

## YELLOWING SYNDROME OF RICE: ETIOLOGY, CURRENT STATUS AND FUTURE CHALLENGES

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### ABSTRACT

*Rice plants showing symptoms of “yellowing” and “tungro-like symptom” were first observed in the Mekong Delta in 1989, and became a serious problem after 1994. Affected rice plants showed light spreading growth habit, light stunting and yellowing or light yellow to dark orange discoloration of the leaves. Sometimes only few tillers per hill appeared infected, without profuse tillering or further growth. In 2005, fifty two leaf samples were tested against RTBV, RTSV, RGSV and RTYV (rice transitory yellowing virus). Only one of the 43 samples exhibiting tungro-like symptoms (yellowing and stunting) were positive to RTSV. Usually infection with RTSV does not exhibit any symptom of yellowing and stunting, suggesting the involvement of other viral agents in the disease. In March of 2006, plants with the yellowing syndrome were collected in farmers’ field in the Mekong Delta. Leaves of plants showing typical yellowing and bronzing of leaves and stunting were collected. Ninety percent of plants collected in March 2006 were found infected with RGSV, while RRSV were detected from more than 70% of the plants, and that about 60% of plants showing the yellowing syndrome were mix-infected with RRSV and RGSV, and one plant was mix-infected with RRSV, RGSV, and RTSV. The disease spread very rapidly to 51,507 hectares within 6 early months in 2006. Some recommendations were mainly to eradicate diseased plants in fields, which may serve as inoculum sources in the following season, “avoiding or escape” high population of BPH during the migration at most sensitivity of rice crop (<20 days), to plant tolerant rice genotypes. Integrated pest management for BPH/virus association is most important strategy for sustainability of rice production in Mekong delta.*

### RICE VIRUSES IN VIETNAM

Rice is the main crop in Vietnam. It is currently cultivated in 4.2 million hectares in the total growing area of 7.32 million hectares. In 2006, rice production in Vietnam was 36 million tones. In 1964, widespread occurrence of yellowing and dwarfing diseases suspected to be caused by viruses were observed in Vietnam. The diseases were called “Vàng Lụi” in Vietnamese language, which means “yellowing and stunting”. The diseases appeared in a very large area in northern part of Vietnam during 1964 to 1970, affecting virtually all varieties planted in about 50,000 hectares (Du 1988). The viral nature of the diseases was not confirmed, but due to the presence of high populations of green leafhoppers (GLH) (*Nephotettix virscens* Distant.) in the affected areas, it was suspected that the diseases were caused either by tungro viruses or by yellow dwarf mycoplasma.

Rice plants showing the “yellowing syndrome” had been reported as early as 1960s in southern regions of Vietnam and given names such as “yellow stunt”, “chlorotic stunt” or “bushy stunt” (Toan 1969). Another occasion of rice disease epidemic supposedly caused by viruses was also recorded in the Mekong Delta in year 1978 to 1980 after the outbreak of brown planthopper (BPH), *Nilaparvata lugens*

on rice variety TN-732. The outbreak resulted in more than 90 % losses of the rice crop. Rice plants in the affected areas showed symptoms such as serrated leaves, twisted and malformed leaves, vein swellings on leaf sheaths and blades, leaf curling and stunted growth. The symptoms on affected plants suggested that the epidemic was caused by rice ragged stunt virus (RRSV), although no serological test was done to confirm the causal agent. The high populations of GLH *Nephotettix* spp., which can transmit tungro viruses, were reported in northern and southern parts of Vietnam (Thuat 1982; Huynh *et al.* 1986). Rice tungro disease was reported in the Central Vietnam but not in the Mekong Delta (Vien *et al.* 1994; Vien *et al.* 1996). Later on, rice plants infected with RRSV were found in the central part of Vietnam after the outbreak of BPH (Ossmat Azzam and Nguyen Van Tuat, personal communication).

