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RICE DWARF VIRUS (RDV) DETECTION IN *Nephotettix nigropictus* (Stål) AND RICE PLANT, AND FIELD REACTION OF RICE CULTIVARS AGAINST RDV IN THE PHILIPPINES

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ABSTRACT

The rice dwarf disease generally occurs in temperate rice growing countries. Its occurrence in the Philippines was reported in 1994 based on transmission studies, electron microscopy of the virus particles, and serological assay of infected plants. A simple nucleic acid amplification technique called the loop-mediated isothermal amplification (LAMP) was applied to detect *Rice dwarf virus* (RDV) in field collected insect vector, *Nephotettix nigropictus* (Stål), individuals and rice plants. Samples were crudely lysated with alkaline lysis method. LAMP successfully detected RDV in both insect vector and plant hosts at 60°C in 60 min. Visual scores of rice dwarf diseased plants in the National Cooperative Trial (NCT) 2014 dry season test showed that rice lines developed for the upland ecosystem obtained the highest infection rate (13.89%); nearly as high as the TN1 check plants (15.21%) while the Multi-Adaptation Trial (MAT) lines obtained the lowest (0.59%). In the 2015 wet season NCT trial, the Traditional rice group recorded the highest percentage of infection (4.81%) followed by those in the direct wet seeded rice (DWSR) category (3.47%) while rice entries in MAT and Transplanted categories obtained the two lowest infection of 1.20% and 1.25%, respectively. The TN1 check variety recorded 2.54% infection. A 0.4% rice dwarf disease incidence was recorded in a field survey in the rice production area of the PhilRice Midsayap Branch station during the 2015 DS cropping. The average number of *N. nigropictus* in the NCT and rice production fields ranged from 0-4.33 for 10 insect net sweeps.

Key words: LAMP, *Nephotettix nigropictus*, *Rice dwarf virus*, virus detection

INTRODUCTION

The rice dwarf disease was first reported in Japan and is considered to be the first known rice virus disease in the world. In addition, several phytopathology historical accounts were attributed to rice dwarf as the first plant virus disease to be transmitted by an insect, first to provide evidence of the multiplication of a plant virus in an insect, and the first virus detected in both plant and insect hosts by electron microscopy (Fukushi, 1934, 1939; Ling, 1972).

The causal agent known as the *Rice dwarf virus* (RDV) is transmitted only by the insect vectors *Nephotettix cincticeps* (Uhler) (Fukushi, 1937), *N. nigropictus* (Stål) (Nasu, 1963), *N. virescens* (Distant) (Xie et al., 1981), and *Recilia dorsalis* (Motschulsky) (Fukushi, 1937). RDV infected plants are stunted with dark green leaves exhibiting chlorotic streaks on the leaf blades. Even when infected at the early stage of growth, the plant survives until harvest but produce no panicles (Fukushi, 1934). RDV is a double-stranded RNA virus belonging to the genus *Phytoreovirus* of the Reoviridae family that replicates in both invertebrate insect vector and plant cells (Boccardo & Milne, 1984).

Plant viruses cause significant economic losses in important crops and no direct method of control is presently available against the causal agent. Thus, diagnostic techniques correlated with the virus-vector interaction are established by transmission studies and symptomatology. In addition, the detection of the virus in the plant and vector is of major importance in virus disease identification and management. Presently, virus detection methods are based on the properties of the virus itself - coat protein and nucleic acid. Coat protein-based virus detection method includes the agglutination test, enzyme-linked immunosorbent assay (ELISA), and immunoblotting. On the other hand, viral nucleic-based techniques as such dot-blot hybridization, polymerase chain reaction (PCR), and the PCR-variant loop-mediated isothermal amplification assay (LAMP) are more sensitive than the coat protein-based methods. These diagnostic methods have higher sensitivity and specificity for a rapid virus diagnosis in disease surveys, epidemiological studies, and breeding programs.

The occurrence of rice dwarf in the Philippines was first noticed in the Philippine Rice Research Institute (PhilRice) branch station in Midsayap, North Cotabato, and was confirmed by insect transmission tests, electron microscopy of clarified sap from infected plants, and serological assays of infected plants using the rapid immunofilter paper assay (RIFA) and the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Among the insect vectors tested, *N. nigropictus* transmitted the virus but not *N. virescens* and *R. dorsalis* (Cabauatan et al., 1994). Since then, no further study on the disease was carried out in the country. This may be due to the inadequate amount or absence of antiserum to assay RDV by ELISA and the limited greenhouse and laboratory facilities in the PhilRice Midsayap station. Nevertheless, the issue on RDV antiserum can now be addressed by the use of LAMP wherein the needed primer is available commercially.

The development and introduction of new rice varieties with greater yield potential, better resistance to insect pests and diseases, and with good eating and/or processing qualities are tested under the National Cooperative Testing (NCT) platform. The outstanding test entries are then recommended to the National Seed Industry Council (NSIC) for release as commercial varieties.

