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Article · March 2018

DOI: 10.1016/j.procbio.2018.02.019

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Production and purification of antibody by immunizing rabbit with rice tungro bacilliform and rice tungro spherical viruses

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ARTICLE INFO

Keywords:
Tungro disease
Rice tungro bacilliform virus
Rice tungro spherical virus
Antibody titer
Enzyme-linked immunosorbent assay

ABSTRACT

Rice tungro disease is the major disease caused by infection with the rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). In this study, New Zealand White rabbits were immunized with pure viruses for the production of antibodies against both species. The production of polyclonal antibodies against Tungro viral disease using ammonium sulfate precipitation and a protein A affinity column and their assessment are described. Two peaks were found from the protein A affinity column. Peak 1 represents the unbound compounds from the extracted serum and peak 2 represents antibody that bound to protein A, which was eluted using elution buffer. Peak 2 was collected for antibody titration. The amount of pure antibody in the titers was quantified by enzyme-linked immunosorbent assay (ELISA) to capture the tungro viruses. Antibody titer was analyzed by the ELISA method. For anti-RTBV, 1.696 mg/mL was highest at the second bleed and anti-RTSV was 2.3225 mg/mL was highest at the first bleed. These antibodies detected the tungro viral disease well and proved to be a potential probe for the detection of rice tungro disease.

1. Introduction

Plant diseases are a serious issue and significantly affect both the quality and quantity of agricultural products [1]. The infection of plants drastically reduces the yield [2] and causes a significant agronomic impact [3]. In addition, infection can cause catastrophes in large agricultural fields, which can lead to famine [4]. In this context, the tungro virus has caused major outbreaks and has caused serious production losses in various countries of South and Southeast Asia.

Generally, farmers detect tungro diseases by visual observation. However, it is quite difficult and not reliable to identify the symptoms through visual observation due to the difficulty in differentiating them from a non-pathogenic disorder such as excess water after a drought, nutritional deficiency, and insect injury, which have similar symptoms [5]. In addition, most farmers take a simple approach using pesticides to control and monitor tungro viral vectors. However, this approach is not effective, and it also affects the health of the operators [6]. Under these circumstances, it is imperative to develop a method to control against the independent or simultaneous occurrence of the two types of viral infections [rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV)] in order to prevent serious outbreaks. Detection of tungro disease requires a fast, simple and sensitive method compared to the current detection strategies, which are tedious and laborious.

The detection of analytes such as biological and chemical pathogens and contaminants plays an important role in the prevention of infection and disease [7]. It is important to develop a method with more specific detection. Recently, immunosensors that depend on antibody-antigen binding have been developed to substitute for traditional analytical techniques. Generally, immunological techniques require a purified antibody with low cost and good quality for long-term usage. Techniques for immunosensors are based on the combination of antibody and antigen specificity in solution or on a solid surface coupled with a transducer [8–11]. Antibodies are indispensable molecules for analyzing protein functions broadly in life sciences [12,13] and are particularly useful for immunohistochemistry, a technique that visualizes the specific tissue and cellular localization of proteins and other antigens. In this context, polyclonal antiserum is a primary source of antibodies due to its low cost and effectiveness in generating high-

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https://doi.org/10.1016/j.procbio.2018.02.019
Received 25 November 2017; Received in revised form 19 February 2018; Accepted 21 February 2018
Available online 02 March 2018
1359-5113/

performance applications [14,15].

There are several techniques for testing the quality, quantity and functional aspects of the pure antibody. Using the enzyme-linked immunosorbent assay (ELISA), the specific binding between an antibody and antigen can be measured using an enzyme-labeled antibody. Antibody titer is the endpoint dilution that exhibits a reaction between the antigen and the pre-immune serum [16,17]. In the current study, New Zealand White rabbits were immunized with two viruses (RTSV and RTBV) for the efficient production of antibodies in order to develop detection systems. The antibodies that were produced were purified and the titers of polyclonal antibody were assessed against the tungro viral disease.

2. Materials and methods

2.1. Materials

Rabbit anti-mouse IgG-conjugated horseradish peroxidase was obtained from Abcam Ltd. (UK). Tetramethylbenzidine (TMB) substrate and blocking milk solution were purchased from KPL Ltd. (London, UK). Bovine serum albumin (BSA), phosphate buffered saline, Freund's complete adjuvant and Freund's incomplete adjuvant were procured from Sigma-Aldrich Co Ltd. (Dorset, UK). A HiTrap protein G column was obtained from Pharmacia Ltd. (Sweden). EZ-link Plus Activated Peroxidase was obtained from Pierce Ltd. (UK). Maxisorb ELISA plates were procured from Nunc (Germany). Six-month-old New Zealand White rabbits were obtained from a local rabbit farm (LTK Sdn. Berhad, Malaysia). The rabbit facility equipped with automated feeding and drinking systems, was located at the Malaysian Agriculture Research Institute, Malaysia. Polyclonal antibody production was conducted at the Malaysian Agriculture Research Institute, Malaysia. Other disposable items used in this study were purchased from Fisher Ltd. (UK). The Malaysia Agriculture Research and Development Institute (MARDI) Ethics Committee approved the procedures performed with animals. We obtained permission from the above committee and they have accepted all the approvals and retain the records.