### **IS YELLOWING SYNDROME AN EMERGING RICE VIRUS DISEASE IN MEKONG DELTA, AND WHERE DID IT START?**

Rice plants showing symptoms of “yellowing” and “tungro-like symptom” were first observed in the Mekong Delta in 1989, and became a serious problem after 1994. Affected rice plants showed light spreading growth habit, light stunting and yellowing or light yellow to dark orange discoloration of the leaves. Sometimes only few tillers per hill appeared infected, without profuse tillering or further growth. In 1997 dry season, the incidence of yellowing syndrome was estimated up to 5-10% in the Mekong Delta, affecting nearly all varieties grown. However, the incidence of yellowing syndrome was up to 50% in some fields, where a high level of nitrogen fertilizer was applied. Diseased tillers had leaves showing interveinal chlorosis, yellowish leaves and stunting, which are typical symptoms of rice tungro disease prevalent in Southeast Asia (Ling 1972). However, in the areas showing the yellowing syndrome in the Mekong Delta, GLH populations were very low. In 1999, Plant Protection Department in the South of Vietnam named it “bệnh vàng lùn” which means “stunting and yellowing syndrome”. Later on, it was also named “grassy stunt virus strain (RGSV)-2” or “bệnh lúa cỏ dòng 2” in Vietnamese language (Pham Van Kim, personal communication).

Local variety Tai Nguyen, which is susceptible to BPH and RGSV, is still cultivated during Mua (monsoon) season in about 400,000 hectares around Ho Chi Minh City, Long An, Ben Tre, Tra Vinh and Soc Trang. Some local rice plants showed typical symptoms of RGSV such as yellowing and stunting, but profuse tillering was rarely found. In year 2000, yellowing syndrome on local varieties was found severely around Ho Chi Minh City together with high population of BPH, it was suspected due to GSV (Huan and Heong 2000). It is assumed that BPH had gradually build up inoculum of viruses causing the yellowing syndrome from its originating source and reached at the present epidemic level in the Mekong Delta. In 1996 rice leaf samples showing symptoms of the yellowing syndrome were examined by enzyme-linked immunosorbent assay (ELISA) using antibodies for several viruses (Table 1). However, no viruses were detected from any of the samples examined. Leaf samples collected from various sites of Vietnam were also examined for the presence of viral agents associated with the yellowing syndrome in 1997. Only one out of 140 leaf samples reacted positive respectively to rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), and two reacted positive to RRSV. This was the first report on the occurrence of rice tungro virus in the Mekong Delta; although only very few plants with typical tungro symptoms (1 out of 140 samples in 1997, and 1 out of 43 samples in 2005) were identified infected with tungro viruses.

The outbreak of RGSV with high BPH populations was observed in rice fields near Ho Chi Minh City in 2000 (Huan and Heong 2000), although the exact nature of causal agents was not revealed. In the following years, the occurrences of rice plants seemingly affected with the yellowing syndrome persisted in the Mekong Delta. Accordingly, another survey was conducted in 2005; fifty two leaf samples were tested against RTBV, RTSV, RGSV and RTYV (rice transitory yellowing virus). Only one of the 43 samples exhibiting tungro-like symptoms (yellowing and stunting) were positive to RTSV. Usually

infection with RTSV does not exhibit any symptom of yellowing and stunting, suggesting the involvement of other viral agents in the disease. Meanwhile seven out of nine samples showing grassy-like symptoms of profuse tillering, yellowing and stunting reacted positive to RGSV, about 70% of which were collected in rice fields surrounded Cuu Long Delta Rice Research Institute (CLRRI), Omon (Table 1). Most of the leaf samples that reacted to RGSV showed grassy-like symptoms. Thus, the overall results confirmed the presence of RTBV, RTSV, RRSV and RGSV in rice areas of Mekong Delta (Du 2005). Tungro viruses, RRSV and RGSV have been a problem in many rice growing areas of Southeast Asian countries. More studies needed to be conducted to understand the epidemiology of yellowing syndrome and the identity of associated viruses in order to prevent the incidence in the Mekong Delta, which is considered as the rice bowl of Vietnam.

**Table 1.** Reactions to rice viruses in leaf samples collected in Vietnam from 1996 to 2005 by enzyme-linked immunosorbent assay.

