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Rice Tungro Disease in Sarawak: Past and Present Status

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ABSTRACT
Rice Tungro Disease (RTD) is one of the viral diseases which devastated rice production in South and Southeast Asia. Due to the severity of the RTD, close monitoring of this disease is important to ensure the disease does not become widespread and could have a disastrous effect to the paddy fields and farmers. In Sarawak, RTD survey is adopted as a continuous effort to monitor rice cultivars. Five divisions (Kuching, Samarahan, Sarikei, Sibu and Miri) in Sarawak were surveyed and were reported to be positive for RTD in 2012. For the following years in 2013 and 2014, RTD-free scenario was observed for all the eleven divisions in Sarawak except for Bario, Miri division. Besides, our PCR results have aligned with the field observation that the symptoms of RTD are no longer observed in those RTD negative sampling area. The effort of RTD surveillance will be continued for the following years to ensure close monitoring of the disease development and minimization of crop lose by taking appropriate eradication actions.

Key words: Paddy, rice, bario, viral disease, surveillance, PCR

INTRODUCTION

Rice (*Oryza sativa* L.) has been the staple food for humans in many countries. Over half of the world's population depends on rice (Bhullar and Gruissem, 2013) as an energy source for daily lives. In Sarawak, rice production is at risk for the attack of destructive Rice Tungro Disease (RTD) and study to look into the progression of the disease need to be conducted. The control of this cancerous disease could be difficult especially during a disease outbreak. This is because intensive planting of rice greatly increases the rate of virus transmission and development of disease outbreak (Hibino, 1983, 1996). This makes rice tungro disease a deadly disease affecting economics, rice yield and farmer income (Herdt, 1988).

The occurrence of RTD is one of the most damaging diseases to rice production in South and Southeast Asia (Jefferson and Chancellor, 2002). The RTD is a disease caused by simultaneous infection of two viruses, Rice Tungro Bacilliform Virus (RTBV) and rice tungro spherical virus (RTSV) whereby both viruses are transmitted by green leafhoppers (*Nephotettix virescens*), GLH (Hull, 1996). The RTBV has a circular, double-stranded DNA genome which is encapsulated in a bacilliform particle (Fauquet *et al*., 2005) and belongs to the family Caulimoviridae (Fauquet *et al*., 2005). The RTSV has a single-stranded polyadenylated plus-sense RNA genome encapsulated in polyhedral particles (Choi, 2008) and belongs to the family Sequiviridae (Fauquet *et al*., 2005). Rice cultivars infected with RTD show typical symptoms such as severe arrested growth, yellowing of the leaves and reduced tillering (Jefferson and Chancellor, 2002). In addition, rice cultivars infected with RTBV alone exhibit noticeable arrested growth and yellowing of the leaves (Hibino *et al*., 1990). However, if infected with RTSV alone, rice cultivars would demonstrate slight retarded growth or no obvious symptoms at all (Hibino *et al*., 1990). The damage is severe particularly infection takes place at early growth stage (Jefferson and Chancellor, 2002). For a susceptible variety, RTD may infect the entire field and bringing total yield loss under favourable condition (BRRI., 1983).

In the report by Yee and Eng (2012), RTD incursion in Sarawak was first discovered in 2012. The disease was discovered from the sampling carried out in paddy fields in five divisions (Kuching, Samarahan, Sarikei, Sibu and Miri) in

Sarawak. The specific locations were Lundu (two cases), Bario (three cases), Pa'lungan (one case), Serian (one case), Selangau (one case), Bintangor (two cases), Durin (one case) and Bawang Assan (two cases). The outcomes also indicated that the indigenous rice cultivars planted in these areas are susceptible to RTD. They pointed out that although the disease symptoms in the field were not obvious; molecular detection by Polymerase Chain Reaction (PCR) method has confirmed the presence of the disease. They further elaborated that the outbreak has shown certain disease distribution pattern. The first disease distribution encompassed the southern area where it was near to the border of Kalimantan (where RTD exists) and the second disease distribution was observed to be occurred along the trunk road connecting all the divisions in Sarawak. The scenario is made possible with the migration of GLH over distances by wind and also human factors. The distribution pattern could be useful for designing preventive strategy to minimize further occurrence of RTD epidemics in other areas.

