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Research article

Rice grassy stunt virus-free and pathogenic rice plants affect the brown planthopper (*Nilaparvata lugens* Stål) life cycle

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Abstract

The developmental duration and performance of the brown planthopper (BPH, *Nilaparvata lugens* Stål), which is an important insect vector, can be influenced by transmission of Rice grassy stunt virus (RGSV) in irrigated rice cultivation in Asian countries including Thailand. In this study, the interaction effect between the statuses (non-viruliferous and viruliferous) of BPH populations on free leaves and RGSV-diseased leaves were investigated using a 2 × 2 factorial in CRD with five replications. The results indicated that there were no significant ($p > 0.05$) interaction between the statuses of BPH vectors and rice plants. In addition, the effects of different RGSV-inoculation periods (10, 20, 30, 40, 50, 60, 70, 80 and 90 days after inoculation) on the developmental periods of each life cycle stage of the non-viruliferous BPH population were investigated following CRD with five replications. The different inoculation periods of rice significantly affected ($p < 0.05$) developmental periods of the first and fourth instar nymph stages as well as on survival periods of both male and female short-winged brachypterous form and long-winged macropterous form. On the contrary RGSV-inoculation periods did not affect ($p > 0.05$) developmental periods of the eggs and other instar stages. These results can be used to improve the effectiveness of insect and rice viral disease control policies and strategies; epidemiological modelling of the virus and vector; outbreak prediction and protection; and agro-ecosystem management systems.

Introduction

The majority of sap-sucking insects such as members of the orders Hemiptera (true bugs: aphids, leafhoppers, and planthoppers), Homoptera (whiteflies) and Thysanoptera (thrips) are important viral transmitted-persistent manner vectors (Hogenhout et al., 2008). The brown planthopper (BPH), *Nilaparvata lugens* (Stål, 1854) (Hemiptera: Delphacidae), is

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a monophagous sap-sucking insect pest of rice plants (Yang et al., 2017), and acts as the major vector of the Rice grassy stunt virus (RGSV or Rice rosette virus, Rice rosette Philippines virus). RGSV was first reported in 1962 and identified in the crops in 1964, which was the early "rice green revolution" period, at the International Rice Research Institute, Los Baños, Laguna in the Philippines, but previously had been noticed in 1959–1960 at the Central Experiment Station, Bureau of Plant Industry, Manila, the Philippines (Ling, 1972; Shikata et al., 1980). Later, it was reported in the other Asian rice-growing areas including Indonesia, Sri Lanka, India, Taiwan, Japan, Malaysia, China and Vietnam (Tantera et al., 1973; Anjaneyulu, 1974; Iwasaki and Shinkai, 1979; Chen, 1984; Chen and Chiu, 1989; Le et al., 2012). However, Hibino (1996) reported that the incidence of RGSV in Asia had declined since 1984 due to the changing proportion of the virus transmission ability of the BPH populations.

In Thailand, RGSV causes rice viral disease which is locally called 'Rohk-kieow-dtia-kaao'. This disease was first noticed in 1966 (Wattanakul and Weerapat, 1967). It was subsequently identified in the crops in December 1982 in local Thai rice varieties, namely, 'Kai-mook-dum' and 'Mo-ra-kot', when rice crops were destroyed by RGSV and BPH outbreaks in Ban-Pho district, Chachoengsao province along with scattered outbreaks in 20–30% of the experimental Thai rice fields in Lam-Luk-Ka district, Pathum Thani province (Disthaporn et al., 1983). In 1986, 1987 and 2007, there have been several scattered outbreaks in irrigated rice fields on the central plain and lower north involving patterns of single and mixed infections with Rice ragged stunt virus (RRSV: Oryzavirus, Reoviridae), and Rice tungro viruses (RTV) (Rice tungro spherical virus [RTSV: Waikavirus, Secoviridae] and Rice tungro baciliform virus [RTBV: Tungrovirus, Caulimoviridae]) (Du et al., 2007).

RGSV is a member of the genus *Tenuivirus* of the family Phenuiviridae and is transmitted by the BPH vector in a persistent propagative manner (Zheng et al., 2014). The specific typical phenotypes are induced by the RGSV infection and BPH behavior interactions and lead to the host plant physiological and phenotypic changes such as excessive tillering (shoot branching), leaf yellowing (chlorosis), small leaf developing, sporadic yellow-brown colored spots on leaves and stunting (Satoh et al., 2013). The life cycle of the BPH vector is incomplete metamorphosis (hemi-metabolism) on host plants and a synchronous pattern, which is related to the biological and environmental conditions in the field (Dader et al., 2017). Research to date has focused on the life cycle under different conditions such as temperature (Manikandan et al., 2015; Guru Pirasanna Pandi et al., 2018), host plant species (Horgan et al., 2018) and planting season (Hu et al., 2019), while other aspects have largely been ignored.

Plant viral infections and the changes in the infected rice plants can potentially influence the survival and developmental periods of the insect vectors (Jiu et al., 2007; Chen et al., 2016). The current study was based on the hypothesis that behavior of insect carrier and virus properties depend on rice plant integrity, such as food plant and host range. The effect of plant virus on the vector reproductive potential is an important key information to understand the ability and transmissibility of infected rice plants. Therefore, the purposes of this study were to assess the influence of RGSV on the performance of its BPH vector. This information will be used to develop the policies and strategies for plant protection and research strategy applications to help the country development.

Materials and Methods

Viral materials

The samples of RGSV-infected rice plants were kindly provided and verified by the Division of Rice Research and Development, Rice Department, Bangkok, Thailand, having been obtained from an irrigated rice field in Nong Suea district, Pathum Thani province, Thailand, during 2018–2019. The diseased rice plant samples were multiplied for routine stock cultures in the greenhouse under conditions of 26±1°C, relative humidity of 70–90% and a photoperiod of 8 hr light to 16 hr darkness; the stock plants were used as the viral plant sources of inoculum to the BPH vectors.

