assay (ELISA) is a common example of the above technique. ELISA can be carried out using leaf extracts and measuring the level of activity of an easily assayable enzyme (alkaline phosphatase or horse radish peroxidase), linked to anti-RTSV or anti-RTBV. As a result, intensity of the color is proportional to the amount of the virus in the infected plant.Apart from that, others immunosensors technique that can be used to detect and measure the typical symptoms of the tungro disease based immunosensors is surface plasmon resonance (SPR). It is one of the most sensitive optical biosensor that being used widely for chemical and biological sensing characterizations [5]. One of the advantages of SPR over other techniques due to no reagent or label for detecting the target antigen is needed. Then, detection to target analyte can be easily achieved since the immobilized biological recognition element (bioreceptors) can be regenerated and reused for continuous or multiple detection. However, this paper will only focus on RTSV for further analysis using ELISA/ SPR.

Material and Method

In this experiment five samples were used for analysis. Four of these samples were Y1286 variety and were infected with RTSV. Three of these infected samples were obtained at random from station MARDI Seberang Perai and labeled as B1, B2, B8 and the fourth one from Horticulture Research Centre, Serdang. The fifth sample was healthy and was obtained from Rice and Industrial Crop Research Centre (RIC). All samples were processed and monitored under controlled environment that have been supervised by MARDI research officers.However, the fifthsample served as thecontrol. The anti-RTSV used in this experiment is from International Rice Research Centre (IRRI), Philippines. The concentration of the antibody used in both ELISA and SPR analyses is 10mg/ml**.**

Sample Preparation for ELISA / SPR:

First of all, the samples were weighed 1.0 g each. Then each sample was ground with liquid nitrogen using mortar and pestle. Next, 2 ml of extraction was added and mixed with the samples. The suspension was then transferred into universal bottle to be used for ELISA/SPR.

Enzyme-linked immunosorbent assay (ELISA):

A sandwich enzyme-linked immunosorbant assay (ELISA) format was developed to detect and screening RTSV from infected plant.The ELISA plate was coated with antibody of RTSV from IRRI (International Rice Research Institute) with amount of 100 µL. Next, it was then incubated 4hr at 37 ^{o}C. The plate was emptied and washed three times with PBS-tween. After that, 100 µL/well of blocking agent was added and the plate was incubated for 1hr. 0.05% bovine serum albumin (BSA) was used in PBS (0.01 M, pH7.4) which acts as blocking agent. After washing as above, the plate was dropped with 100 µL of extract leaf sample from infected leaf followed with incubated overnight at 4 ^{o}C. After that, the microplate titer was added with 100 µL/well of antibody with concentration 1mg/ml in PBS followed with incubated for 2hr at 37 ^{o}C. After washing as above, 100 µL/well of goat anti-rabbit IgG-AP antibody conjugate specific to the intermediate antibody at 1:1000 dilutions was added into microplate titer followed by incubation for 30 min at 37°C. After washing as above, substrate PNP was added with 100µL/well followed with incubation process for 5-15 min at 37 ^{o}C. Absorbance at 405 nm was read for this analysis.

Reactivity using Immunosensors based Surface Plasmon Resonance (SPR):

Clean gold SPR discs was used and immersed overnight in 1.0 mM solution of 11-MUA in ethanol. Afterwards discs were rinsed with 70% ethanol, followed by degas distilled water and gently dried using blower before placing in SPR chamber. Freshly 11-MUA-modified discs were placed in the SPR chamber and conditioned in running PBS buffer. Next, degas distilled water was injected on the activated disc surface to check for leakage between the two measurement channels. Then immobilization process started when freshly 11-MUA-modified discs was placed in the SPR chamber and conditioned in PBS buffer. After conditioning, the acid group of this molecule is activated by incubation with1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS).The thiol groups of 11-MUA was activated by treating with a mixture of 100 mM EDC and 100 mM NHS for each well. Subsequently, Anti-RTSV was injected on the activated disc

surface. The SPR angle shift was recorded after a disc was washed with PBS buffer. In the interaction phase, the healthy sample and infected sample was loaded to visualize the interaction between RTSV and anti-RTSV interaction.