Sampling date	Number of samples	Number of samples positive for <sup>1</sup>					
		RTBV	RTSV	RRSV	RGSV	RDV	RTYV
1996 April	23	0	0	0	0	0	nt
1997 January	140	1	1	2	0	nt	0
2005 January							
Plants with tungro like symptom	43	0	1	nt	0	nt	0
Plant with grassy like symptom	9	0	0	nt	7	nt	0

(<sup>1</sup>) RTBV-rice tungro bacilliform virus, RTSV-rice tungro spherical virus, RRSV-rice ragged stunt virus, RGSV-rice grassy stunt virus, RDV-rice dwarf virus, RTYV-rice transitory yellowing virus, (nt) not tested

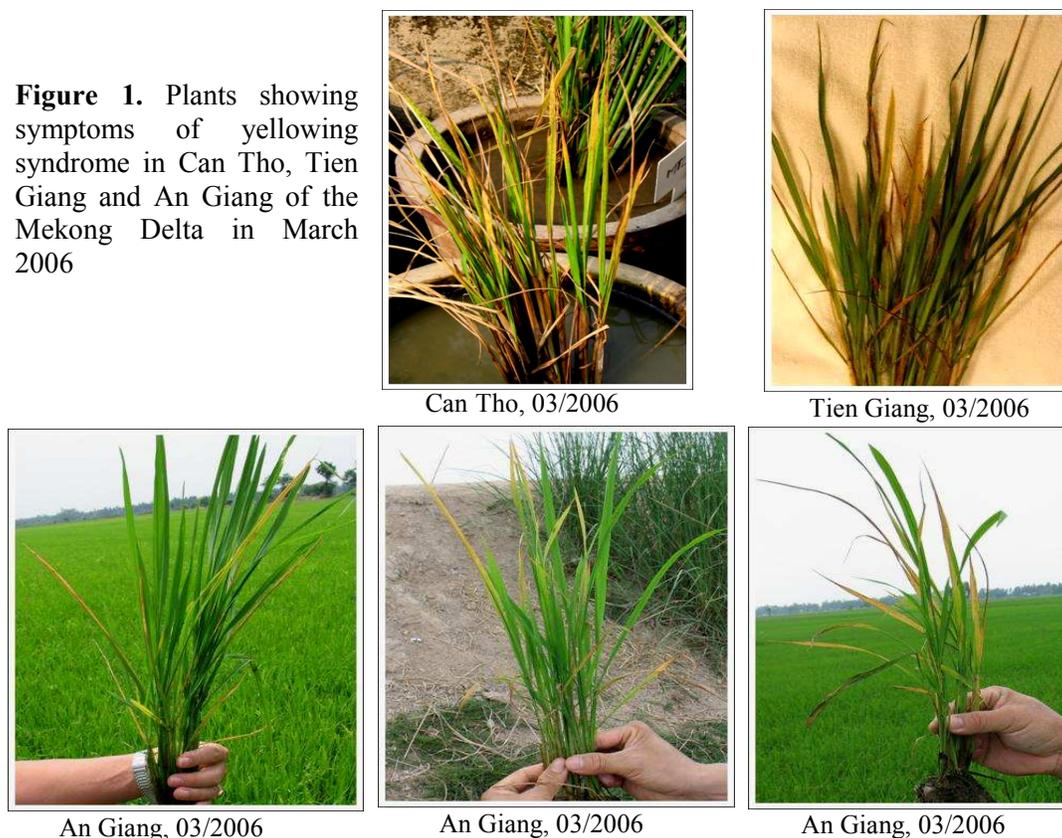
## IS YELLOWING SYNDROME CAUSED BY MIXED (DUAL) INFECTION OF RICE VIRUSES?

The epidemiological characteristics of the yellowing syndrome indicated the involvement of viruses and insect vectors, although the exact causes of the disease are not well elucidated by the previous attempts. Another attempt for the identification of viruses associated with the yellowing syndrome was made with the improved procedure for sap extraction from leaf samples in order to achieve better detection of viruses. In March of 2006, plants with the yellowing syndrome were collected in farmers' field in Mekong Delta (Tien Giang, Can Tho and An Giang provinces). Leaves of plants showing typical yellowing and bronzing of leaves and stunting were collected (Fig. 1).