2.2. Source and collection of tungro viruses

In this analysis, MR81 and Y1286 varieties were used as plants for infection by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Both seeds were planted in 5 soil-filled pots, uniformly watered and fertilized to enhance seed growth. A plastic (Mylar) cage was used 20 days after planting. The upper portion of the cage was covered with a cotton ball after introducing 5 insect vectors (green leafhopper). *Nephotettix virescens* were introduced into each cage for 24–48 hours to initiate the inoculation. To maintain their health and expand their lifespan, the vectors were transferred to cultivar TN1. After 50–60 days of incubation and infection, infected rice plants exhibited rice tungro disease symptoms and were ready to use for the isolation and purification of rice tungro viruses.

The virus was purified according to a previous method by Omura et al. [18] with modifications. The artificially infected plant (100 g) was homogenized in 0.1 M sodium citrate, 0.01 M EDTA and 5% Celluclast using a heavy blender. Then, the sample was filtered through 2 layers of muslin cloth. The filtrate was added to a conical flask and shaken in an incubated shaker at 30 °C for 3 h. Afterwards, the sample was filtered again using 2 layers of muslin cloth, and then again, the filtrate was added to a conical flask and shaken in an incubated shaker at 40 °C for 3 h. Then, the sample was centrifuged at 19,000*g* for 15 min (Beckman Coulter Avanti J-26 XP1) with J-14 rotors, and the supernatant was collected. The supernatant was mixed with 7% polyethylene glycol (PEG8000), 0.2 M NaCl, 1% Triton X-100 at room temperature; after 1 h the mixture was centrifuged at 23,000*g* for 3 h at 4 °C. The pellet was resuspended in 5 mL of a mixture containing 0.1 M sodium citrate and 0.01 M EDTA. The mixture was stored overnight at −20 °C. Later, the supernatant was centrifuged at 15,000*g* for 15 min at 4 °C. The supernatant was then layered onto a 10% sucrose cushion and was centrifuged at 68,000*g* for 2.5 hours at 4 °C (Beckman Coulter Optima XPN-100 Ultracentrifuge) with a swing-out rotor. The pellet was collected and was resuspended in 0.5 mL of 0.1 M sodium citrate and 0.01 M EDTA. Finally, the suspension was centrifuged for 11,000*g* for 10 min at 4 °C (Rotina 420, Hettich).

2.3. Immunization procedure

Immunization of rabbits with the purified virus was achieved by the modification of a protocol described by Omura et al. [18]. Two different rabbits were used for immunization with RTBV or RTSV. The first and second injections were performed with 250 μg/mL and 500 μg/mL, respectively. Both injections were given intravenously for the first and second week after pre-immune blood collection. Subsequent injections were given subcutaneously with an emulsion consisting of 1.0 mg/mL in 1 mL of Phosphate Buffered Saline (PBS) and an equal volume of incomplete Freund's adjuvant (IFA). The injections were repeated one month after the third injection, substituting IFA with complete adjuvant (CFA). All injections with both viruses consisted of a virus suspension with an optical density of 1.0 at A_{260}. Afterwards, the rabbit was bled for antibody determination 1 week after the last injection.

2.4. Antibody purification

Antisera against Tungro viruses (RTBV/RTSV) were diluted in distilled water (1:10 v/v) and then precipitated using 80% saturated ammonium sulfate while stirring to obtain serum proteins. The serum mixture was centrifuged (5810 R, Eppendorf, Appendix A7) at 20,000*g* for 15 minutes at 4 °C. The pellet was resuspended in 0.01 M PBS, dialyzed three times and then passed through a protein G HiTrap column connected to an AKTA purifier (Pharmacia Ltd., Sweden) described in the following section. Fractions yielding the highest absorbance at OD_{280nm} were collected (IgG Stock). Then, IgG titers for anti-RTBV/RTSV were determined by indirect ELISA.

2.5. Fractionation of IgG using a protein G column

The purification steps were performed with the protein G column using AKTA-Prime plus following the manufacturer's instructions (Pharmacia). This instrument is a compact one-step lab-scale protein purification system combined with Prime View 5.0 software. First, the partially pure IgG sample (5 mL) was filtered (using a 0.45 μm filter) to eliminate the cell debris. The conditions for the IgG sample were adjusted to the ionic strength of the binding buffer by dialyzing against 0.02 M Phosphate (pH 7.0). The protein G column (5 mL) was first equilibrated with 5 column volumes of binding buffer before loading the IgG sample. After loading, the column was washed thoroughly with 5–10 column volumes of the binding buffer to eliminate unbound materials, and this process was continued until there was no protein present in the eluent (determined by UV absorbance at 280 nm). The IgG sample was eluted with 5 column volumes of elution buffer (0.1 M glycine-HCl, pH 2.7) at a flow rate of 2 mL/min, and then the column was re-equilibrated with binding buffer (5–10 column volumes) to stabilize the binding of protein G to the ligand. The IgG fractions were immediately neutralized with neutralization buffer (100 μL of 1 M Tris-HCl/mL of fraction, pH 9.0) to adjust the appropriate final pH of the IgG.