Result and Discussions

The immunoassay format was used for analysis fivesamples which are to determine the sensitivity of method of ELISA and SPR analysis to screen the RTSV. During validation of both assays using the same control (healthy sample), the SPR was shown to be considerably more sensitive than the ELISA method even though both used same concentration of anti-RTSV (10mg/ml). The result of both ELISA and SPR analysis are shown in Fig. 2(a) and Fig. 2(b) respectively. As shown in Fig. 2(a), the absorbanceforthe four samples(B1, B2, B3 and infected sample) compared to the control (healthy sample) show no significant differences. At 405nm, the absorbance of the healthy sample is 0.130. The absorbencies of B1, B2, B8 and infected sample at 405nm are 0.148, 0.137, 0.135, and 0.145 correspondingly. In contrast, the result of SPR analysis shows significant differences between the control sample and the other four samples with the response values obtained for the four samples more than the value of the control sample. The response of the control sample is 62.095. Meanwhile the responses of B1, B2, B8 and infected sample are 286.725, 243.774, 158.537, and 128.891 respectively. Specific binding was measured in Response Units (RU) as a function of time following referencing with binding on the reference antibody surface.

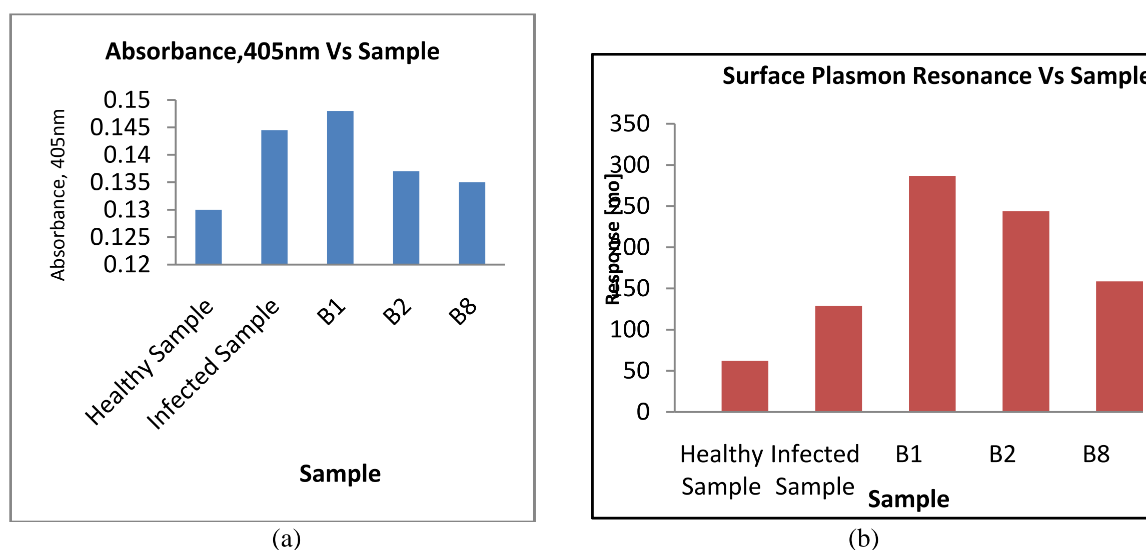
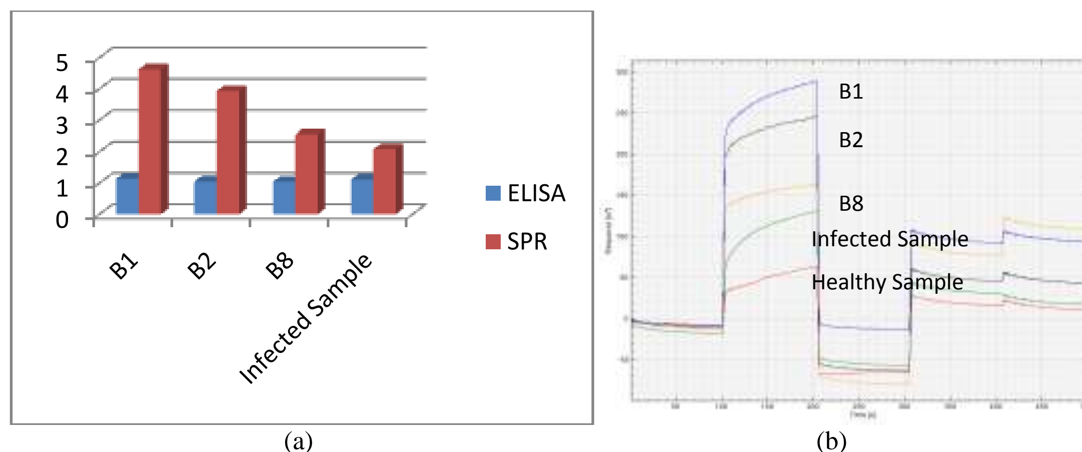


Fig. 2: (a) Determination of absorbance value of antibody using Enzyme Link Immunosorbent Assay (ELISA) (b) Response of the SPR measurement.

