

## Feed the Future: Innovation Lab for Integrated Pest Management Trip Report

**Country(s) Visited:** Cambodia

**Dates of Travel:** Sept 12 to 17, 2016

### Travelers' Names and Affiliations:

- Gilda Jonson (GJ), Senior Associate Scientist, Genetics and Biotechnology Division, International Rice Research Institute (IRRI), Philippines
- Parameas Kong (PK), Research Assistant, Plant Protection Office, Cambodia Agriculture Research and Development Institute (CARDI).

### Purpose of Trip:

- To examine the distribution of rice viral diseases in provinces located east of Phnom Penh to Cambodia - Vietnam border, and
- To assess the facilities for identification of rice virus diseases in CARDI and other institutes in Cambodia.

### Sites Visited:

GJ and PK visited four provinces, Takeo, Kampong Cham, Prey Veng, and Svay Rieng (Fig. 1), and collected rice leaves suspected to be infected with viruses. Rice leaves were collected from a total of 14 sites in the four provinces (**Fig.1**). Two districts in an opposite direction (southern and northern part) per province were selected so that the sampling sites represent the entire province.



**Fig 1.** Location of four provinces surveyed for rice viral diseases.

### Methods:

Leaf samples were collected in one to two sites per district (**Table 1**). At a site, leaf samples were collected from 20 plants that were suspected to be infected with

viruses. All collected leaves were brought to IRRI, Philippines and tested for the presence of *Rice tungro bacilliform virus* (RTBV), *Rice tungro spherical virus* (RTSV), *Rice grassy stunt virus* (RGSV), *Rice ragged stunt virus* (RRSV) by enzyme-linked immunosorbent assays (ELISA) using antibodies specific to the respective viruses. Examination of leaves for the infection with rice orange leaf phytoplasma (ROLP) was done by a polymerase chain reaction (PCR) using DNA oligonucleotides specific to the ROLP genome.

**Table 1.** Sites and plants that leaf samples were collected from.

Date visited	Province	Site ID number	Commune	District	Cultivar/variety	Growth stage
Sep 13	Takeo	1, 2	Potsor	Batie	Phlsar Khgney	Tillering
		3, 4	Sambuor	Treang	Srov Krahorm	seedbed - Tillering
Sep 14	Kampong Cham	5	Domrel	O'reang Auv	Unknown	Tillering
		6	Khvet Thom	Prey Chhor	Unknwon	Tillering
Sep 15	Prey veng	7, 8	Prey Kandieng	Peam Ror	IR-504	Early tillering
		9, 10	Baray	Baray	Somali, IR-504	Tillering
Sep 16	Svay Rieng	11, 12	Chambak	Svay Chhroum	Unknown	Tillering
		13, 14	Khset, Svay Tayean	Kampong Rou	Unknown	Tillering

### Description of Activities:

- **Sep 13 in Takeo Province**

GJ and PK visited two sites in each of commune Potsor, Batie District and commune Sambuor, Treang District. The two sites in the same commune were about 200-500m apart. Rice plants in the sampling sites in both districts in Takeo had been planted by broadcasting, and were at tillering stage. Asynchronous planting was common in the sampling sites. Rice plants cultivated were traditional cultivars called Phlsar Khgney and Srov Krahorm.

GJ and PK collected leaves from plants that were showing leaf discoloration and stunted growth, which are typical symptoms caused by virus infection. Examination of the samples for virus infections by ELISA confirmed that two plants in the site 4 in Sambour, Treang were infected with tungro viruses. One plant was found to be infected with RTBV, and the other was found to be co-infected with RTSV and RTBV. RTSV and RTBV are transmitted by green leafhopper (GLH).

- **Sep 14 in Kampong Cham Province**

Leaf samples were collected at a site (site 5) in commune Domrel, O'reang Auv District near the Cambodian border with Vietnam, and at another site (site 6) in commune Khvet Thom, Prey Chhor District, which is located at the central part of Kampong Cham province. Examination of the samples for virus infections by ELISA confirmed that two plants at the site 5 were co-infected with RTSV and RTBV.