Insect vector materials

The putative viruliferous and non-viruliferous BPH populations (*N. lugens* Stål) were collected using light traps in the same rice field containing the viral rice plant materials. Then they were maintained in insect rearing cages (40 cm × 40 cm × 60 cm) and reared on rice plant seedlings (*Oryza sativa* L. variety RD-7; 7–10 cm in height and 6–9 d after germination [DAG]) for routine stock cultures under greenhouse conditions. To obtain the experimental BPH populations, the mature females were generally transferred for oviposition. Then, after 48 hr, the RD-7 seedlings were replaced to ensure sufficient nutrition and the infested rice plant seedlings with the BPH eggs were cultured in insect-free cages for continuous production of the stock culture of non-viruliferous BPH populations. Various stages of BPH offspring (third generation, F_3) were used in the study.

Rice grassy stunt virus transmission and test sample preparation

After the molting of the third instar nymph, the non-viruliferous BPH populations were used for RGSV inoculation. Fifty individual instar nymphs were fed on infected rice plant samples for 24 hr of the acquisition feeding period (AFP) and the transmitted BPH populations were transferred and reared on the viral-free *O. sativa* L. var. RD-7 rice plant seedlings for 3 d of the latent period (LP). Next, Taichung Native 1 (TN1) rice plant seedlings were inoculated with the viruliferous-BPH status populations for 24 hr of the inoculation access period (IAP) and the inoculated rice plant seedlings were treated and grown under greenhouse conditions. After that, the rice plant leaf test samples were collected at different viral infectious periods (10 days interval) during day 10 to day 90 after inoculation (DAI). Then the leaf samples were cut into 5–7 cm long pieces, before placing and storing for at least 2 hr in Petri dishes containing wet cotton to retain moisture. Subsequently, the non-viruliferous and viruliferous BPH populations of various developmental stages, which had been starved for 2 hr of fasting period (FP), were placed onto the rice plant leaf test samples using a fine brush and were regularly checked for developmental patterns and survival rate. The following parameters were estimated: period of egg fertility to hatching success, individual developmental stages of instar nymph through molting success, and longevity of adults to mortality success.

Experimental design and statistical analysis

The interaction effect between the BPH status (non-viruliferous BPH populations on free leaves and on RGSV-diseased leaves, and viruliferous BPH populations on free leaves, and on RGSV-diseased leaves) were investigated by using a 2 × 2 factorial in CRD with five replications. Simultaneously, the effects of different RGSV-inoculation periods or days after inoculation of rice plants (DAI: 10, 20, 30, 40, 50, 60, 70, 80 and 90) on the survival and developmental durations of each life cycle stage of the non-viruliferous BPH population were investigated following CRD with five replications. The data were analyzed by using analysis of variance facilitated by the IBM SPSS 16.0 software package (IBM; USA). Mean values were compared using Duncan's multiple range test, and significance was tested at $\alpha = 0.05$.

Results

Rice grassy stunt virus external symptom detection on rice plants

The test results of TN1 rice plants infested with the viruliferous BPH populations are shown in Table 1. The symptoms continued after infection and began to express the typical and clear symptom appearances between 6–10 DAI and 10–30 DAI. In addition, the RGSV infection occurred at the early vegetative and reproductive phases of rice plants and showed specific disease symptoms (becoming more evident post-infection) than viral-free rice plants including stunting of shoots, excessive tillering (branching), leaf yellowing (chlorosis) with short narrowing and numerous small, irregular pale-brown to dark-brown sporadic spots of various shapes and sizes on leaf blades and leaf veins and leaf tip dying. The ripening phase of the infected rice plants showed delayed flowering and produced a few or small incomplete panicles, small dark-brown colored incomplete panicles and dark-brown unfilled grains (Fig. 1). The experimental RGSV-infected rice plants were similar to those that occurred in naturally infected rice plants.

Table 1 Symptoms on rice plants caused by Rice grassy stunt virus

Growth phase and stage	DAI	External symptoms	Symptomatic proportion
Vegetative phase	6–11	Early stunting	8/10 (80%)
	6–15	Conspicuous yellowish-white striping leaves	8/10 (80%)
	7–15	Early pale-green or pale-yellow leaves	8/10 (80%)
	10–30	Numerous small, irregular dark-brown sporadic spots of various shapes and sizes	8/10 (80%)
	15–45	Excessive tillering and erect leaves	10/10 (100%)
Reproductive phase	50–70	Leaf-yellowing (chlorosis)	10/10 (100%)
		Leaf tips dying	10/10 (100%)
Ripening phase	80–100	Delayed flowering	10/10 (100%)
		Producing few or small incomplete panicles	10/10 (100%)
		Abortive kernels and unfilled grains	10/10 (100%)

DAI = days after inoculation; tested rice plants ($n = 10$) and symptoms were observed under greenhouse conditions in the Division of Rice Research and Development, Rice Department, Bangkok, Thailand.

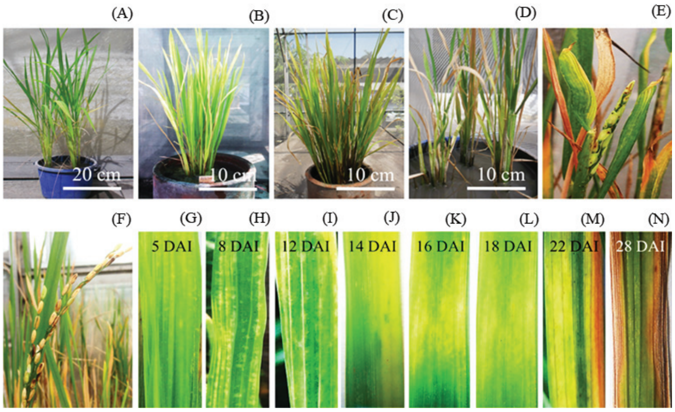

Fig. 1 Symptoms of Rice grassy stunt virus (RGSV)-infected cultivar TN1 rice plants: (A) viral-free TN1 rice plants (45 d after germination, DAG); (B) stunted or dwarfed rice plant growth with pale-green and yellowing erect leaves, short-narrow and uneven-margined leaves; (C) stunted and excessive tillering or branching at nodes at 18–22 d after inoculation (DAI); (D) yellowing and drying leaves; (E and F) delayed flowering, incomplete panicle, malformed flag leaves, abnormal young rice seed and unfilled grains; (G–N) Rice leaf abnormality infected with RGSV at 5 DAI, 8 DAI, 12 DAI, 14 DAI, 16 DAI, 18 DAI, 22 DAI and 28 DAI, respectively