The main objective of this study is to monitor the severity of RTD epidemics in Sarawak since its first report in 2012. Due to the severity effect of the RTD, close monitoring of the disease is important to ensure the disease does not become widespread and could have a disastrous effect to the paddy fields and farmers. Other than that, forecast of the disease outbreak could be made as well. Thus, RTD survey becomes a continuous effort for Agriculture Research Centre Sarawak.

MATERIALS AND METHODS

Plant materials: Survey on RTD was conducted during the main planting season in all the different divisions in Sarawak in the final quarter 2013 until August, 2014. The areas reported with history of positive RTD cases were revisited for sampling. Besides, new paddy fields were sampled as well. Leaf samples with symptoms of RTD were labelled, cut into fine pieces and kept dry in containers containing silica gel.

DNA extraction: For RTBV analysis, leaf samples collected were subjected to total genomic DNA extraction using modified CTAB method (Doyle and Doyle, 1990). Briefly, leaf tissues were pulverized using liquid nitrogen prior the addition of 1 mL of CTAB extraction buffer per 0.2 g of tissue powder and was incubated at 65°C for 30 min. The homogenate was added with equal volume of CIA (24:1) and was centrifuged at 12,000 rpm for 10 min. The upper aqueous layer was recovered with 2/3 volume of cold isopropanol at (-20°C) and the mixture was centrifuged at 12,000 rpm for 10 min. The DNA pellet was then washed with 1 mL of 70% ethanol, dried and re-suspended in 50 µL of deionized water.

RNA Extraction and reverse transcription: For RTSV, RNA was extracted using TRIzol® (Life technologies, USA) reagent. Briefly, leaf tissues were pulverized using liquid nitrogen prior the addition of 1 mL of extraction buffer per 0.2 g of tissue powder and was incubated at room temperature for 10 min. The homogenate was added with equal volume of CIA (24:1) and was centrifuged at 12,000 rpm for 10 min. The upper aqueous layer was recovered with 2/3 volume of cold isopropanol at (-20°C) and the mixture was centrifuged at 12,000 rpm for 10 min. The DNA pellet was then washed with 1 mL of 70% ethanol, dried and re-suspended in 50 µL of deionized water. The RNA extracted was converted into cDNA using Fermentas™ RevertAid Reverse Transcriptase (Life technologies, USA) according to the manufacturer's instructions.

RTBV and RTSV PCR analysis: The PCR conditions and thermal cycling parameters were tested for optimal amplification. The PCR mixture were composed of 1X PCR buffer (Promega, USA); 1.5 mM $MgCl_2$ (Promega, USA); 0.2 mM dNTPs (Promega, USA); 25 pmol forward and reverse primer, 1 unit *Taq* DNA polymerase (Promega, USA) and 100 ng DNA template.

Primers for PCR amplification were synthesized by Integrated DNA Technologies (Singapore). Primers were as follows: sense, 5'-AGAAATGGTATCAGAGCGATGTTC-3' and antisense, 5'-TCCTTAGGTCTAGCTTGTTGT-3' for RTBV; sense, 5'-GATTTTGGAAGAAGCCTATCGTGTT-3' and antisense, 5'-GATCTGCTTGGCGCCCACTGCCAAA-3' for RTSV. The PCR reaction mixture were subjected to initial denaturing at 94°C for 2 min followed by 30 sec of denaturing at 94°C, 30 sec of annealing at 27°C and 30 sec of elongation at 72°C and 5 min of final elongation at 72°C, with total of 35 cycles. The PCR products were electrophoresed on a 1.5% agarose gels. All the PCR reactions were done in triplicate. Negative control, positive control, no template control were included to rule out false positive or false negative result. The PCR product was sent to First BASE Laboratories (Malaysia) for DNA sequencing in order to confirm the identity of the amplified product.

RESULTS

The PCR results for some of the samples collected and tested were shown in Fig. 1. The state of infection by the viruses was shown by the absence or presence of the specific PCR products. As shown in Fig. 1, PCR band observed at about 1000 bp (gel picture on the right) and DNA sequencing result demonstrated samples tested positive for RTSV. As for RTBV, PCR products observed at about 500 bp (gel picture on the left) and DNA sequencing result confirmed the presence of RTBV in the sample tested.