Leaves of collected plants were cut into pieces of about 2 mm long, pulverized with liquid nitrogen, then diluted 10 times (w/v) with 0.02M phosphate buffer (pH 7.4) containing 0.15M NaCl and 0.05% Tween 20. Samples were examined for the infection with RTBV, RTSV, RGSV and RRSV by ELISA according to the method as described by Bajet *et al.* (1985). Leaf sap from non-infected rice variety Taichung Native 1 (TN1) was used as negative control and those from TN1 infected with RTBV/RTSV, RGSV and RRSV were used as positive control. Absorbance values over twice the mean of four healthy controls were considered to be positive for virus infection.

The results showed that close to 90% of plants collected in March 2006 were found infected with RGSV, while RRSV were detected from more than 70% of the plants, and that about 60% of plants showing the yellowing syndrome were mix-infected with RRSV and RGSV, and one plant was mix-infected with RRSV, RGSV, and RTSV (Table 2).

**Figure 1.** Plants showing symptoms of yellowing syndrome in Can Tho, Tien Giang and An Giang of the Mekong Delta in March 2006



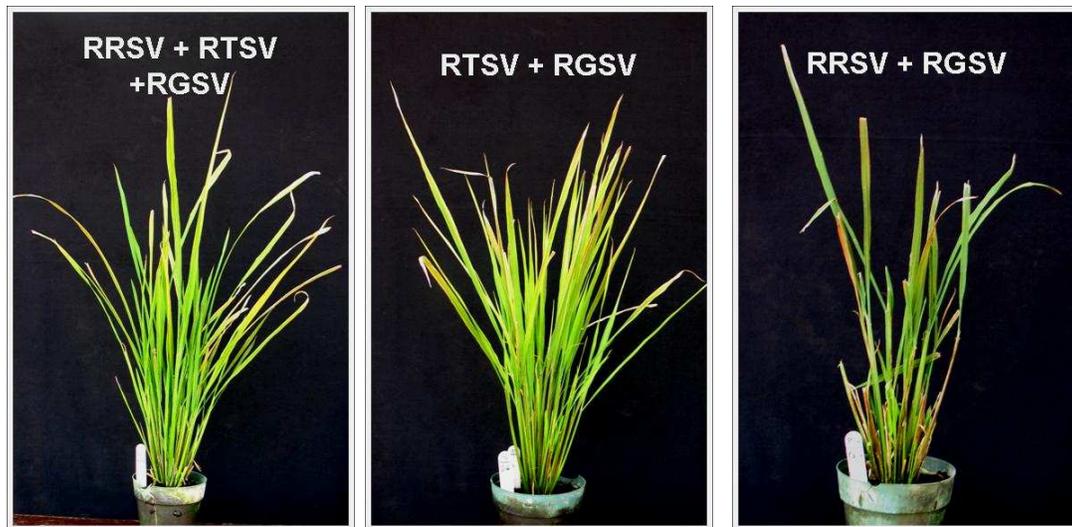
**Table 2.** Reaction to rice viruses in leaf samples collected in the Mekong Delta, March 2006 by enzyme linked immunosorbent assay.

Sample no.	RTBV	RTSV	RGSV	RRSV	Sample no.	RTBV	RTSV	RGSV	RRSV
An Giang 1			+	+	Tiền Giang 1				+
An Giang 2			+	+	Tiền Giang 2				+
An Giang 3			+	+	Tiền Giang 3			+	+
An Giang 4			+	+	Tiền Giang 4			+	+
An Giang 5			+	+	Tiền Giang 5			+	+
An Giang 6			+		Tiền Giang 6			+	+
An Giang 7			+		Tiền Giang 7			+	+
An Giang 8			+		Tiền Giang 8			+	
An Giang 9		+	+	+	Tiền Giang 9			+	+
An Giang 10				+	Tiền Giang 10			+	+
An Giang 11			+	+	Tiền Giang 11			+	+
An Giang 12			+	+	Cần Thơ			+	
An Giang 13			+	+					