2.6. Indirect ELISA for the determination of antibody titer

The pure antibody was examined by the indirect ELISA method to determine the antibody titer using a microplate reader (Versamax). A higher antibody titer indicated a better quality of fractionated antibody. To perform the indirect ELISA, the surface microtiter plates were

initially treated with 100 µL of antigen (purified RTBV/RTSV) and incubated overnight at 4 °C. The plate was washed three times with TPBS (PBS with 0.05% Tween 20, 200 µL/well) using a microtiter washer. The unbound sites on the microtiter well surface were blocked by treating with 1% bovine serum albumin (BSA) diluted in PBS (250 µL/well) for 30 min at 37 °C. The plate was washed three times with TPBS. Next, different amounts of purified antibodies (0.2, 0.04, 0.008, 0.0016, 0.00032, 0.000064, 0.0000128, and 0.00000256 mg/mL) in a volume of 100 µL were added and the plate was incubated for another 2 h at 37 °C. After washing the plate three times with TPBS, a secondary antibody solution (Goat anti-rabbit IgG, 100 µL) was added to the plate. Then, the plate was incubated for 30 min at 37 °C and washed three times with TPBS. Finally, the TMB substrate solution was added to the plate (100 µL/well) and the reaction was measured at 405 nm. The intensity of the color was proportional to the amount of antibody present.

2.7. Protein Assay

The antibody samples were correlated with a protein assay using BSA as a standard. The BSA standard was prepared by serial dilution starting from a stock standard solution (3 mg/mL, 0.003 g of BSA diluted with 1 mL PBS). The serial dilution consisted of 3, 2.5, 2, 1.5, 1, 0.5, 0.25 and 0.1 mg/mL. The protein determination was conducted on a titer plate. The samples from the purified antibodies were analyzed, including pre-immune through bleed 4. An amount of 25 µL from the sample was added to the microtiter plates. Then, 200 µL of the substrate was added at the appropriate ratio (9 Reagent A:1 Reagent B) into the well. All samples including antibody samples and BSA standards were pipetted into separate wells on the microtiter plate. The sample and reagent were mixed thoroughly using the microplate shaker and incubated for 30 min at 37 °C in the dark. Next, the assay was read at 560 nm using the microplate reader. All assays were performed in triplicate. The protein standard curve was plotted against absorbance at 560 nm. The concentration of purified antibody from all bleeds was determined from the standard curve.

2.8. Antibody sensitivity performance with positive and negative samples

The sensitivity of antibody performance was determined with test samples, which consisted of purified virus and infected and healthy plant samples. In this experiment, bleed 1 from RTSV and Bleed 2 from RTBV were chosen because they exhibited the highest antibody titer as determined from several dilution assays. In addition, a fixed sample concentration of 200 µg/mL for the purified virus and 0.5 g/mL for the infected and healthy samples was chosen and placed onto the microplate for the analysis. All analyses were done using the indirect ELISA method. Microtiter plates were coated first with 100 µL from purified RTBV/RTSV, and infected and healthy leaves were incubated overnight at 4 °C. The plate was washed three times with TPBS and the unoccupied sites on the microtiter well surface were blocked with 1% BSA (diluted in PBS, 250 µL/well) and incubated for 30 min at 37 °C. The plate was then emptied and washed three times with TPBS. Next, the purified antibodies, which consisted of anti-RTBV/RTSV (0.2 mg/mL, 100 µL) were added and the plate was incubated for another 2 h at 37 °C. The plate was then washed three times with TPBS and the secondary antibody goat anti-rabbit IgG solution was added (100 µL) to the plate. Then, the plate was incubated for 30 min at 37 °C and washed three times with TPBS. Finally, the TMB substrate solution was added to the plate (100 µL/well), and the reactions were measured at 405 nm. The color intensity of the solution was proportional to the amount of antibody in the solution.

3. Results & discussion

Rice is considered the most vital food crop throughout the world. Food security as an essential national policy is an objective for overall progress, and food security is highly associated with rice security. Most countries are willing to support programs focused on rice because rice is a staple of most populations [19,20]. In the major rice-growing countries, outbreaks of rice disease remain the primary threat to sustainable rice production. As one of the most destructive diseases in paddy plantations, tungro disease has been recognized as a nutritional disorder that is widely distributed in South and South East Asia [21].

