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NEW YELLOWING SYNDROME CAUSED BY RICE TRANSITORY YELLOWING VIRUS ON RICE IN SOME AREAS OF MEKONG DELTA

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ABSTRACT

During winter-spring and summer crops 2011, a yellow syndrome on rice was found in some areas in Mekong Delta. Samples were collected and different experiments were done following Koch postulate. Results including symptomatology, inoculation, detection by RT-PCR and transmission electron microscopy and analysis of sequences obtained from PCR products were all supporting the conclusion of the new yellowing syndrome is caused by *Rice transitory yellowing virus*, RTYV (synonym: *Rice yellow stunt virus*, RYSV) belonging to the genus Nucleorhabdovirus, the family Rhabdoviridae and green leafhopper is the vector insect.

Keywords: Rice, RTYV, RT-PCR, green leafhopper, Mekong delta.

I. INTRODUCTION

Rice transitory yellowing virus (RTYV) was first reported in Taiwan in 1965, in Thailand and Japan in 1986 while at about the same period another disease called rice yellow stunt reported in some provinces of Southern China caused by Rice yellow stunt virus (RYSV). Both diseases are vectored by green leafhopper (GLH). The complete genomic sequence of RTYV was deposited in GenBank in 2003 with the accession number AB011257 (Huang et al., 2003). Hiraguri and co-authors sequenced the complete genomic sequence of RYSV (accession number: AB516283) and proved that RTYV and

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RYSV are just the two isolates of the same virus (Hiraguri et al., 2010) and we use the name RYSV in this paper.

In Vietnam, the yellow syndrome on rice had been found in some mountainous provinces in the North during 50th and again in early 80th years. The symptoms on infected rice plants were similar to the description by Taiwan scientists and hence called 'vang-lui' meaning 'yellow stunt' or 'vang-la-di-dong' meaning 'transitory yellowing'. Inoculation experiments using different rice hoppers revealed that GLH was the vector transmission. Virus particles of bullet-shape with about the same size of RYSV described previously was shown under the transmission electron microscopy at the National Institute of Hygiene and Immunology (NIHE) in 1985 (Trung, 1985).

Summer crops 2009 - 2010, a never described yellow syndrome had seriously damage rice production throughout Hiep-Hoa district of Bac-Giang province, especially in 2009 and 2010. RYSV was defined to be the causal factor and green leafhopper is the effective vector insect. The virus was found randomly at very low incidence in summer crops 2011.

Winter-spring and summer crops 2011, a new yellowing syndrome on rice was observed in some areas in Mekong delta. Farmers were confused these symptoms with the 'yellow stunt' disease caused by RGSV and RRSV out breaking in recent years and therefore their attempt to control the disease was not effectively. This paper describes steps following Koch postulate to reveal that this new yellowing syndrome was caused by RYSV and GLH is the vector insect.

II. METHODOLOGY

Field survey was done in Tien Giang and Long An provinces at the end of Winter-Spring and early Summer crop 2011 following standard methods of PPRI.

Pictures of typical symptoms at different stage of rice plant development were taken and compared with previous description. Leaf samples were treated as drying method for further detection by one-step RT-PCR (Anh et al., 2009) using specific primers. The obtained sequence of PCR products was compared with GenBank sequences (BLAST: *http://blast.ncbi.nlm.nih.gov*) and analyzed by MEGA-5. The virus particles were revealed using Jeol Jem 1010 with transmission electro-microscopy (TEM) method at NIHE, Vietnam.

The inoculation experiments were done at greenhouse of PPRI. Two sources of GLH (*Nephotettix virescent*) and brown plant hopper (BPH: *Nilaparvata lugens*) were used: Healthy insects rearing in greenhouse and insects collected in field. Healthy insects were put in an insect-proof cage containing infected rice plant collected in field with typical symptoms for virus acquisition during 10 days before group transmission (10 insects/10 rice x 24h) using healthy rice (var. Taichung-native-1, TN1). The same transmissions were done using insects (adult) collected in diseased field. Rice seedling after inoculation was then transplanted into other insect-proof cages for further observation of symptom development. Insect groups after inoculation were preserved separately in absolute alcohol for further detection by RT-PCR.

Some infected rice plants after inoculation and the corresponding insects used for the inoculation were then tested by one-step RT-PCR and sequencing following the same procedure to confirm the causal pathogenic factor.

III. RESULTS AND DISCUSSION

3.1. Description of new symptom observed on rice plant

Observation in an area at Thai Binh Trung Co., Vinh Hung Dist., Long An Prov., different fields of about the same seedling date within 15th to 25th March 2011 were all suffered from a migrating brown planthopper (BPH) at about 15 - 20 days after seedling (DAS). However, all field which were sprayed hoppercides immediately at the time of migrating BPH were heavily infected and much heavier than fields sprayed about 2 weeks after the migration of BPH. This at least means that BPH is not the vector of this yellowing syndrome. Field survey has revealed that the incidence of this yellowing syndrome were very much high in some areas in Long An but randomized at low level in other provinces (table 1).