The NCT trial in the PhilRice Midsayap branch station is one of the seven test sites spread across the Philippines. Among the different rice insect pests and diseases under the NCT consideration, tungro disease appraisal is always included because it is commonly observed in this station in both dry and wet seasons. Lately, however, stunted plants with dark green leaves and whitish streaks were also observed. This led the authors to use the advanced molecular virus detection LAMP technique (Natomi et al., 2000) to confirm the presence of RDV in the rice plant and insect vector *N. nigropictus*. In addition, visual evaluation of the NCT test lines to rice dwarf infection based on the characteristic symptoms of the disease was conducted during the 2014 dry season (DS) and 2015 wet season (WS) cropping. The prevalence of RDV-viruliferous *N. nigropictus* and rice dwarf incidence in the rice production fields of the station during the 2015 DS cropping were appraised to provide additional information on the current RDV situation in the place where the disease was initially reported in the Philippines.

MATERIALS AND METHODS

Loop-Mediated Isothermal Amplification (LAMP) Assay

LAMP is a sensitive assay for the detection of the target virus that even one copy of a target gene can trigger amplification that requires extreme aseptic techniques to avoid false positive results. Hence, stringent quality measures and controls were observed for the tests to obtain accurate LAMP results. Aseptic techniques were applied to the designated work areas and negative controls using HPLC water as no template reactions were incorporated in every assay procedure.

LAMP assay was used to detect RDV in rice plant tissues and the insect vector *N. nigropictus*. Diseased plant tissues were randomly collected from the NCT test and Plant Breeding fields in PhilRice Midsayap Station (PhilRice MS) based on the characteristic symptoms of RDV while the healthy plant tissues used as check were taken from caged plants in the greenhouse of the PhilRice Central Experimental Station (PhilRice CES), Science City of Muñoz, Nueva Ecija. On the other hand, *N. nigropictus* were collected from the PhilRice MS fields where the disease is known to occur while the control specimens of the insect were collected from the PhilRice Bicol Station (PhilRice BS) where the disease is not reported to be present.

To optimize the LAMP assay, modifications were made that involved tests using different incubation temperatures and incubation time due to variable results obtained from the initial trials. Different incubation temperatures from 60-65^{o}C with an increment of one degree C were tested. Incubation time using a heat block was observed within 30, 45, and 60 min. After incubation, one mL of

10x Sybr Green dye was added for visualization. A Viewpoint LED Transilluminator (Manila Health Tech, Inc.) with LED bulbs of 460-490nm wavelength and a BBL Crystal™ Panel Viewer were used in the UV and white light visualization of results, respectively. The appearance of green fluorescence in the reaction indicated positive result and orange indicated negative. The LAMP assays were conducted in the laboratory of the Plant Breeding and Biotechnology Division, PhilRice CES, Science City of Muñoz, Nueva Ecija.

LAMP primer set

The LAMP primer set designed by Le et al. (2010) with GenBank accession No. D13773 was adopted. This is a set of four specifically designed primers for RDV which are capable of recognizing six distinct regions on the respective target DNA region. The sequences coding for structural proteins of the virus are as follows:

RDV F3 5'-ATTCCAGCCGGGGCATAT-3'
RDV B3 5'-CCCACCACCAAGTGAGAAC-3'
RDV FIP 5'-AACGCCAGCTATTGTCGTTCCAGGGCATCAGTGCTAAGTGT-3'
RDV BIP 5'-CTACTGCAACTGCCGCAGACGTCCGTTTGGACAGGGAGG-3'

Nucleic acid extraction

The total RNA was crudely extracted from infected and healthy leaf samples following the method described by Wang et al. (1993), with minor modifications. From the field-collected diseased leaf samples, about one-cm long piece was cut and placed in microcentrifuge tube where 50µL of 0.5N NaOH was added. The leaf was crushed thoroughly by a sterilized plastic rod (Golden Bat (Far East) Inc., Quezon City, Philippines) and 20µL of crushed sample was aliquoted and transferred into a new microcentrifuge tube. Then, 150µL of Tris-HCl, pH 8.0 was added and mixed by lightly tapping the tube and placed in a tray with crushed ice or in freezer for later use.

Field-collected *N. nigropictus* samples were morphologically examined and identified. The crude RNA was extracted by crushing each insect with a rod in 50µL of 0.5N NaOH in a microcentrifuge tube. Then, 5-10µL of lysate was added with 150µL of Tris-HCl, pH 8.0, and kept in cold condition for LAMP assay.

Field collection of test samples for LAMP assay

Insect vector test samples. Samples of the insect vector *N. nigropictus* (Figure 1) were collected from the PhilRice MS using a 30-cm insect net after repeated sweepings in fields with rice crop at late tillering stage. In addition, *N. nigropictus* were also collected from rice seedlings growing in three areas of the field where harvested rice was threshed. The collected insects were sorted and the *N. nigropictus* were individually placed in microcentrifuge tubes and labeled. For comparison, *N. nigropictus* were also collected from the PhilRice BS in Ligao, Albay where the rice dwarf disease is not known to be present. Adult *N. nigropictus* were used in the LAMP assay.