In the case of tungro disease, the symptoms are often detected late, which makes it difficult to take any further action [6]. In addition, this disease has been considered a nutritional disorder of rice since 1950. At this time, the control of tungro disease remains a challenge, although many studies have been conducted to eradicate and prevent its outbreak in rice fields [22]. As a result, a monitoring system is essential to provide more rapid detection and to prevent a serious outbreak. Due to the extreme importance of securing the production of rice, it is necessary to develop a simple and effective tool for tungro disease detection. In this study, we attempted to produce pure polyclonal antibodies capable of detecting rice RTBV and RTSV with the ultimate aim of rice crop protection.

3.1. Antibody production

Initially, before obtaining a high amount of pure IgG, the purified RTBV/RTSV were injected into New Zealand White rabbits with a mixture of Freund's adjuvant, an inexpensive strategy for the production of polyclonal antibodies. Freund's adjuvant is an oil-based paraffin and has been utilized for triggering the immune system to generate high antibody titers. This adjuvant not only activates the immune system, it also enables the antigen to be released gradually into the injection point [23].

Purification of specific antibodies against the virus was performed by two primary steps. First, serum containing antibody was precipitated with saturated ammonium sulfate. The partially purified antibody was then passed through a protein A affinity column to obtain a pure IgG antibody against RTBV/RTSV. Based on the studies of Page and Thorpe [24], the best and most effective techniques for a high yield of pure IgG can be achieved by using protein A or G chromatography, as they are efficient in aseptic purification strategies.

The graphs representing the chromatograms of IgG fractions eluted from the protein A affinity column for RTSV and RTBV displayed two peaks (Figs. 1 and 2). Peak 1 showed that the antibody was successfully bound to the protein A column matrix, whereas peak 2 showed that the bound antibody had been successfully eluted. This finding suggested that the elution buffer used in this experiment was appropriate for purifying the antibodies. The purified antibody fractions were collected from the fractions that exhibited the highest absorbance at a wavelength of 280 nm. The IgG fractions (from peak 2) for both chromatograms displayed the highest absorbance and were collected. The fractions collected for RTBV were fractions 23–29 and for RTSV were fractions 26–30. All collected fractions were stored at −20 °C for future use.

3.2. Optimal antibody activity determination using an ELISA for RTBV/RTSV

The activity of the produced polyclonal antibody against the antigen was tested by an indirect ELISA protocol using a serial dilution of virus. This is important for monitoring the progress of the immune response of the rabbits and confirms that they were sufficiently immunized. The results in Figs. 3 and 4 were obtained from different concentrations of anti-RTSV/anti-RTBV (0–0.2 mg/mL) by five serial dilutions immobilized with a constant virus concentration (200 µg/mL). Both figures show the complete pattern of antibody levels in rabbits within three months of the immunization schedule.

Different absorbance readings in each micro-well plate were plotted. Control experiments using the purified pre-immune serum for

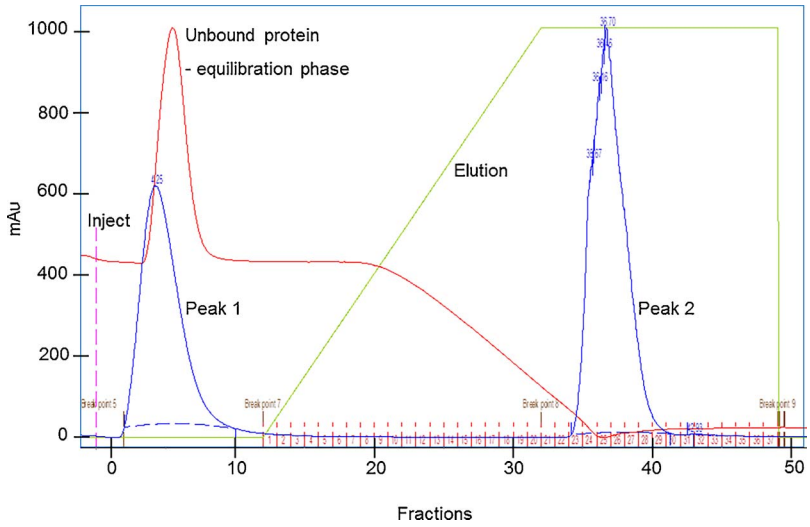

Fig. 1. Chromatogram profile of anti-RTBV IgG eluted from the protein A affinity column and AKTA purifier system. During elution, two major peaks (1 & 2) were obtained. The unbound elution is displayed.

both antibodies showed non-significant binding levels. Based on the optimal antibody activity in the range of 0 to 0.2 mg/mL, anti-RTSV/anti-RTBV displayed a high absorbance and differential reading, which was achieved against the pre-immune serum. It is evident that a significant binding level occurs due to the increase in boosting injections from 250 µg/mL to 1.0 mg/mL into rabbit.