- **Sep 15 in Prey Veng Province**

At the time of visit, a majority of rice cops in Prey Veng province were already harvested, and some fields were left fallowed for the next planting. GJ and PK collected leaf samples at two sites (sites 7 and 8) in commune Prey Chhor, Peam Ror District in a northern part of the province, and other two sites (sites 9 and 10) in

Baray District, which is at central part of Prey Veng. Plants at the four sites were hybrid rice variety IR-504 and traditional cultivar Somali at tillering stage. Examination of the samples for virus infections by ELISA confirmed that the four sites were affected with tungro viruses (Fig. 2 and Table 2). Some plants at the site 7 were also found to be infected with RGSV, or mix-infected with RGSV, RTSV, and RTBV. RGSV is transmitted by brown planthopper (BPH).

**Table 2.** Rice plants infected with RGSV, RTBV, and RTSV in Prey Veng

Site ID	Number of plants examined	Number of plants confirmed to be infected with			
		RTBV	RTSV + RTBV	RGSV	RGSV + RTBV + RTSV
7	20	0	3	1	1
8	20	1	1	0	0
9	20	2	9	0	0
10	20	1	0	0	0



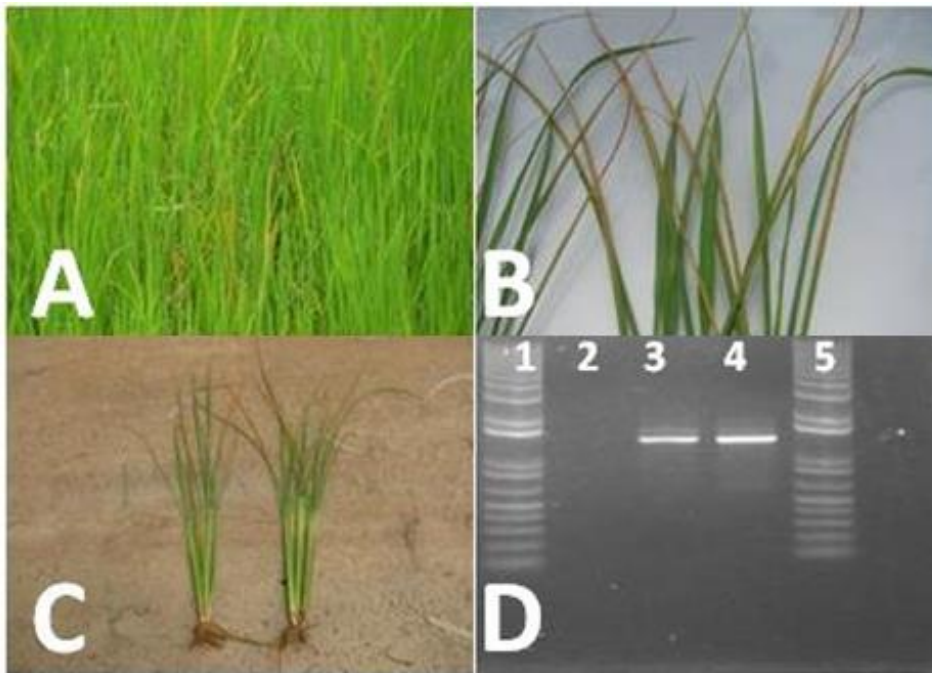
**Fig. 2.** Rice plants suspected to be infected with viruses in Prey Veng Province, Cambodia

- **Sep 16 in Svay Rieng Province**

Leaf samples were in four sites: two (sites 11 and 12) in commune Chambak, Svay Chhroum, District, and the other two (sites 13 and 14) in commune Khset and Svay Tayean, Kampong Rou District. All plants were late maturing unknown traditional cultivars at tillering stage. All plants had been planted by broadcasting, except those in the site 12, which was transplanted. Examination of the samples for virus infections by ELISA confirmed that plants in the site 11 are affected with RTSV and RTBV.

Plants with symptoms suspected to be caused by ROLP are common in Svay Rieng province, especially in the site 12 (**Fig. 3 A**). The discoloration of leaves to orange color starting from the tip progressing downward and accompanied by rolling of leaves (Fig. 3 Band C) were commonly observed. All the leaf samples collected in the site 12 were negative for RTBV or RTSV. To confirm the ROLP infection, we conducted a PCR for ROLP using DNA extracted from the leaves collected in the site 12. The PCR resulted in amplification of a DNA fragment with the expected size of