Developmental periods of brown planthopper eggs and nymphs and survival periods of adults

The developmental periods of eggs and 1st to 5th instars of the BPH vector released into the rice plant leaf are shown in Table 2. There were no significant (p = 0.257 – 0.700) interaction between the statuses of adult BPH females and the statuses of rice plants (infected or viral free) on BPH developmental periods (Table 2) and adult BPH survival periods (Table 3). Likewise, neither BPH status nor rice plant status affected the developmental periods of the BPH from eggs to the fifth instar. The significant effect of BPH status was shown only on the survival period of the female short-winged brachypterous form (p = 0.012) whereby the viruliferous BPH tended to have longer survival period than the non-viruliferous.

In addition, the different RGSV-inoculation periods did not have significant effects on a majority of BPH egg and nymph developmental periods except for the first (p = 0.045) and the fourth instar (p = 0.043) (Table 4). Interestingly, survival periods of males and females adult BPH were significantly affected by days after inoculation (p < 0.001 – 0.033) (Table 5). However, the external morphological characters of BPH in the egg stage (Fig. 2), instar-nymph stage (Fig. 3) and adult stage (Fig. 4) were not different between the non-viruliferous and viruliferous BPH infested on the viral-free and infected rice plants.

Discussion

The results of the evaluation of the continuity of RGSV-infected rice host plant symptomatology showed variation in their morphology on the growth phases and stages of rice plants. The dominant features were severe stunting and failure to elongate, yellow-leaf discoloration, short-narrow leaves with excessive tillering and failure to head, few or small incomplete panicles and dark-brown to black unfilled grains. In addition, the infected rice leaf blades and leaf veins often had a conspicuous yellowing pattern due to chlorophyll degradation (Piao et al., 2017), with the leaf-yellowing probably caused by plant-nutritional deficiency, genetic variation of hosts, environmental stresses, pest or disease damage or alterations in the chloroplast development after infection.

The chlorosis patterns were yellowish-white striping, pale-green to pale-yellow discoloration with numerous, small, irregular yellow-brown or dark-brown sporadic spots of various shapes and sizes, leaf-yellowing and drying-up from tips, necrosis and eventual wilting. The newly developmental infected leaves may be mottled or striped. Hibino et al. (1985) reported that the chlorosis pattern caused by RGSV infection was similar to Tungro-like symptoms caused by RTV infection. These infected leaves turned pale-green to dark-green when nitrogen fertilizer was applied.

Table 2 The developmental periods of egg and instar nymph stages of brown planthopper (BPH) reared on free- or RGSV-diseased rice plants

BPH status (Factor A)	Rearing status (Factor B)	BPH developmental period (d)					
		Egg stage	Instar nymph stage				
			First	Second	Third	Fourth	Fifth
Non viruliferous-BPH	On free leaves	10.000±0.707^a	3.500±0.354^a	2.200±0.274^a	3.100±0.224^a	2.900±0.224^a	2.900±0.224^a
	On RGSV-diseased leaves	9.800±0.447^a	3.200±0.274^a	2.000±0.000^a	3.200±0.274^a	2.800±0.274^a	2.800±0.274^a
Viruliferous-BPH	On free leaves	9.600±0.548^a	3.200±0.274^a	2.200±0.447^a	3.100±0.224^a	2.900±0.224^a	2.800±0.274^a
	On RGSV-diseased leaves	9.600±0.548^a	3.200±0.274^a	2.100±0.224^a	3.100±0.224^a	2.900±0.224^a	2.900±0.224^a
	BPH status (A)*	0.257	0.274	0.700	0.644	0.644	1.000
	Rearing status (B)*	0.700	0.274	0.257	0.644	0.644	1.000
	A × B*	0.700	0.274	0.700	0.644	0.644	0.384

RGSV = Rice grassy stunt virus

Values (mean ± SD) in the same column superscripted by different lowercase letters are significantly (p < 0.05) different.

* probability for F-value from analysis of variance

Table 3 The survival periods of adult stages of brown planthopper (BPH) reared on free- or RGSV-diseased rice plants

BPH status (Factor A)	Rearing status (Factor B)	Adult BPH survival period (d)			
		Short-winged brachypterous form		Long-winged macropterous form	
		Male	Female	Male	Female
Non viruliferous-BPH	On free leaves	12.600±0.418^{a}	14.400±0.548^{a}	13.400±0.548^{a}	15.800±0.837^{a}
	On RGSV-diseased leaves	12.900±0.224^{a}	14.800±0.447^{ab}	13.600±0.418^{a}	15.800±0.447^{a}
Viruliferous-BPH	On free leaves	12.800±0.447^{a}	15.200±0.447^{b}	13.800±0.274^{a}	16.100±0.894^{a}
	On RGSV-diseased leaves	12.800±0.447^{a}	15.200±0.447^{b}	13.800±0.758^{a}	15.800±1.095^{a}
	BPH status (A)*	0.781	0.012	0.224	0.699
	Rearing status (B)*	0.409	0.360	0.679	0.699
	A × B*	0.409	0.360	0.679	0.699

RGSV = Rice grassy stunt virus

Values (mean ± SD) in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

* probability for F-value from analysis of variance

Table 4 The developmental periods of egg and instar nymph stages of brown planthopper (BPH) reared on rice plants at different RGSV-inoculation periods