Table 1 and 2 showed the RTD results for samples collected from different divisions in the year 2013 and year 2014, respectively. Although RTD was observed during the main planting season in 2013, resampling for some of these

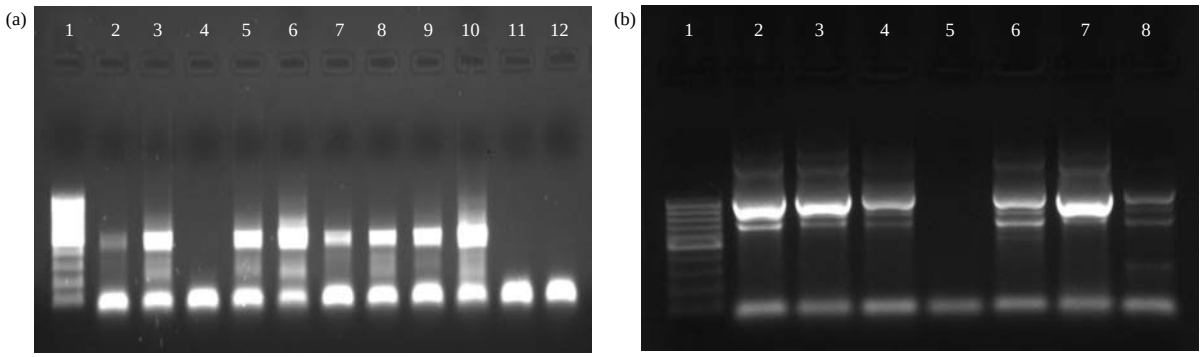

Fig. 1(a-b): RTD PCR amplification results of some of the samples collected from Bario, (a): PCR analysis for RTBV, distinct PCR bands observed in Lane 2-10 (except Lane 4) at approximately 500 bp confirmed the presence of RTBV. Lane 1: 100 bp Marker, Lane 2-9 (Field Owner), 2: Leju, 3: Parir, Libut 4: Peter Matu, 5: Sina Uding, 6: Balan Radu, 7: Johny Kapong, 8: Giak Bala, 9: Hendrick Ibuh and Lane 10: Positive control, Lane 11: Negative control, Lane 12: No template control, (b): PCR analysis for RTSV, bright bands observed in Lane 2-8 (except Lane 5) at approximately 1000 bp confirmed the presence of RTSV. Lane 1: 100 bp Marker, Lane 2-8 (Field owner): 2: Leju, 3: Parir Libut, 4: Sina Uding, 5: Balan Radu, 6: Johny Kapong, 7: Giak Bala and 8: Hendrick Ibuh

Table 1: RTBV PCR detection from leaf samples collected from paddy fields from different divisions in Sarawak in 2013

Division	No. of field survey	RTD symptoms observed	RTBV positive	RTSV positive
Miri (Bario)	24	7	11	ND
Miri (Pa'lungan)	3	3	2	ND
Sibu (Bawang Assan)	7	1	0	ND
Samarahan (Tebedu)	2	0	0	ND
Sri Aman (Tg. Bijat)	10	2	0	ND

ND: Not done

Table 2: RTBV and RTSV PCR detection from leaf samples collected from paddy fields from different divisions in Sarawak from January, 2014 to August, 2014

Division	No. of field survey	RTD symptoms observed	RTBV positive	RTSV positive
Bintangor	2	0	0	ND
Sibu (Bawang Assan)	1	0	0	ND
Betong (Lubok Nibong)	8	0	0	ND
Samarahan (Asajaya)	3	0	0	ND
Kuching (Lundu)	10	0	0	ND
Sri Aman (Tg. Bijat)	16	0	0	ND
Miri (Bario)	11	11	10	10

ND: Not done

places with RTD history was not carried out. This was due to flooding that hampered our sampling activity. Nonetheless, resampling in the areas with RTD history would be conducted during the coming planting season for the year 2015, including places which have been reported to be free from RTD.