Since single infections of rice plants with any of RRSV, RGSV and RTSV usually do not result in the typical symptoms of yellowing syndrome, we examined the symptoms of plants artificially inoculated with the combinations of RGSV, RRSV and RTSV. Isolates of RTSV, RRSV, and RGSV and colonies of GLH *Nephotettix virescens* Distant and BPH *Nilaparvata lugens* Stahl maintained in TN1 at the virology greenhouse of the International Rice Research Institute were used in all transmission experiments. Seedlings of TN1 were used as test plants in all insect inoculations. For RTSV transmission, virus-free adult green leafhoppers were given access to RTSV-infected TN1 plants for four

days prior to inoculation. For RRSV and RGSV, second instar BPH nymphs were given four days access to RRSV-infected and RGSV-infected TN1 plants separately. Then they were separately reared on healthy TN1 seedlings for six days for virus incubation in the insect.

Symptoms on plants mix-infected with these viruses were markedly different from those of singly infected plants (Fig. 2). Tillers of plants mix-infected with RGSV and RRSV were shorter and thinner, while the leaves showed bronzing and occasional yellowing. Symptoms on plants mix-infected with RGSV and RTSV were similar to those of tungro disease, showing discoloration of leaves to pale yellow to pale orange and occasional leaf bronzing. These symptoms appeared to be similar to those observed for plants with the yellowing syndrome in fields (Fig. 1). Collectively these results suggest that the yellowing syndrome might be caused by the combination of RGSV with RRSV and/or RTSV.



**Figure 2.** Symptoms of plants mix-infected with rice viruses

#### **RTSV-RRSV-RGSV**

Plants infected with these three viruses are similar to plants doubly infected with RTSV-RGSV but the yellowing is less intense. Plants simultaneously infected with RRSV and RGSV had more severe symptoms than plants infected with RTSV first then followed by both RRSV and RGSV together two weeks later. The leaves were broader and profuse tillering is somewhat delayed. Vein swellings (galls) and leaf distortions can be observed.

#### **RTSV-RGSV**

Double infection with these viruses shows tungro – like symptoms. Emerging leaves exhibit very distinct interveinal chlorosis. The leaves later turn pale yellow to pale orange and the interveinal chlorosis becomes more pronounced. Tiller number is more than the normal plant but lesser and bigger than plants infected with RGSV alone. The leaves are wider and spreading instead of narrow and erect in RGSV-infected plants. Brownish discoloration (bronzing) may also be observed on some leaves. The plant usually dies before maturity.

#### **RRSV-RGSV**

Doubly infected plants show more severe stunting than singly infected plants. Usually RRSV symptoms appear first. The tillers are dark green, shorter, thinner, and erect. Small diminutive tillers (smaller than RGSV-infected plants) appear later. Bronzing is also observed in young leaves, which becomes more distinct as the leaf matures. Occasionally some older leaves show yellowing.

### OUTBREAK OF YELLOWING SYNDROME CAUSED BY RICE VIRUSES TRANSMITTED BY BPH IN THE MEKONG DELTA

Following the outbreak of yellowing syndrome occurred in March 2006, the disease spread very rapidly to 51,507 hectares within 6 months (Huan 2006). Hundred twenty thousands hectares of summer-autumn rice crop has been devastated by the disease and BPH. It is difficult for rice farmers to continue next crop (2007 dry season). This urges the local authorities to take all preventive measures possible against BPH and virus infection. Survey was conducted in eastern parts of provinces in the Mekong delta to examine the extent of virus infection on seedlings and transplanted plants, and the level of viruliferous BPH. Sites of survey were Binh Phuoc, Dong Nai (eastern), around Ho Chi Minh, Long An, Tien Giang, Tra Vinh (Mekong Delta). Results revealed the presence of RGSV, RRSV and RTSV in the leaf samples collected (Table 3). Overall dual infection with RRSV and RGSV was observed in more than a half of plants examined. The rates of dual infection with RRSV and RGSV were especially high in Binh Phuoc. Dual infection with RGSV and RTSV was also detected only in plant samples collected in Tien Giang and Binh Phuoc. About a half of BPH collected was found carrying RRSV or RGSV, although the proportion of BPH carrying both viruses was low.

**Table 3.** Rice viruses serologically detected from rice plants and BPH at different sites of the Eastern part and the Mekong Delta (August 2006).