Days after inoculation (DAI)		BPH developmental period (d)					
		Egg stage	Instar nymph stage				
			First	Second	Third	Fourth	Fifth
Vegetative growth							
10	Initial tillering stage	9.800±0.447^{a}	3.200±0.447^{ab}	2.100±0.224^{a}	3.000±0.000^{a}	3.000±0.000^{ab}	2.900±0.224^{a}
20	Early tillering stage	10.000±0.000^{a}	3.600±0.418^{bc}	2.100±0.224^{a}	3.100±0.224^{a}	2.800±0.274^{a}	3.000±0.000^{a}
30	Middle tillering stage	9.600±0.548^{a}	3.100±0.224^{a}	2.200±0.447^{a}	3.200±0.274^{a}	3.000±0.000^{ab}	3.000±0.000^{a}
40	Final tillering stage	10.000±0.000^{a}	3.200±0.27^{ab}	2.100±0.224^{a}	3.300±0.447^{a}	3.000±0.000^{ab}	3.000±0.000^{a}
Reproductive growth							
50	Initial panicle stage	10.000±0.000^{a}	3.400±0.224^{abc}	2.200±0.274^{a}	3.200±0.274^{a}	3.100±0.224^{bc}	3.200±0.274^{a}
60	Middle panicle stage	10.000±0.707^{a}	3.700±0.27^{c}	2.400±0.418^{a}	3.200±0.447^{a}	3.100±0.224^{bc}	3.200±0.274^{a}
70	Flowering stage	10.200±0.447^{a}	3.200±0.27^{ab}	2.400±0.418^{a}	3.400±0.418^{a}	3.100±0.224^{bc}	3.100±0.224^{a}
Ripening growth							
80	Milk stage	10.000±0.000^{a}	3.400±0.224^{abc}	2.500±0.500^{a}	3.500±0.354^{a}	3.300±0.274^{c}	3.200±0.274^{a}
90	Initial dough stage	10.000±0.707^{a}	3.400±0.224^{abc}	2.300±0.447^{a}	3.500±0.354^{a}	3.100±0.224^{bc}	3.100±0.224^{a}
	p-Value	0.660	0.045	0.577	0.266	0.043	0.216

RGSV = Rice grassy stunt virus

Values (mean ± SD) in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 5 The survival periods of adult stages of brown planthopper (BPH) reared on rice plants at different RGSV-inoculation periods

Days after inoculation (DAI)		Adult BPH survival period (d)			
		Short-winged brachypterous form		Long-winged macropterous form	
		Male	Female	Male	Female
Vegetative growth					
10	Initial tillering stage	12.600±0.548^{a}	14.600±0.548^{ab}	13.600±0.418^{a}	16.000±0.707^{bc}
20	Early tillering stage	13.000±0.000^{ab}	15.200±0.447^{bc}	13.600±0.418^{a}	16.200±0.837^{c}
30	Middle tillering stage	13.200±0.447^{ab}	15.600±0.548^{c}	13.800±0.837^{ab}	16.100±0.224^{c}
40	Final tillering stage	13.200±0.447^{ab}	15.200±0.837^{bc}	14.000±0.000^{abc}	16.100±0.742^{c}
Reproductive growth					
50	Initial panicle stage	13.600±0.418^{b}	15.000±0.000^{abc}	14.200±0.447^{abcd}	15.800±0.447^{bc}
60	Middle panicle stage	13.200±0.570^{ab}	15.200±0.447^{bc}	14.200±0.447^{abcd}	15.600±0.418^{bc}
70	Flowering stage	13.600±0.548^{b}	14.600±0.548^{ab}	14.500±0.500^{bcd}	14.600±0.548^{a}
Ripening growth					
80	Milk stage	13.500±0.500^{b}	14.500±0.500^{ab}	14.800±0.447^{d}	15.200±0.570^{ab}
90	Initial dough stage	13.500±0.500^{b}	14.400±0.548^{a}	14.600±0.548^{cd}	14.700±0.447^{a}
	p-Value	0.033	0.011	0.002	< 0.001

RGSV = Rice grassy stunt virus

Values (mean ± SD) in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

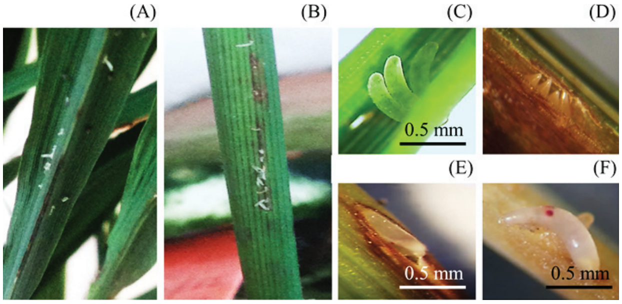

Fig. 2 Embryonic development of brown planthopper (BPH) egg: (A and B) inserted BPH eggs into midrib of leaves on ventral side; (C) groups of inserted banana-shaped eggs (2–5) in rice plant tissues (1 d after oviposition, DAO); (D and E) BPH eggs inside with dark brown-incision on leaf sheath tissues; (F) eggs covered with a dome shape (flat) or egg cap that protects eggs from environmental harm and also showing red-eye spot (3–5 DAO)

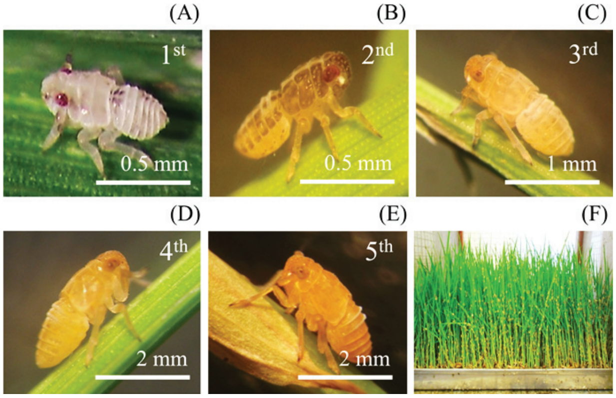

Fig. 3 Development of brown planthopper (BPH) instar nymph stages: (A) first instar nymph stage (3 d after hatching, DAH); (B–E) developmental stages of second to fifth instar nymph at 5 DAH, 8 DAH, 11 DAH and 15 DAH, respectively, with red and pale-brown to dark-brown eye spots, and pale-brown to dark-brown body of cuticle-colored layer; (F) BPH nymphs feeding on rice plant seedlings