In addition to that, the SPR shown to tolerate higher measurement levels that ELISA method. As shown Fig.3 (a), the differential ratio for each sample from ELISA and SPR was determined. Therefore, based on result obtained the differential ratio using SPR and ELISA between B1, B2, B8 and infected plantwas 3.48, 2.88, 1.51 and 0.97.As a result the higher differential between both methods shown that SPR has higher sensitivity compare to ELISA method. Apart from that, the interaction between samples was shown on Fig 3 (b). Based on result, a set of 5 samples can be analyzed simultaneously in one time that includes capturing and full kinetic analysis on the sample. The interaction of each sample with same antibodies has shown the different response that correlated well with the immunoassay resultsfor differential between healthy and infected sample for this analysis.

Presented here is study conducted by Fernando et al on Recombinant viral proteins for use in diagnostic ELISAs to detect virus infection, the author claimed that ELISAs provide a valuable tool in the detection and diagnosis of virus infection. The ability to produce recombinant viral proteins will ensure that future ELISAs are safe, specific and rapid. This latter point being the most crucial advantage in that even if a virus cannot be cultured, provided gene sequence is available, it is possible to rapidly respond to emerging viruses and new viral strains of existing pathogens. Indeed, ELISAs based on peptides (corresponding to epitopes) also hold great promise, as in this case no cloning or expression of a recombinant protein is required. Both recombinant protein and peptide based systems lend themselves to large scale production and purification. He also claim that the approaches can also be used to distinguish recombinant vaccines from parental or wild type viruses which can easily be employed both human and plant alike. He ague that Recombinant proteins are phosphorylated in insect

cells and at least in the case of the corona virus infectious bronchitis virus (IBV) nucleocapsid protein the sites of phosphorylation are the same as in mammalian or equivalent cells . Obviously, protein expression in species-specific cells is optimal. However, typical protein yield can be substantially lower than either in *E. coli* or insect cells. This is probably reflected inthe fact that both *E. coli* and Sf9 cells can be grown easily in suspension culture whereas this can be problematic formammalian cells.



(a) (b)

Fig. 3: (a) The differential ratio between ELISA and SPR method(b) Interaction between healthy sample, infected sample and random sample.

Conclusion:

ELISA and SPR immunoassay formats were developed for the screening purpose .Both technique can be used for the initial identification and screening of RTSV in a sample. The various formats were compared on the basis of their analytical performance using the same assay components. The developed SPR immunoassay format is the best immunoassay format in terms of highest analytical sensitivity and widest detection range compared to ELISA to detect RTSV in a sample also we presented a brief review on recombinant viral proteins for use in diagnostic ELISAs to detect virus infection.

Acknowledgement

The authors wish to thank Malaysian Agricultural Research and Development Institute (MARDI),Institute of Nano electronic Engineering (INEE), University Malaysia Perlis, Universiti Putra Malaysia, for giving financial and technical support to conduct this research.

References

Azzam, O. and T.C.B. Chancellor, 2002. The biology, epidemiology, and management of rice tungro disease in Asia. Plant Dis., 86: 88-100.
Cabauatan, P.Q. and H. Hibino, 1985. Transmission of rice tungro bacilliform and spherical viruses by *Nephotettixoirescens*Distant.Philippine Phytopath, 21: 103-109.
Herdt, R.W., 1991. Research Priorities for Rice Biotechnology. In: G.S. Khush and G.H. Toenissen (Eds), RiceBiotechnology, Biotechnology in Agriculture No.6, pp. 19-54, IRRI, CAB International, UK.
Hibino, H., M. Roechan and S. Sudarisman, 1978. Association of two types of virus particles with PenyakitHabang(tungro disease) of rice in Indonesia. Phytopathology, 68: 1412-1416.
Homola, J., S.S. Yee, G. Gauglitz, 1999. Surface plasmon resonance sensor: review. Sens. Actuators, B54: 3-15.
Kelly-Anne Spencer, Fernando A. Osorio, Julian A. Hiscox, 2007. Recombinant viral proteins for use in diagnosticELISAs to detect virus infection, Vaccine, 25: 5653-5659.