Table 1. Incidences of the new yellowing syndrome in Mekong delta, April 2011

Province	LONG AN			TIEN GIANG		DONG THAP		HAU GIANG	
District	Vinh Hung			Chau Thanh (2 fields in Long Dinh com.)		OM 50404	OM 50404	OM 4218	OM 4218
Rice cultivar	VD 20	OM 4900	OM 50404	OM 50404	OM 50404	OM 50404	OM 50404	OM 4218	OM 4218
Total number of observed plants	180	647	190	2,217	2,088	1,259	1,211	482	825
Symptomized plants	177	581	182	156	214	198	65	52	86
Diseased incidence (%)	98.3	89.8	95.8	7.04	10.25	15.7	5.4	10.8	10.4

Note: + = fgr gen; Y = Aromatic; N = non-aromatic

3.2. Causal factors

3.2.1. Symptoms in field

Symptoms in the infected fields in Tien Giang and Long An were very much similar to the RYSV infected plant described previously and very much similar to what observed in Bac Giang province in Summer crops 2009 and 2010 (Figure 1). Symptoms appeared commonly by the end of tillering until flowering stage. Infected plants are mildly stunting, reducing number of tiller. Infected leaves are yellow or yellow mottle to orange and usually opening outwards while the leaf collars are stayed closer to each other. Symptomized leaves are shown upwards from lower leaves. These symptoms are somehow similar to of RYSV and tungro disease (Hibino, 1996). However, the yellowing symptom appears upwards from lower leaves is typical for RTYV but not tungro disease.

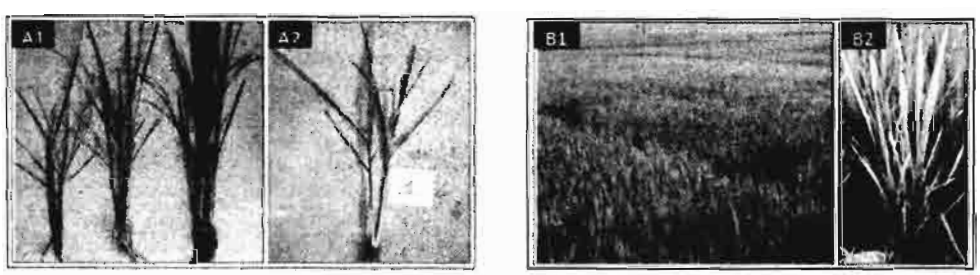

Figure 1. Typical symptoms of infected plants in Long An Summer crop 2011(A1, A2) and typical symptoms observed in Hiep Hoa district of Bac Giang province Summer crop (B1, B2).

3.2.2. Virus particle morphology under electron microscopy

Pictures of virus particles were taken under a Jeol Jem 1010 electro-microscopy using TEM method at NIHE, Vietnam on the infected rice plant collected in Thanh Hoa district of Long An province. The results showed that virus particles on tested samples are similar in shape and about the same size to what observed on samples collected in Bac Giang Summer crop 2010 and as described previously by Japanese scientists (Hibino, 1996).

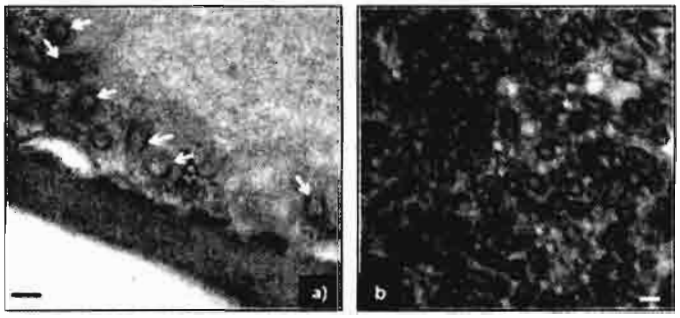

Figure 2. Virus particles taken on samples collected in Long An province, May 2011 (Bar = 100 nm)

3.2.3. Detection by RT-PCR

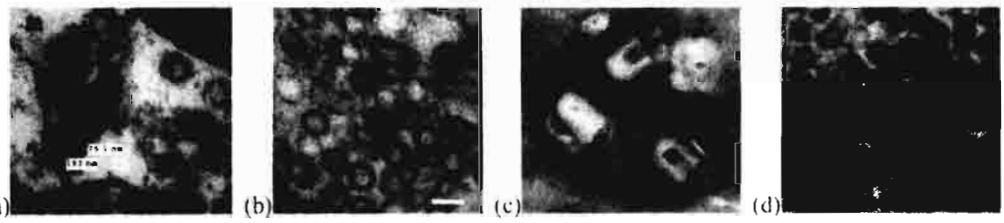

Figure 3. Virus particles taken on samples collected in Bac Giang province, Summer crop 2010 (a and b) and the bullet-shape particles of RYSV (180-210 x 94nm) with the hollow in the middle at the bottom (c and d) (Bar = 100 nm).