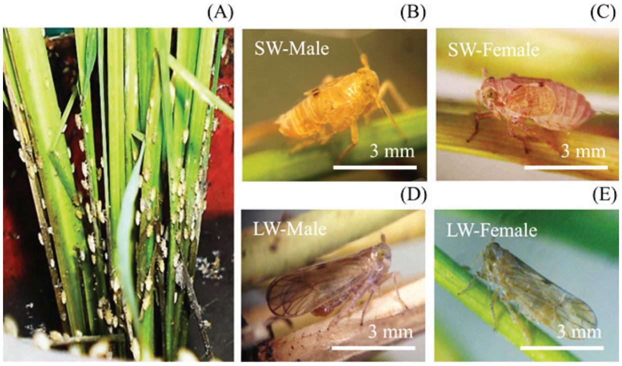

Fig. 4 Wing dimorphism of brown planthopper (BPH) adult stages: (A) large numbers of BPH at base of rice plants at water level; (B and C) adult stages of short-winged (SW) brachypterous form of male and female, respectively; (D and E) adult stages of long-winged (LW) macropterous form of male and female, respectively

The specific physiological abnormalities and height reduction phenomenon associated with multiplication of RGSV in the rice plant cells has been reported by Coloquio and Koganezawa (1992) wherein the high viral loads were observed in the leaf and shoot sheaths (5–10 DAI) and roots (35–63 DAI) of the infected rice. In addition, Satoh et al. (2013) reported that the RGSV-infected rice plants disturbed the plant biochemical process and the metabolic and physiological systems, which led to harmful effects on rice plant health from the lack of basic, essential materials for rice plant growth and development.

A plant virus can induce various plant responses such as systemic necrosis, pigmentation and structural changes, imbalanced or excessive accumulation of the photo-assimilates that inhibit the host photosynthesis and lead to vector physiology, sap-feeding and survival behavior (He et al., 2015; Zhao et al., 2016; Chen and Wei, 2020). However, little is known about the interactive effects of plant viruses on life parameters and the reproduction of insect vectors on rice host plants. The interactive effects of the RGSV-infected rice host plant and the BPH vector on the performance of the vector's life cycle studied in the current research showed no significant interaction associated with survival rate between the non-viruliferous and viruliferous BPH populations or between the viral-free and RGSV-infected rice plants on the lifespan of each individual stage. In addition, Ingwell et al. (2012) reported that the effects of viral infection on biochemical and physiological changes of the host plant can induce plant secondary metabolites which attract the transmission of insect vectors. Although the insect's life cycle was used as an indicator of the viral impact on the performance of vectors, understanding of the survival rate and the effects of viral-pathogens on vectors was limited by the diversity of virus-host interactions.

The vector lifespan is essential for persistent RGSV transmission allowing more time for stylet salivation. The infected rice plants have higher potential to produce the viruliferous status of BPH vector and to serve as viral sources for longer periods than viral-free rice plants. These phenotypic changes of host plants involve the alteration of vector orientation, quality or palatability and transmission. Egg deposition and laying behavior by insect vectors onto the host plants changed in the genotypic and variation of plant responses. In particular, the plant phytochemical profiles and plant volatile compounds may affect the oviposition-site selection by the adult BPH females (Hilker and Fatouros, 2015; Griese et al., 2017). In the current study, it was hypothesized that the RGSV-infected plant responses lead to protection of insect eggs against generalist predators and unsuitable environments. The egg developmental duration and the developments of an instar nymph and adult stages were similar to reports of studies on the white-backed planthopper (WBPH: *Sogatella furcifera* Horváth, 1899; Hemiptera, Delphacidae) and Southern rice black-streaked dwarf virus (SRBSDV: Fijivirus, Reoviridae) by He et al. (2014), and Zhang et al. (2014). However, the longevity period of the adult BPH vector was slightly shorter when reared on RGSV-infected rather than viral-free rice plants, which was consistent with Tu et al. (2013), Lei et al. (2014) and An et al. (2015).

In general, the BPH populations needed a host and suitable food plants for sap-feeding, living and completion of the life cycle, which were related to biological and ecological fitness (Lu et al., 2007), especially nitrogen (N) in rice plants through the leaf nitrogen concentration (Mattson, 1980). N is the most important plant nutrient and a major component of chlorophyll (Chl) and proteins, which play a key role in the process of photosynthesis (Bassi et al., 2018). Besides, considerable variation in the Chl and N contents regularly led to alterations in the degree of rice leaf greenness, leaf size and shape, rice growth phases and development, yield and quality (Long et al., 2013). Higher BPH infestations on rice plant caused hopper burn resulting in the moisture content in the rice host plants decreasing from about 84% to 72% (Santa, 1959) and from 76% to 62% during ingestion (Cagampang et al., 1974). It also led to rice leaf yellowing (chlorosis), and drying up of rice plants (hopper burn) (Bae and Pathak, 1970). However, the lower infestations reduced the number of tillers and panicles and total rice grain weight. In addition, unfilled rice grains were resulted from BPH infestation during the reproductive phase, but not during the vegetative phase (Rubia-Sachez et al., 2003).

The surviving BPH population has a longer life span when reared on a high N content in the rice host plant, with the instar nymphs reducing their developmental period by 2–3 d (Rashid et al., 2016). The short developmental periods induce the short-winged brachypterous form of the adult stage, with higher female reproductive rates and a shorter pre-oviposition period resulting in laying more eggs (Lu et al., 2004). In addition, they lived longer with high rates of aggregation and population buildup, feeding preference, honeydew excretion, tendency for outbreak and viral transmission of serious diseases. These phenomena have been found in sap-feeding pests such as small brown planthopper (SBPH: *Laodelphax striatellus* Fallén, 1826; Hemiptera, Delphacidae), WBPH (*S. furcifera* Horváth, 1899), green rice leafhopper (GRLH: *Nephotettix cincticeps* Uhler, 1896; Hemiptera, Cicadellidae), rice stem maggot (RSM: *Chlorops oryzae* Matsumura, 1915; Diptera, Chloropidae), Asiatic rice borer or striped rice stem borer (*Chilo suppressalis* Walker, 1863; Lepidoptera, Crambidae) and rice leaffolder (RLF: *Cnaphalocrocis medinalis* Guenée, 1854; Lepidoptera, Crambidae) (Ma and Lee, 1996).