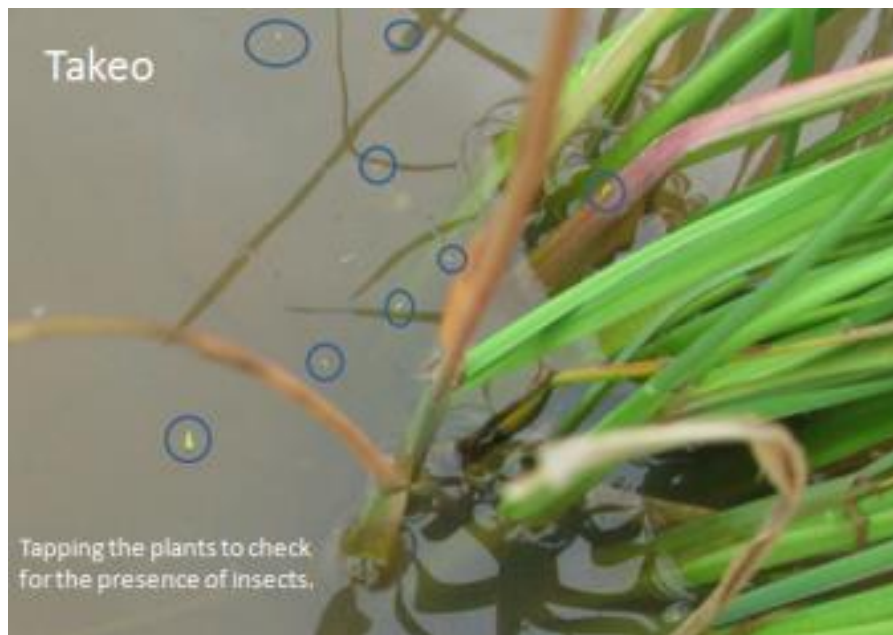
1.2 kbp (Fig. 3 D, lanes 2 and 4), confirming the infection of plants in the site 12 with ROLP.



**Fig.3. A, B, and C:** Plants with symptoms caused by ROLP in Svay Rieng Province, **D:** PCR detection of ROLP-specific DNA fragment from DNA of the plants suspected to be infected with ROLP

**Observations, suggestions, recommendations, and follow-up Items:**

- The cultivation pattern of rice in the four provinces seemed not to follow synchronous planting, which is recommended to manage the occurrence of rice virus diseases. We observed that the site 9 in Prey Veng where relatively a high percentage of tungro-infected plants (**Table 2**) was adjacent to a land ready for planting. Therefore, it is highly likely that GLH carrying tungro viruses move from the site 9 to the adjacent land once new seedlings are planted in the adjacent land, and cause tungro infection in the new seedlings. We recommend re-visiting to the site 9 to see whether such spread of tungro disease actually occurs.
- The levels of GLH were relatively high in most sites we visited (**Fig. 4**). The presence of insects such as GLH and BPH in the sites affected by viruses and ROLP should be monitored and controlled regularly to avoid chronic crop losses from viruses and ROLP. We also recommend planting of varieties resistant to GLH, BPH, and tungro viruses to prevent future incidences of virus and ROLP diseases.



**Fig. 4.** GLH infesting rice plants in Takeo province.

- Other prevalent rice diseases observed during the trip were bacterial leaf streak and brown spot (**Fig. 5**).



**Fig. 5.** Bacterial leaf streak and brown spot commonly observed during the field survey in Takeo, Kampong Cham, Prey Veng, and Svay Rieng Provinces of Cambodia.

- During survey period, majority of rice planted were traditional cultivars having a long maturity period. To avoid lodging, reducing the height by cutting at maximum tillering was being practiced by farmers. The farmers seemed not to realize that such practice is injuring the plants and making them prone to bacterial diseases.
- GJ visited CARDI and met Dr. Sathya Khay, Head of Plant Protection Division (PPD) of CARDI. PPD has a unit of thermal cycler, although it has never been used. GJ recommended Dr. Khay to request an electrophoresis unit, and a gel-documentation unit, so that PPD can conduct diagnosis of virus and phytoplasma diseases by PCR or reverse transcriptase-PCR (RT-PCR). IRRI can provide DNA

primers for specific detection of RTBV, RTSV, RGSV, RRSV and ROLP upon request.

- GJ also visited Plant Pathology laboratory of Royal University of Agriculture in Phnom Penh. Their lab is not equipped at all to conduct any work for virus diagnosis by ELISA or RT-PCR.

**List of contacts made**

<b>Name</b>	<b>Title/Organization</b>	<b>Contact Information (Address, phone, email)</b>
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