Different primer pairs were used to detect *Rice grassy stunt virus* (RGSV), *Rice tungro spherical virus* (RTSV) and RYSV from the samples collected in the disease field (table 2 and figure 4).

Table 2. List of primers used for virus detection

Primer name	Primer sequence (5' -> 3')	Size (nt)	Position in the gene sequence	Product size
RGSV-s3	AGAATTTTATGTCACTTAG	19	RNA3: 152 - 170	732 bp
RGSV-as3	TATCCAGATTTCAGGTGC	18	RNA3: 866 - 883	
RTSV-s	CAGGATAGTAGYGTTGCAGCAGCT	24	RTSV_Vt6: 2488 - 2511	833 bp
RTSV-as	GCACTCACCATTATCTT	17	RTSV_Vt6: 3304 - 3320	
RYSV-F2	GCCAGTGCAGATGCCTTATTAGTC	24	909-932	444 bp
RYSV-R2	GGTCTCTGCCTCTGTCATTCGTTC	24	1329-1352	

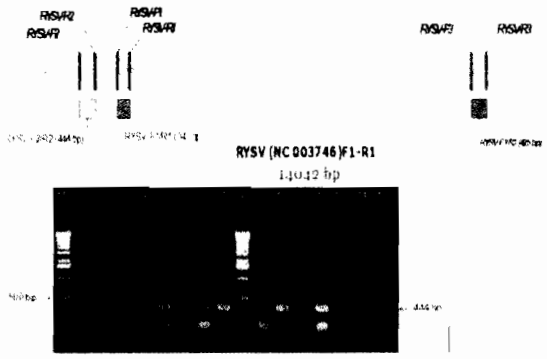

Figure 4. Schematic diagram of position and size of spesific primers and their PCR products available at PPRI for detection of RYSV (left); and a PCR results (right) of the detection of RYSV from samples collected in Thai Binh Trung Com., Vinh Hung Dist., Long An Prov.(Farmer Nguyen Van-Giau, cultivar VD20, seedling 20/3/2011) (12/15 samples positive to RYSV, primer RYSV-F2/R2, 444 bp). M: 1Kb marker (Invitrogen); 1 - 15: numbering of samples. Numbers to the left and right of the gel are the size of corresponding band of 1 Kb marker and the expecting size of the PCR product, respectively.

Among 46 samples collected in Tien Giang and Long An provinces, RT-PCR was done all for RYSV and tungro (RTSV) while only 22 samples containing some suspected symptoms were done additionally for RGSV. There were 45/46 samples positive to RYSV, in which 42 samples were mono-infection with RYSV, 1/22 samples co-infection with RYSV plus RGSV and 2/46 samples co-infection with RYSV plus tungro virus (RTSV). There was only 1/22 samples mono-infection with RGSV and no sample mono-infection with tungro disease (table 3).

Table 3. Virus detection by RT-PCR on infected samples collected in Tien Giang and Long An provinces in Summer crop 2011 (PPRI's Viro-Lab, May 2011)

Items	RGSV	Tungro	RYSV	RGSV + Tungro	RGSV + RYSV	Tungro + RYSV
Number of tested samples	22	46	46	22	22	46
Number of positive samples	1	0	42	0	1	2
Disease incidence (%)	4.5	0	91.3	0	4.5	4.3

3.2.4. Sequencing of PCR products and analysis

Some PCR products positive to RYSV were sent for direct sequencing by a company in France (MilleGen) for both orientations. The analysis of obtained sequences by BLAST (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*) showed that both isolates of Tien Giang and Long An are of 98% identity to the corresponding sequences of RYSV, the Chinese (AB516283) and Japanese (AB011257) isolates (data not shown).

Multiple alignment by muscle method integrated in the MEGA-5 software of three Vietnam isolates and the two reference isolates (AB516283 and AB011257) showed 2 new polymorphic sites, one on the Tien Giang and the other on the Long An isolate (red arrows), 3 polymorphic sites at which all Vietnam isolates are identical but different to one of the two reference isolates (blue arrows), and 3 polymorphic sites at which all Vietnam isolates are identical but different to both referent isolates (green arrows) (figure 5).