Based on the above results, there were no significant interaction between the statuses of vectors and rice plants on the developmental periods of eggs and nymphs, and survival periods of adults. In addition, the different RGSV-inoculation periods had significant effects on the developmental periods of the first and fourth instar nymphs and survival periods of adults. The results have several possible explanations. We have three hypotheses. First, the persistent infection is continually presents in the host cells and goes throughout the life of the insect vector without changing the external morphology of insect. This hypothesis is related to the insect-immune regulatory systems (Chen et al., 2019), RNA interference (RNAi) pathway, autophagy, and apoptosis (Chen and Wei, 2020), thereby affecting the viruliferous insect vectors which act as asymptomatic transmitters. Second, RGSV did not pass through the insect egg, resulted in the status of non-infectious BPH populations after hatching. This hypothesis is related to the mechanism of transovarial transmission of persistent-plant virus in insect vector (Hogenhout et al., 2008). The coat proteins of RGSV (RGSV-CP) did not interact with vitellogenin (Vg), which is the precursor of major yolk protein (MYP) of insect ovariesy (Liao et al., 2017). Therefore, RGSV cannot effectively overcome the transovarial transmission barriers and spread into the insect ovaries. Several virus-vector systems such as RRSV-BPH (*N. lugens* Stål, 1854) (Huang et al., 2015) and SRBSDV-WBPH (*S. furcifera* Horváth, 1899) similarly operate (Zhang et al., 2014). Third, the infectious status of host plants changes plant compounds and morphologies, resulting in the plant-feeding behavior of insects (Wang et al., 2018). Thus, it could be speculated that the performance of insect vectors on host plants might vary according to, the morphology, the status of nutritional quality and secondary metabolites in the host plants. All of these may cause the plant-virus-vector interactions in discontinuous trend under the various environmental conditions for the developmental periods of instar nymph stages and survival periods of adult stages. However, the confirmation and validation of these explanations require additional research in the fields.

Rice host plant quality defines the host plant components, and clearly is a potential threat to insect behavior, that can positively or negatively influence survival and the developmental period patterns, depending on both the environmental characteristics and internal physiological processes. The changes in host plant quality for insect herbivores, which are induced by plant damage from insect behavior and viral infection, resulted in reducing the pre-survival period, increasing the duration of development and disturbing the feeding behavior of the insects. The persistent propagative virus could increase host plant qualities. It had a positive impact on vector fitness through the down-regulation of pathways involved in defense responses, resulting in reduced plant cell wall composition, with altered amino acids and secondary metabolite production of plant sap (Gutiérrez et al., 2013). However, the vector insects preferred infected host plants. Thus, it could be speculated that the current results were due to altered plant nutrition or to the direct negative effect of the virus on its vector, but not the emergence of the instar nymphs.

The survival rate of the BPH stage decreased and was influenced by the food sources and variation in the temperature (Manikandan et al., 2015). Srinivas et al. (2016) and Sucheta and Mayabini (2012) reported that the most favorable temperature ranges for egg laying by adult BPH females in the development of the instar nymph and adult stages were 27–33°C and 25–35°C, respectively, and completely stopped above 40°C. Alagar et al. (2007) reported that such BPH behavior on susceptible TN1 rice plants produced higher numbers than on the resistant genotypes of rice varieties such as PTB33, ASD7, ADT45, CO43 and KAU1661, respectively. In addition, the rice host plant quality defined the plant components and was clearly a potential threat to insect vector behavior that produced positive or negative performance in survival rates and developmental period patterns and depended on both the environmental characteristics and the internal physiological processes.

The changes in host plant quality were induced by plant damage from the insect-feeding behavior and viral infection, with these phenomena not only reducing the pre-survival period but also increasing the developmental duration. In addition, the rice viral transmissibility of the viruliferous BPH status regularly decreased during the insect's lifespan, which was correlated with a decrease in the viral acquisition and did not disappear entirely. Although the longer developmental periods of the instar nymphs and survival periods of the adults benefited the viral acquisition and transmission and might increase the rice infection rate, in general, the rice viral infections of insect vectors and rice plants were unfavorable to BPH vector population expansion. Therefore, the host-interactions of BPH and the rice plant, as revealed by this study, demonstrated that the infection with RGSV was caused by the inconstancy of developmental and survival periods of each BPH life cycle stages. Developmental research approaches are required to apply this information using systematic studies in the eco-management of rice fields, so that the results can be generalized for future use.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Alagar, M., Suresh, S., Samiyappan, R., Saravanakumar, D. 2007. Reaction of resistant and susceptible rice genotypes against brown planthopper (*Nilaparvata lugens*). Phytoparasitica 35: 346–356. doi.org/10.1007/BF02980697

An, X.K., Hou, M.L., Liu, Y.D. 2015. Relation between the viral load accumulation of Southern rice black-streaked dwarf virus and the different developmental stages of *Sogatella furcifera* (Hemiptera: Delphacidae). J. Econ. Entomol. 108: 917–924. doi.org/10.1093/jee/tov065

Anjaneyulu, A. 1974. Identification of grassy stunt, a new virus disease of rice in India. Curr. Sci. 43: 416–417.

Bae, S.H., Pathak, M.D. 1970. Life history of *Nilaparvata lugens* (Homoptera: Delphacidae) and susceptibility of rice varieties to its attacks. Ann. Entomol. Soc. Am. 63: 149–155. doi.org/10.1093/aesa/63.1.149

Bassi, D., Menossi, M., Mattiello, L. 2018. Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. Sci. Rep. 8: 2327. doi.org/10.1038/s41598-018-20653-1

Cagampang, G.B., Pathak, M.D., Juliano, B.O. 1974. Metabolic changes in the rice plant during infestation by the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). Appl. Entomol. Zool. 9: 174–184. doi.org/10.1303/aez.9.174

Chen, C.C. 1984. Rice virus diseases transmitted by brown planthopper in Taiwan with special reference to rice wilted stunt. Formos. Entomol. 4: 83–91. doi.org/10.6660/TESFE.1984017

Chen, C.C., Chiu, R.J. 1989. Transmission of Rice wilted stunt virus by brown planthopper (*Nilaparvata lugens* Stål). Taichung DAIS. 23: 3–10.