Sample type/ Location	Number of samples	Percentage of detection				
		RGSV	RRSV	RTSV	RGSV+RRSV	RTSV+RGSV
<b>Rice seedlings</b>						
- Binh Chanh, HCM city	63	97	37	0	34	0
- Ben Luc, Long An	27	94	67	0	61	0
		95.5	52	0	47.5	0
<b>Direct seeded/ Transplanted plant</b>						
- Tien Giang	8	71	57	27	57	13
- Binh Phuoc	21	90	81	10	81	5
- Tra Vinh	5	100	20	0	20	0
- Dong Nai	1	100	100	0	100	0
		90	65	9	65	5
<b>BPH at different sites</b>						
	35	66	41	0	8	0

Collectively, the results from repeated surveys strongly suggest that RGSV and RRSV are associated with the yellowing syndrome of rice, although it is less clear whether the mixed infection of the two viruses is a requisite for the development of symptoms often observed in fields. The result also indicates that BPH population could be monitored for the presence of the two viruses by a serological method. Observations in fields showed that seedlings which are 20 days older already exhibit symptoms of RGSV and RRSV infection. Thus, when infected seedlings were transplanted in fields, they could serve as inoculum sources to further spread the disease.

### HOW TO MANAGE THE YELLOWING SYNDROME?

Three months after the outbreak of infection with RGSV and RRSV, the areas affected by the viruses increased rapidly. The ministry of Agriculture and Rural Development (MARD) of Vietnam held a meeting on 12 June 2006 to discuss with scientists and local authorities for the solution. Several possible management strategies based on the biological relationship between BPH and the viruses were discussed. The major vector of RGSV and RRSV is *Nilaparvata lugens* Stal, although these viruses are also transmitted by *N. bakeri* Muir and *N. muiri* China. RGSV and RRSV are persistent (propagative) in the vectors but not transmitted *via* eggs. Epidemiology of the disease also should be considered in the design

of appropriate management strategies. Intensive cropping system of rice cultivation in the Mekong Delta is quite different from that in other Asian rice countries. Farmers in the Mekong Delta continuously cultivate two to three rice crops per year (sometimes seven crops per two years). Practices such as asynchronous planting, high density planting for direct seeding, high nitrogen application, use of short duration varieties, and overuse of pesticides are common in the area. Since the yellowing syndrome appeared to be highly associated with the level of BPH in fields, it is also important to monitor BPH populations to determine exact timing of migration. Along with the numbers of BPH observed in fields, it is critical to examine the percentage of BPH carrying the viruses because sometimes the level of virus incidence is not correlated with the level of BPH population in fields. Migration is already known to occur during harvesting time, and thus prediction of seasonal wind direction should be taken into account for proper management of BPH-transmitted viruses. In addition, the eradication of virus sources, and selection of rice varieties are also important to prevent the yellowing syndrome. During the outbreak in 2006, more than 50,000 hectares of infected rice field are believed to serve as the sources of inoculum, and all varieties grown in the areas are susceptible to RGSV and RRSV. Thus, considering the observations so far, the followings are to be suggested to farmers to manage the yellowing syndrome;

1. Eradication of diseased plants in fields, which may serve as inoculum sources in the following season.
2. Synchronous planting in the affected areas, because the common practice of staggered planting (rice plants in different stages) promotes continuous availability of the host for viruses and vectors, serving as inoculum source to spread the disease from one crop to another.
3. Planting of tolerant varieties to viruses, such as OM4498, OM5930, OMCS2000, OM576, IR50404, CL8.
4. Avoid the use of susceptible varieties, specially such as OM1490, Jasmine 85, OM2517, OM2518.
5. Use light trap to monitor BPH migration in order to apply the crop escape or avoidance strategy. At seedling stage within 20 days after seeding, plants are very susceptible to virus infection. Crop establishment using direct seeding could be done during migration time (5-7 days average) or just after the migration ends. For transplanting, seedlings should be covered by nylon mosquito net during night time and removed in the morning. Seedlings should be transplanted right after the migration ends.
6. Insecticides can be applied to lower BPH population. However, RRSV and RGSV can be transmitted by BPH within 5-15 minutes in night time after landing on rice field, thus even insecticide application may not help. Spraying right after 1<sup>st</sup> hatching at 35 to 40 days is very important. Viruses persist in the body of the brown plant hopper until death, thus BPH could carry viruses, migrate from one area to another and serve as an inoculum source to newly established crop.
7. Farmers should follow practice of “Three Reductions and Three Gains” to keep rice plant more healthy. Use of less nitrogen fertilizers can make the cultivation condition unfavorable for pest occurrence.