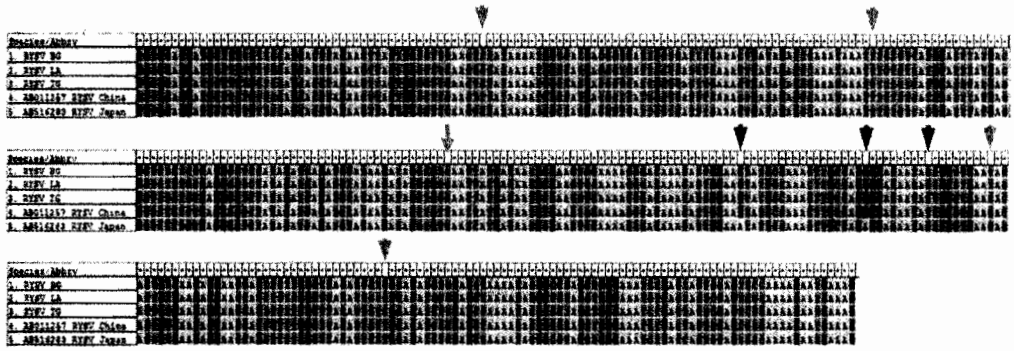

Figure 5. Schematic diagram of polymorphic position (arrows) on the multiple alignment by MEGA-5 with muscle method on 3 Vietnam isolates and 2 reference isolates (AB011257 and AB516283)

3.3. Transmission vector insect

One of the most important points in the Koch postulate for diagnosis of a virus pathogen is the transmission vector. Different inoculations were done at greenhouse of PPRI in Hanoi during June and July 2011. The result shown in table 4 confirmed that green leafhopper was the transmission vector insect of the disease. Both healthy GLH rearing in the greenhouse of PPRI and GLH collected on diseased fields can transmit the virus.

Table 4. Results on the inoculation experiments using healthy insect rearing in greenhouse and insect collected in diseased fields at Vinh Hung Dist., Long An Prov. *(PPRI, June and July 2011)*

Inoculation method	Insect species	No. of inoculated plants	Symptomized		Latent period on rice (days)
			(plant)	(%)	
Healthy insect acquired the virus for 10 days before inoculated on healthy seedling TN1	BPH	50	0	0	-
	WBPH(*)	50	0	0	-
	GLH	50	11	22.00	10 - 28
Adult insects collected in the diseased fields inoculated on healthy seedling TN1	BPH	30	0	0	-
	WBPH	30	0	0	-
	GLH	30	5	16.67	12 - 27

Note: (*) White-back planthopper

3.4. Detection of RTYV on inoculated rice plant and vector insect

Some inoculated rice plants showing typical symptoms and corresponding insects were tested by RT-PCR and sequencing following the same procedure as done in the section 3.2.3 and 3.2.4 and gave similar results.

IV. CONCLUSION

Based on the analysis and comparison of symptoms, morphology and size of virus particle by TEM, RT-PCR detection, sequence analysis and inoculation experiments we conclude that the new yellowing syndrome on rice in some areas of Mekong delta during Summer crop 2011 was caused by a virus named *Rice yellow stunt virus* (RYSV), synnomym: *Rice transitory yellowing virus* (RTYV), belonging to the genus *Nucleorhabdovirus* of the family *Rhabdoviridae*, green leafhopper is the transmission vector insect.

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METHANE (CH_4) AND NITROUS OXIDE (N_2O) EMISSION FROM RICE CULTIVATION IN VIETNAM: A REVIEW

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ABSTRACT

Rice is Vietnam's main food product, accounting for around 45% of gross production of food crops. Vietnam is a sustainable rice supplier, the world's fifth-largest rice producer and the second-largest (after Thailand) rice exporter in the world. Recognizing the importance of the role of rice production in the national economy and food security, the reduction of the major greenhouse gases (GHGs) (CH_4, N_2O...) from paddy fields has been paid great attention by the Government of Vietnam and is part of The National Target Program to Respond to Climate Change. The GHGs emission from rice cultivation and its negative effects have been recently studied. The main research results of methane (CH_4) and nitrous oxide (N_2O) emission from paddy field in Vietnam linked to global warming are summarized in this article. We also provide a comprehensive review of some potential GHGs emissions mitigation options.

Key words: Greenhouse gases, methane, nitrous oxide, emission, rice.

I. INTRODUCTION

Over the last 20 years Vietnam has been transformed from a net rice importer to the second largest rice exporter in the world with more than 30 million tons of rice produced annually. Rice production alone contributed 45% of the gross value of agricultural output and about 90% of total annual food production (GSO, 2011). The Red River Delta and the Mekong River Delta are considered the

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