DISCUSSION

The RTD disease surveillance in Sarawak has been a continuous effort by Agriculture Research Centre, Department of Agriculture Sarawak since the year 2012 when it was first reported. This disease is caused by a complex of two viruses which differ in terms of structure and genetic composition. In the infected paddy field, physical observation alone is not reliable because other biotic and abiotic factors would demonstrate similar symptoms. Therefore, detection methods using the PCR technique is one of the preferred choices due to its accuracy and sensitivity in virus detection. According to Takahashi *et al*. (1993), PCR technique was 10^4 times better sensitivity than was enzyme-linked immunosorbent assay (ELISA). As mentioned, RTD is a disease due to joint infection of two different viruses, namely RTBV and RTSV (Hull, 1996). The RTSV functions as a helper virus in this case and RTBV cannot be transmitted in its absence (Latif *et al*., 2013). The viral complex of both viruses causes the devastated outcome when infection happens (Hibino, 1983). As reported by Hibino *et al*. (1978), two types of virus particle were found in rice plants affected by tungro disease in Indonesia. Plants showing severe symptoms had both types, but those with moderate symptoms had only B particles. Thus, RTBV analysis was first conducted followed by RTSV in this study.

There were a number of major outbreaks of tungro in Asia which have destroyed more than 4000 hectares of rice field recorded for the past 20 years. These major outbreaks were: Malaysia (20,365 ha) (Chen and Othman, 1991), Indonesia (71,000 ha) (Manwan *et al*., 1985, 1987; Daradjat *et al*., 1999) and India (260,000 ha) (Hibino, 1987; Ramasamy and Jatileksono, 1996).

As mentioned earlier, there were places reported to be RTD positive in the year 2012. Those places were Lundu (two cases), Bario Asal (three cases), Pa' lungan (one case), Serian (one case), Selangau (one case), Bintangor (two cases), Durin (one case) and Bawang Assan (two cases). For the year 2013 and 2014, different scenario was observed for all of the divisions in Sarawak except for Bario and Pa'lungan in Miri division (Table 1 and 2). The places with the history of RTD

were sampled again but this time negative results were observed. This implies that RTBV virus was no longer present in these previously infected fields. Our PCR analyses exactly coincide with the report made by farmers. As stated by farmers, the symptoms of RTD were no longer observed. One of the possible explanations that the RTD was no longer present in the areas other than Bario Asal and Pa'lungan was because the paddy planting activity in these areas were less intensive as compared to Bario Asal and Pa'lungan which practiced staggered planting. Thus the chance of spreading disease is reduced.

The recurrence incidence of tungro disease in Bario could be explained from the history of tungro disease occurrence in other part of the world. Large-scale tungro disease outbreak was recorded in India, Thailand, Indonesia, Malaysia and Philippines from the 1960s to the mid-1970s (Jefferson and Chancellor, 2002). According to Swaminathan (1984), the outbreak was due to an increased in the population of the leafhoppers which spread the tungro viruses. They explained the huge increased in the area planted with modern high-yielding and early-maturing rice varieties that delivered significant increases in rice grain yield has provided a conducive environment for the development of the vector. Moreover, the short latent period of the virus combined with fast acquisition by the vector have causing rapid spread of the disease (Ishii-Eiteman and Power, 1997).

In Bario, ratoon crops were not removed during the off-season. Insect net sweeps were set up and high populations of the green leafhoppers, the vectors of RTD were caught. These ratoon crops act as a continuous source of inoculum for the green leafhoppers to transmit viruses. For Pa'Lungan, which is geographically near to Bario Asal, would be at high risk of exposure to RTD followed by the infection.

CONCLUSION

During the main planting season for the year 2013 until August 2014, field survey was carried out as an effort to monitor RTD in Sarawak. The results of field survey have revealed the number of RTD cases detected in that given timeframe. On top of that, it helps in our understanding of the RTD situation in Sarawak which could ease the disease control when it is necessary. Other than enhancing the understanding of the situation, it also helps to improve management strategies for RTD in Sarawak. Thus, RTD surveillance must be continued so that the disease can be closely monitored and appropriate action can be taken to ensure that crop loss is minimized.

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