### **LOOKING TO THE FUTURE**

At present, the virus infection transmitted by BPH in rice fields are lower incidence than in 2006. In Vietnam, virus incidence historically occurred in the 1960's and 1970's; it caused a serious problem of food shortage at that time. Again in 2006, rice production was estimated 800,000 tons lost due to rice viruses. Although some farmers are still relying on application of pesticides to manage the disease, however, escape strategy is accepted by most of rice farmers. This proved to be very successful in controlling virus infection in intensive cropping systems of the Mekong Delta. In near future, more studies need to be conducted to better understand the epidemiology of the virus disease, the virus-vector interaction and the interrelationship among hosts, vectors and viruses. Most of all, the development of rice varieties resistant to viruses and BPH is urgent. Integrated pest management based for BPH/virus association is most important management strategy for sustainability of rice production in the Mekong Delta.

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**Triệu chứng bệnh Vàng lùn của lúa: Tác nhân gây bệnh, hiện trạng và thử thách**

Triệu chứng “Vàng lá” và “Vàng lá giống như bệnh do Tungro” gây ra được ghi nhận đầu tiên ở Đồng bằng sông Cửu Long trong năm 1989. Sau năm 1994, triệu chứng trên trở nên nghiêm trọng trên cây lúa. Lúa bị bệnh có biểu hiện rõ rệt phát triển nhiều chồi, hơi thấp (lùn) cây và vàng hoặc vàng nhạt rồi chuyển thành vàng cam trên lá, đôi khi có vài chồi trên bụi bị bệnh và bụi lúa bị bệnh không có biểu hiện của sự ra nhiều chồi và chuyển màu vàng cam rất giống với bệnh Tungro. Trong năm 2005, 52 mẫu lá bị bệnh có phản ứng với các dạng virus RTBV, RTSV, RGSV và RTYV (rice transitory yellowing virus). Chỉ có 1 mẫu (giống bệnh Tungro) trong số 43 mẫu có phản ứng dương tính với RTSV. Thông thường khi cây lúa bị nhiễm với RTSV thì không cho thấy triệu chứng vàng và lùn, do vậy khả năng nhiều dạng virus khác cùng gây nhiễm. Trong năm 2006, lúa bị bệnh vàng lùn ở ĐBSCL được sưu tập, lá bị bệnh có triệu chứng điển hình vàng và vàng sậm màu nâu gỉ .90 % có phản ứng với bệnh Lùn lúa cỏ (RGSV), và 70 % có phản ứng với virus gây bệnh Lùn xoắn lá, 60% có phản ứng với cả 2 viruses (mix-infected) với RRSV và RGSV, có 1 mẫu có phản ứng với cả 3 loại viruses RRSV, RGSV, and RTSV. Bệnh lây lan rất nhanh lên đến 51.507 hectares chỉ trong 6 tháng đầu của năm 2006. Vài khuyến cáo kịp thời đưa ra nhằm ngăn chặn sự phát triển của dịch bệnh do viruses gây ra: Hủy ruộng lúa bị bệnh nặng, vì đó là nguồn cung cấp viruses cho rầy nâu lây lan, áp dụng phương pháp “gieo sạ đồng loạt và né rầy” “avoiding or escape” trong giai đoạn đầu của cây lúa (<20 ngày tuổi), trồng giống chống chịu rầy nâu và bệnh, áp dụng các biện pháp canh tác phù hợp hạn chế bệnh. Một trong những phương pháp quan trọng trong quản lý rầy nâu và bệnh virus là ứng dụng các biện pháp tổng hợp (IPM) giải pháp góp phần ổn định lâu dài cho sản xuất lúa ĐBSCL.