Based on the optimal antibody activity of RTSV and RTBV, bleed 1 and bleed 2 were selected due to their high activity compared to the subsequent bleeds. However, the optimal patterns of antibody activity were quite different from the study conducted by Noraini & Azura [15], in which a higher activity of antibodies was achieved with a longer immunization period. Consequently, the last bleed with more specific IgG produced the highest level of antibody in their study and was thus chosen for analysis. In addition, the results also indicate that the optimal concentration of anti-RTBV/anti-RTSV for the specific binding of antibody is in the range of 0.2 to 0.000064 mg/mL; therefore, the smallest value (0.000064 mg/mL) that still produces optimal binding ability can be chosen for economic reasons and to ensure that the cost of the test will be competitive. However, in the following experiments on microplates with both polyclonal antibodies, a concentration of 1 mg/mL was used to clearly assess the results.

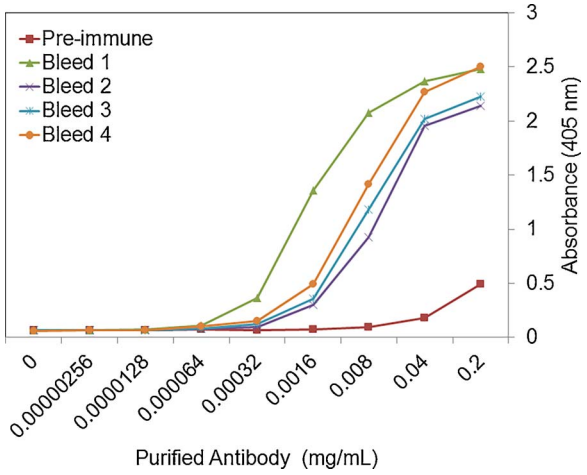

Fig. 3. Antibody titers from the concentrations of 0 to 0.2 mg/mL against RTSV with the samples of pre-immune bleeds from 1 to 4. Absorbance was measured at 405 nm.

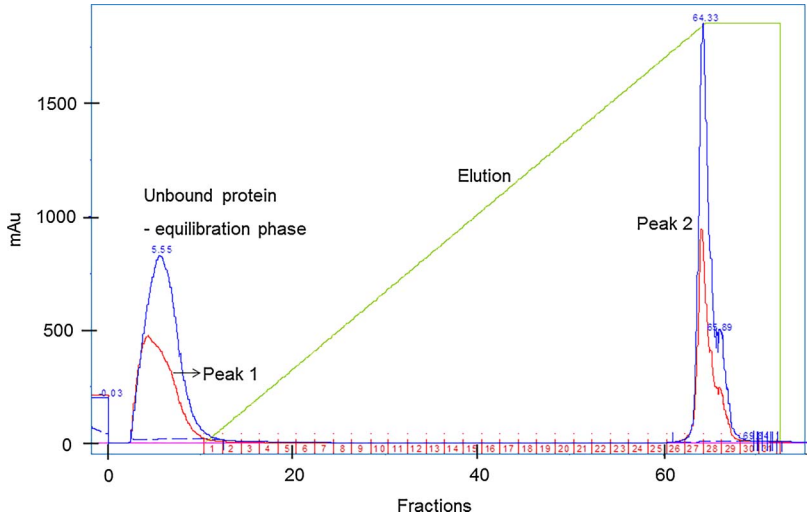

Fig. 2. Chromatogram profile of anti-RTSV IgG eluted from the protein A affinity column and AKTA purifier system. During elution, two major peaks (1 & 2) were obtained. The unbound elution is displayed.

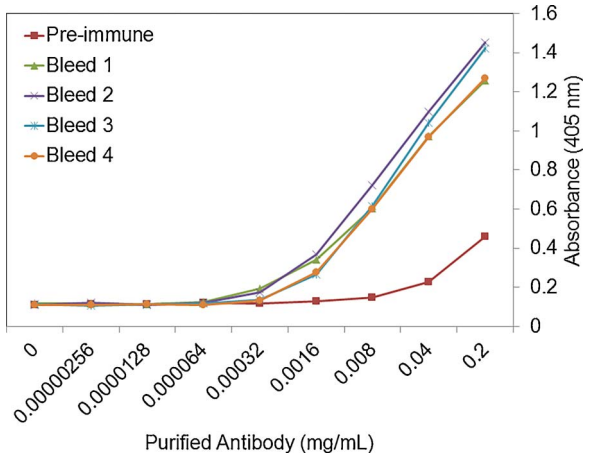

Fig. 4. Antibody titers from the concentrations of 0 to 0.2 mg/mL against RTBV with the samples of pre-immune bleeds from 1 to 4. Absorbance was measured at 405 nm.

3.3. Protein levels in antibody production

The concentration of antibody was determined using the bicinchoninic acid (BCA) assay method. Based on the titer determination, the activity obtained from antibody bleeds 1 for RTSV and bleed 2 for RTBV showed the highest reading. Therefore, this antibody preparation was used for further study. Based on this result (Fig. 5a), RTBV showed the highest absorbance at bleed 1 compared to other bleeds. However, this result contradicts the antibody activity determination, where the highest antibody activity occurred at bleed 2. In this case, despite its slightly lower protein content, bleed 2 was chosen for antibody assessment due to its higher antibody activity. Probably a small amount of antibody used in the analysis had a stronger antibody activity, and hence it has the advantage of long-term usage.