Chen, Q., Wei, T. 2020. Cell biology during infection of plant viruses in insect vectors and plant hosts. Mol. Plant-Microbe Interact. 33: 18–25. doi.org/10.1094/MPMI-07-19-0184-CR

Chen, Q., Zheng, L., Mao, Q., et al. 2019. Fibrillar structures induced by a plant reovirus target mitochondria to activate typical apoptotic response and promote viral infection in insect vectors. PLoS Pathog. 15: e1007510. doi.org/10.1371/journal.ppat.1007510

Chen, Y., Lu, C., Li, M., Wu, W., Zhou, G., Wei, T. 2016. Adverse effects of Rice gall dwarf virus upon its insect vector *Recilia dorsalis* (Hemiptera: Cicadellidae). Plant Dis. 100: 784–790. doi.org/10.1094/PDIS-06-15-0713-RE

Coloquio, E.L., Koganezawa, H. 1992. Nonspecific reaction in ELISA of viruses in rice roots. Int. Rice. Res. Newsl. 17: 11.

Dader, B., Then, C., Berthelot, E., Ducousso, M. Ng, J.C.K., Drucker, M. 2017. Insect transmission of plant viruses: Multilayered interactions optimize viral propagation. Insect Sci. 24: 929–946. doi.org/10.1111/1744-7917.12470

Disthaporn, S., Chettanachit, D., Putta, M. 1983. Unknown virus-like rice disease in Thailand. Int. Rice. Res. Newsl. 8: 12.

Du, P.V., Cabunagan, R.C., Cabauatan, P.Q., Choi, H.S., Choi, I.R., Chien, H.V., Huan, N.H. 2007. Yellowing syndrome of rice: Etiology, current status, and future challenges. Omonrice 15: 94–101.

Griese, E., Dicke, M., Hilker, M., Fatouros, N.E. 2017. Plant response to butterfly eggs: Inducibility, severity and success of egg-killing leaf necrosis depends on plant genotype and egg clustering. Sci. Rep. 7: 7316. doi.org/10.1038/s41598-017-06704-z

Guru Pirasanna Pandi, G., Chander, S., Singh, M.P., Pathak, H. 2018. Impact of elevated CO_2 and temperature on brown planthopper population in rice ecosystem. Proc. Natl. Acad. Sci. India, Sect. B Biol. Sci. 88: 57–64. doi.org/10.1007/s40011-016-0727-x

Gutiérrez, S., Michalakis, Y., Van Munster, M., Blanc, S. 2013. Plant feeding by insect vectors can affect life cycle, population genetics and evolution of plant viruses. Funct. Ecol. 27: 610–622. doi.org/10.1111/1365-2435.12070

He, W.-B., Li, J., Liu, S.-S. 2015. Differential profiles of direct and indirect modification of vector feeding behaviour by a plant virus. Sci. Rep. 5: 7682. doi.org/10.1038/srep07682

He, X., Xu, H., Gao, G., et al. 2014. Virus-mediated chemical changes in rice plants impact the relationship between non-vector planthopper *Nilaparvata lugens* Stål and its egg parasitoid *Anagrus nilaparvatae* Pang et Wang. PLoS One. 9: e105373. doi.org/10.1371/journal.pone.0105373

Hibino, H. 1996. Biology and epidemiology of rice viruses. Annu. Rev. Phytopathol. 34: 249–274. doi.org/10.1146/annurev.phyto.34.1.249

Hibino, H., Cabauatan, P.Q., Omura, T., Tsuchizaki, T. 1985. Rice grassy stunt virus strain causing tungrolike symptoms in the Philippines. Plant Dis. 69: 538–541. doi.org/10.1094/PD-69-538

Hilker, M., Fatouros, N.E. 2015. Plant responses to insect egg deposition. Annu. Rev. Entomol. 60: 493–515. doi.org/10.1146/annurev-ento-010814-020620

Hogenhout, S.A., Ammar, E.-D., Whitfield, A.E., Redinbaugh, M.G. 2008. Insect vector interactions with persistently transmitted viruses. Annu. Rev. Phytopathol. 46: 327–359. doi.org/10.1146/annurev.phyto.022508.092135

Horgan, F.G., Peñalver Cruz, A., Bernal, C.C., Ramal, A.F., Almazan, M.L.P., Wilby, A. 2018. Resistance and tolerance to the brown planthopper, *Nilaparvata lugens* (Stål), in rice infested at different growth stages across a gradient of nitrogen applications. Field Crops Res. 217: 53–65. doi.org/10.1016/j.fcr.2017.12.008

Hu, G., Lu, M.-H., Reynolds, D.R., et al. 2019. Long-term seasonal forecasting of a major migrant insect pest: The brown planthopper in the Lower Yangtze River Valley. J. Pest Sci. 92: 417–428. doi.org/10.1007/s10340-018-1022-9

Huang, H.-J., Bao, Y.-Y., Lao, S.-H., et al. 2015. Rice ragged stunt virus-induced apoptosis affects virus transmission from its insect vector, the brown planthopper to the rice plant. Sci. Rep. 5: 11413. doi.org/10.1038/srep11413

Ingwell, L.L., Eigenbrode, S.D., Bosque-Pérez, N.A. 2012. Plant viruses alter insect behavior to enhance their spread. Sci. Rep. 2: 578. doi.org/10.1038/srep00578

Iwasaki, M., Shinkai, A. 1979. Occurrence of rice grassy stunt disease in Kyushu, Japan. Jpn. J. Phytopathol. 45: 741–744. doi.org/10.3186/jjphytopath.45.741

Jiu, M., Zhou, X.-P., Tong, L., Xu, J., Yang, X., Wan, F.-H., Liu, S.-S. 2007. Vector-virus mutualism accelerates population increase of an invasive whitefly. PLoS One. 2: e182. doi.org/10.1371/journal.pone.0000182

Le, T.T., Luong, V.D., Ngo, T.T.N., Pham, V.K. 2012. Induced systemic resistance against Rice grassy stunt virus—a promising field for ecological rice production. J. Viet. Env. 2: 48–53. doi.org/10.13141/jve.vol2.no1.pp48-53

Lei, W., Liu, D., Li, P., Hou, M. 2014. Interactive effects of Southern rice black-streaked dwarf virus infection of host plant and vector on performance of the vector, *Sogatella furcifera* (Homoptera: Delphacidae). J. Econ. Entomol. 107: 1721–1727. doi.org/10.1603/EC13569

Liao, Z., Mao, Q., Li, J., et al. 2017. Virus-induced tubules: A vehicle for spread of virions into ovary oocyte cells of an insect vector. Front. Microbiol. 8: 475. doi.org/10.3389/fmicb.2017.00475

Ling, K.C. 1972. Rice virus diseases. International Rice Research Institute (IRRI). Los Baños, the Philippines.