In addition, RTSV showed the highest absorbance at bleed 1 (Fig. 5b). This result has similarities with the results obtained for optimal antibody activity. The amount of antibody from bleed 2 (RTBV) and bleed 1 (RTSV), based on the protein standard curve, was 1.696 mg/mL and 2.3225 mg/mL, respectively. All the results were interpreted from the standard curve. A linear graph of absorbance against different concentrations of protein was obtained from 0 to 3 mg/mL with a regression value of 0.9983.

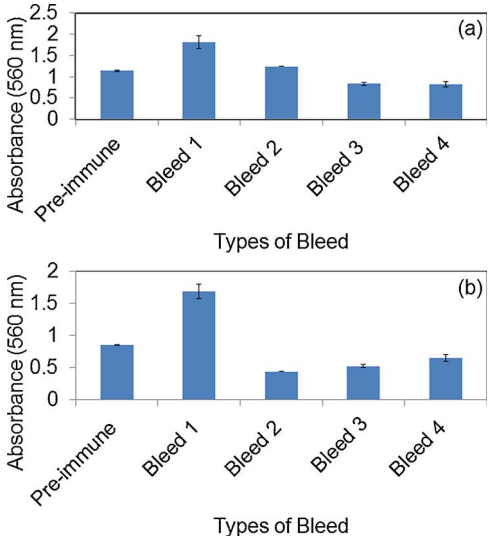

Fig. 5. Protein absorbance maxima at 560 nm for the samples of pre-immune bleeds from 1 to 4. The BSA standard curve for the determination of protein content of antibody used the BCA method. (a) RTBV and (b) RTSV.

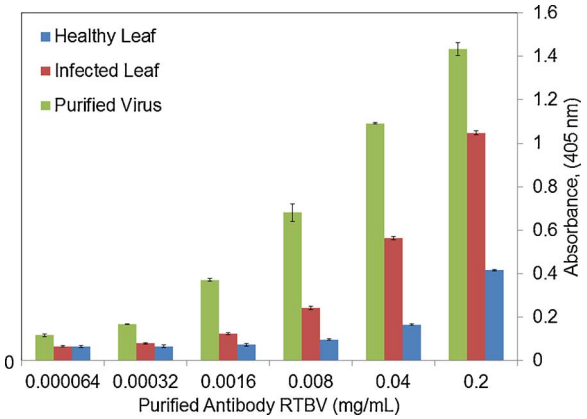

Fig. 6. Sensitivity performance of pure anti-RTBV from the concentrations of 0.064 to 200 µg/mL against the purified virus from healthy and infected leaves. Absorbance was measured at 405 nm.

3.4. Antibody sensitivity performance with positive and negative samples

An ELISA using the indirect method was developed to compare the sensitivity of the performance limit for anti-RTBV/anti-RTSV analysis. The experiment was based on different samples that reacted with the antibody for healthy leaf, infected leaf, and purified virus. The optimal performance limit was investigated using several dilutions from the highest titer of bleed 2 for anti-RTBV and bleed 1 for anti-RTSV against different samples.

The performance activities of anti-RTBV and anti-RTSV (Figs. 6 and 7) decreased with increasing dilution factors. The increment in dilution factors may reduce the interference or prevent the specific binding of both antibodies and the respective antigens. According to the results, the absorbance values of samples from healthy and infected leaves were significantly different at wavelengths of 405 nm. At an anti-RTBV concentration of 0.2 mg/mL, absorbance values for the healthy leaf, infected leaf and purified virus were 1.434, 1.048 and 0.416, respectively, which were significantly higher than the absorbance of other concentrations. However, at the same concentration (0.2 mg/mL), the performance sensitivity of anti-RTBV was similar in pattern to the performance sensitivities of anti-RTSV, which were 0.778, 1.761 and 2.482 for healthy leaf, infected leaf, and purified virus, respectively. At 0.000064 mg/mL (lowest antibody concentration) both antibodies showed a similar trend of performance sensitivity due to a low affinity with specific binding. The generation of antibodies, especially against the pathogens as shown in the present study, can be used for several downstream applications. Currently, antibodies against pathogens are widely accepted in biosensing applications [25–30], serving as

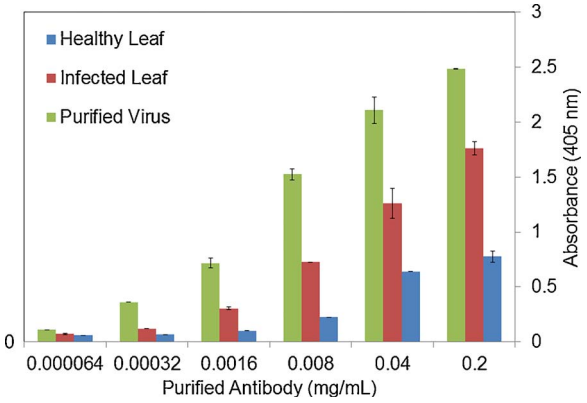

Fig. 7. Sensitivity performance of pure anti-RTSV from the concentrations of 0.064 to 200 µg/mL against the purified virus from healthy and infected leaves. Absorbance was measured at 405 nm.

potential probes for developing point-of-care devices for medical diagnosis.

4. Conclusion

In this research, a polyclonal antibody produced against RTSV and RTBV was successfully purified by a protein A affinity column. Optimal antibody activities for RTSV and RTBV from bleed 1 and bleed 2 were determined and compared to the subsequent bleeds. A polyclonal antibody produced against RTSV/RTBV in this research has potential to be used as a probe for the detection of tungro diseases with an optimal and economical concentration of antibody of 1.696 mg/mL (RTBV) and 2.3225 mg/mL (RTSV). The current study revealed additional information on the antibodies and tested different bleeds. The optimal antibody fraction was demonstrated with infected plant materials. The main contribution of this research is the development of an antibody for an early warning of tungro disease infection. Another significant contribution is its use for industrial applications, particularly in the agricultural sector, to sustain rice production and prevent economic losses worldwide. Furthermore, novel plant varieties that are resistant to pathogens can be generated.

Acknowledgments

Authors express their thanks to the Malaysia Agriculture Research and Development Institute, Universiti Putra Malaysia, and Universiti Malaysia Perlis for providing financial and technical support to conduct this research. We acknowledge the support from Research grant IPB (Vot No. 9442500).

References

[1] S. Sankaran, A. Mishra, R. Ehsani, C. Davis, A review of advanced techniques for detecting plant diseases, Comput. Electron. Agric. 72 (2010) 1–13, http://dx.doi.org/10.1016/j.compag.2010.02.007.
[2] A. Meunkaewjinda, P. Kumsawat, K. Attakitmongcol, A. Srikaew, Grape leaf disease detection from color imagery using hybrid intelligent system, 2008 5th Int. Conf. Electr. Eng. Comput. Telecommun. Inf. Technol. vol. 1, (2008) 56–59, http://dx.doi.org/10.1109/ECTICON.2008.4600483.
[3] M.M. López, E. Bertolini, A. Olmos, P. Caruso, M.T. Gorris, P. Llop, et al., Innovative tools for detection of plant pathogenic viruses and bacteria, Int. Microbiol. 6 (2003) 233–243, http://dx.doi.org/10.1007/s10123-003-0143-y.
[4] S. Arivazhagan, R.N. Shebiah, S. Ananthi, S.V. Varthini, Detection of unhealthy region of plant leaves and classification of plant leaf diseases using texture features, Agric. Eng. Int. CIGR J. 15 (2013) 211–217.
[5] P.D. Nath, L. Kenyon, V.I. Bartolome, G. McLaren, O. Azzam, Simple serological assays for detecting rice tungro viruses, Food Agric. Immunol. 12 (2000) 139–151, http://dx.doi.org/10.1080/095401000404085.
[6] S. Mohd Daud, H.J. Jozani, F. Arab, A review on predicting outbreak of tungro disease in rice fields based on epidemiological and biophysical factors, Int. J. Innov. Manag. Technol. 4 (2013) 447–450, http://dx.doi.org/10.7763/IJIMT.2013.V4.439.
[7] F. Ricci, G. Adornetto, G. Palleschi, A review of experimental aspects of electrochemical immunosensors, Electrochim. Acta. 84 (2012) 74–83, http://dx.doi.org/10.1016/j.electacta.2012.06.033.
[8] S.C.B. Gopinath, K. Awazu, M. Fujimaki, K. Shimizu, Evaluation of Anti-A/Udorn/307/1972 antibody specificity to influenza viruses using waveguide mode sensor, PLoS One 8 (2013) e81396.
[9] S.C.B. Gopinath, T.H. Tang, Y. Chen, M. Citartan, T. Lakshmipriya, Current aspects in immunosensors, Biosens. Bioelectron. 57 (2014) 292–302.
[10] M.N.A. Uda, C.M. Hasfalina, S. Faridah, S. Noraini, U. Hashim, A.B. Ariffin, et al., Comparative Study Between Elisa And Surface Plasmon Resonance (Spr) For Rice 9 (2013), pp. 5568–5571.
[11] M.N.A. Uda, T. Adam, C.M. Hasfalina, S. Faridah, I. Zamri, U. Hashim, et al., Reviewed Immunosensor Format Using Nanomaterial for Tungro Virus Detection 832 (2014), pp. 410–414, http://dx.doi.org/10.4028/www.scientific.net/AMR.832.410.
[12] Y. Poogodi, G.L. Suresh, N. Gopinath, S.C.B. Chang, T. Inoue, S. Inoue, Dynamic change of NCAM polysialylation on human neuroblastoma (IMR-32) and rat pheochromocytoma (PC-12) cells during growth and differentiation, J. Biol. Chem. 277 (2002) 28200–28211.