Long, J.R., Ma, G.H., Wan, Y.Z., Song, C.F., Sun, J., Qin, R.J. 2013. Effects of nitrogen fertilizer level on chlorophyll fluorescence characteristics in flag leaf of super hybrid rice at late growth stage. Rice Sci. 20: 220–228. doi.org/10.1016/S1672-6308(13)60138-9

Lu, Z., Heong, K.L., Yu, X., Hu, C. 2004. Effects of plant nitrogen on ecological fitness of the brown planthopper, *Nilaparvata lugens* Stål. in rice. J. Asia Pac. Entomol. 7: 97–104. doi.org/10.1016/S1226-8615(08)60204-6

Lu, Z., Yu, X., Heong, K.L., Hu, C. 2007. Effect of nitrogen fertilizer on herbivores and its stimulation to major insect pests in rice. Rice Sci. 14: 56–66. doi.org/10.1016/S1672-6308(07)60009-2

Ma, K., Lee, S. 1996. Occurrence of major rice insect pests at different transplanting times and fertilizer levels in paddy field. Korean J. Appl. Entomol. 35: 132–136.

Manikandan, N., Kennedy, J.S., Geethalakshmi, V. 2015. Effect of temperature on life history parameters of brown planthopper (*Nilaparvata lugens* Stål). Afr. J. Agric. Res. 10: 3678–3685. doi.org/10.5897/AJAR2015.10094

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11: 119–161. doi.org/10.1146/annurev.es.11.110180.001003

Piao, W., Han, S.H., Sakuraba, Y., Paek, N.C. 2017. Rice 7-hydroxymethyl chlorophyll a reductase is involved in the promotion of chlorophyll degradation and modulates cell death signaling. Mol. Cells. 40: 773–786. doi.org/10.14348/molcells.2017.0127

Rashid, M.M., Jahan, M., Islam, K.S. 2016. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Sci. 23: 119–131.

Rubia-Sachez, E., Suzuki, Y., Arimura, K., Miyamoto, K., Matsumura, M., Watanabe, T. 2003. Comparing *Nilaparvata lugens* (Stål) and *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae) feeding effects on rice plant growth processes at the vegetative stage. Crop Prot. 22: 967–974. doi.org/10.1016/S0261-2194(03)00112-1

Santa, H. 1959. Damages of rice plants caused by planthoppers. Plant Protection (Tokyo). 13: 307–310.

Satoh, K., Yoneyama, K., Kondoh, H., et al. 2013. Relationship between gene responses and symptoms induced by Rice grassy stunt virus. Front. Microbiol. 4: 313. doi.org/10.3389/fmicb.2013.00313

Shikata, E., Senboku, T., Ishimizu, T. 1980. The causal agent of Rice grassy stunt disease. Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci. 56: 89–94.

Srinivas, M., Rama, N., Varma, G., Jagadeeshwar, R. 2016. Effect of temperature on rice brown planthopper (*Nilaparvata lugens* Stål). The J. Res. PJTSAU. 44: 114–117.

Sucheta, R., Mayabini, J. 2012. Effect of high temperature on the multiplication of brown planthopper *Nilaparvata lugens* (Stål). Oryza 49: 288–291.

Tantera, D.M., Satomi, H., Roechan, M. 1973. Grassy stunt disease of rice in Indonesia. Contr. Centr. Res. Inst. Agric. Bogor. 2: 1–8.

Tu, Z., Ling, B., Xu, D., Zhag, M., Zhou, G. 2013. Effects of Southern rice black-streaked dwarf virus on the development and fecundity of its vector, *Sogatella furcifera*. Virol. J. 10: 145. doi.org/10.1186/1743-422X-10-145

Wang, Q., Li, J., Dang, C., Chang, X., Fang, Q., Stanley, D., Ye, G. 2018. Rice dwarf virus infection alters green rice leafhopper host preference and feeding behavior. PLoS One. 13: e0203364. doi.org/10.1371/journal.pone.0203364

Wattanakul, L., Weerapat, P. 1967. Virus diseases of rice in Thailand. In: Proceedings of the Symposium on the Virus Diseases of the Rice Plant. Los Baños, the Philippines, pp. 79–85.

Yang, L., Han, Y., Li, P., Wen, L., Hou, M. 2017. Silicon amendment to rice plants impairs sucking behaviors and population growth in the phloem feeder *Nilaparvata lugens* (Hemiptera: Delphacidae). Sci. Rep. 7: 1101. doi.org/10.1038/s41598-017-01060-4

Zhang, J., Zheng, X., Chen, Y., Hu, J., Dong, J. Su, X., Zhang, Z. 2014. Southern rice black-streaked dwarf virus infection improves host suitability for its insect vector, *Sogatella furcifera* (Hemiptera: Delphacidae). J. Econ. Entomol. 107: 92–97. doi.org/10.1603/EC13204

Zhao, J., Zhang, X., Hong, Y., Liu, Y. 2016. Chloroplast in plant-virus interaction. Front Microbiol. 7: 1565. doi.org/10.3389/fmicb.2016.01565

Zheng, L., Mao, Q., Xie, L., Wei, T. 2014. Infection route of Rice grassy stunt virus, a Tenuivirus, in the body of its brown planthopper vector, *Nilaparvata lugens* (Hemiptera: Delphacidae) after ingestion of virus. Virus Res. 188: 170–173. doi.org/10.1016/j.virusres.2014.04.008