[13] S.C.B. Gopinath, P.K.R. Kumar, Biomolecular discrimination analysis by surface plasmon resonance, Analyst 139 (2014) 2678–2682.
[14] L. Naik, K. Lata, R. Sharma, B. Mann, Y.S. Rajput, Production of polyclonal antibody for oxytetracycline and their use in lateral flow assay, J. Microbiol. Immunol. Biotechnol. 1 (2014) 8–17.
[15] S. Noraini, N. Azura, Production of polyclonal antibody against tetracycline using KLH as a carrier protein 66 (2011), pp. 61–66.
[16] S. Faridah, N. Azura, R. Hazana, G. A.R, Z. Norzaili, A. Azima, et al., Electrochemical sensors for detection of tetracycline antibiotics Unbound free tetracycline and tetracycline conjugates were removed during the washing step Direct competitive ELISA method Carbon working electrode was connected to the electrochemical ayse, Malaysia Soc. Anim. Prod. 15 (2012) 67–80.
[17] M.N.A. Uda, C.M. Hasfalina, A.A. Samsuzanaa, S. Faridah, I. Zamri, B.S. Noraini, et al., immunosensor development formatting for tungro disease detection using nano-gold antibody particles application 20057 (2017), p. 20057, http://dx.doi.org/10.1063/1.4975290.
[18] T. Omura, Y. Saito, T. Usugi, H. Hibino, Purification and serology of rice tungro spherical and rice tungro bacilliform viruses, Japanese J. Phytopathol. 49 (1983) 73–76, http://dx.doi.org/10.3186/jjphytopath.49.73.
[19] A.K. Tyagi, A. Mohanty, Rice transformation for crop improvement and functional genomics, Plant Sci. 158 (2000) 1–18, http://dx.doi.org/10.1016/S0168-9452(00)00325-3.
[20] B.P.M. Swamy, A. Kumar, Genomics-based precision breeding approaches to improve drought tolerance in rice, Biotechnol. Adv. 31 (2013) 1308–1318, http://dx.doi.org/10.1016/j.biotechadv.2013.05.004.
[21] M.N.A. Uda, C.M. Hasfalina, A.A. Samsuzana, S. Faridah, I. Zamri, B.S. Noraini, et al., Comparison study of two different isolation and purification method for rice tungro bacilliform virus (RTBV), Agric. Agric. Sci. Procedia 2 (2014) 107–112, http://dx.doi.org/10.1016/j.aaspro.2014.11.016.
[22] Q. Yao, Z. Guan, Y. Zhou, J. Tang, Y. Hu, B. Yang, APplication of support vector machine for detecting rice diseases using shape and color texture features, Int. Conf. Eng. Comput. (2009) 79–83, http://dx.doi.org/10.1109/ICEC.2009.73.
[23] D.L. Trott, E.M. Hellesrad, M. Yang, M.E. Cook, Additions of killed whole cell bacteria preparations to freund complete adjuvant alter laying hen antibody response to soluble protein antigen, Poult. Sci. 91 (2008) 912–917.
[24] M. Page, R. Thorpe, IgG Purification in Immunochemical Protocols, John D. Pound, Humana Press, 2008, pp. 95–111.
[25] R. Adzhri, M.F. Md Arshad, A.R. Ruslinda, S.C.B. Gopinath, M. Fathil, C. Ibau, M.N. Nuzaihan, Enhanced sensitivity mediated ambipolar conduction with p-type tio2 anatase transducer for biomarker capturing, Sens. Actuators A Phys. 259 (2017) 57–67.
[26] R. Fatin, A.R. Ruslinda, U. Hashim, M.K. Md Arshad, R.M. Ayub, S.C.B. Gopinath, HIV-1 Tat biosensor: current development and trends for early detection strategies, Biosens. Bioelectron. 78 (2016) 358–366.
[27] T. Lakshmipriya, S.C.B. Gopinath, T.H. Tang, Biotin-streptavidin competition mediates sensitive detection of biomolecules in enzyme-linked immunosorbent assay, PloS One 11 (2015) e0151153.
[28] K. Nomura, S.C.B. Gopinath, T. Lakshmipriya, N. Fukuda, X. Wang, M. Fujimaki, An angular fluidic channel for prism-free surface-plasmon-assisted fluorescence capturing, Nature Commun. 4 (2013) 2855.
[29] S.C.B. Gopinath, K. Awazu, M. Fujimaki, K. Shimizu, Neu5Acα2, 6 Gal and Neu5Acα2, 3 Gal receptor specificities on influenza viruses determined by a waveguide-mode sensor, Acta Biomater. 9 (2013) 5080–5087.
[30] S.C.B. Gopinath, K. Awazu, M. Fujimaki, Detection of influenza viruses by a waveguide-mode sensor, Anal. Methods 2 (2010) 1880–1884.