Rice plant test samples. Rice plants in the field exhibiting the rice dwarf symptoms of severe stunting and presence of white streaks on the leaf blades (Figure 1) were sampled. With the use of hand gloves, a half portion of the second youngest leaf of suspected infected plant was obtained and placed in a labeled glassine bag.

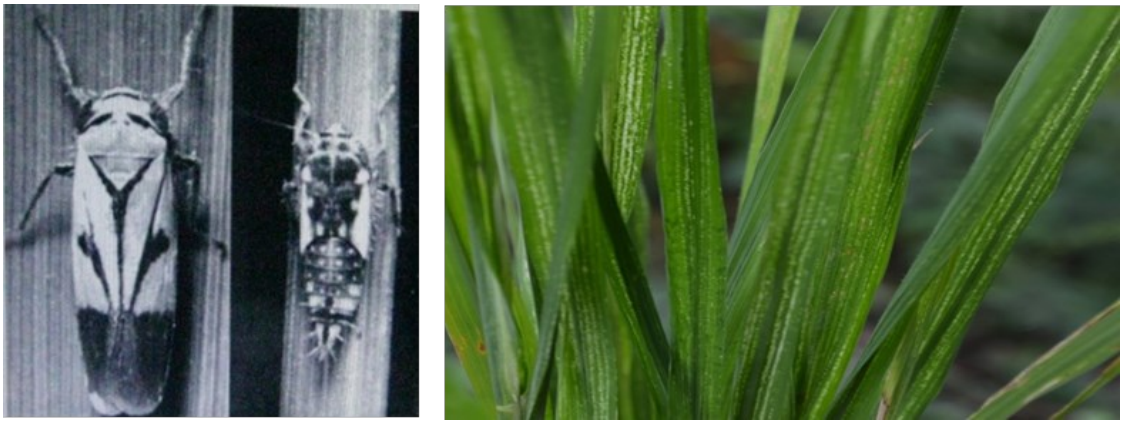

Figure 1. Adult and nymph *Nephotettix nigropictus* (left) and rice dwarf diseased plant with prominent streaks along the leaf blades (right).

All collected insect vector and plant samples were kept in an air-conditioned room before transport by air the following day to the PhilRice CES for the LAMP assay.

RDV Incidence in the National Cooperative Test (NCT) entries

Ten to 20 plants of each test entry in the NCT trial in the PhilRice MS were meticulously scored visually for presence of whitish streaks on the leaf blades and plant stunting to determine the percentage rice dwarf infected plants at 54 days after transplanting (DAT) during the 2014 DS trial and at 50 DAT during the 2015 WS trial. The test entries were composed of elite lines to be recommended for commercial release to farmers under different categories as follows: Transplanted, Direct Wet-Seeded Rice (DWSR), Multi-Adaption Trial (MAT), Special Purpose, Hybrid, Upland, and Rainfed. The rate of RDV infection was compared to the Taichung Native 1 (TN1) check variety.

The abundance of *N. nigropictus* in the NCT test fields as well as in a neighboring weedy field with no apparent rice plant was estimated by conducting three 10-sweep strokes of an insect net. No insecticide application was conducted in the NCT test fields.

Disease field survey

The incidence of rice dwarf disease in the rice production fields in Area 64 of the PhilRice MS was visually assessed in nine contiguous rice paddy fields planted with NSIC Rc 222 at late tillering stage during the 2015 DS cropping. In each rice paddy field, three quadrants, each with 25 rice hills, were visually recorded for plant stunting and the presence of white streaks on the leaf blades to estimate the disease incidence. The abundance of *N. nigropictus* in each field was also estimated by 10 sweep strokes of an insect net. Insecticide application was regularly conducted in these fields.

RESULTS AND DISCUSSION

The set of LAMP primers (Le et al., 2010) successfully detected RDV in both the leaf samples and in the insect vector *N. nigropictus*, at 60°C for 60 min (Figure 2). Also, the RDV strain used by Le et al. (2010) was similar to the Philippine strain. However, the reason on how a temperate rice virus disease like rice dwarf occurred in southern Philippines is not known.

Previous surveys on the abundance of *N. virescens* on volunteer rice plants showed their presence on seedlings that emerged from spilled rice seeds in threshing sites (Tiongco et al., 1992). In this study, 10 of the 15 *N. nigropictus* (average = 66.67%) collected from seedlings growing in three spots where threshing of harvested paddy rice was conducted, were infected with RDV (Table 1). This highlights the importance of spilled seeds in the threshing site as they can later emerge to seedlings and may serve as consequent secondary host for the insect when the main crop is not available after harvest. When traditional thresher is used, threshing sites occur in isolated clumps in the field. Seedlings may emerge around the threshing site even if the culms were burned. However, seedlings from spilled seeds after harvest by the combine harvester are generally

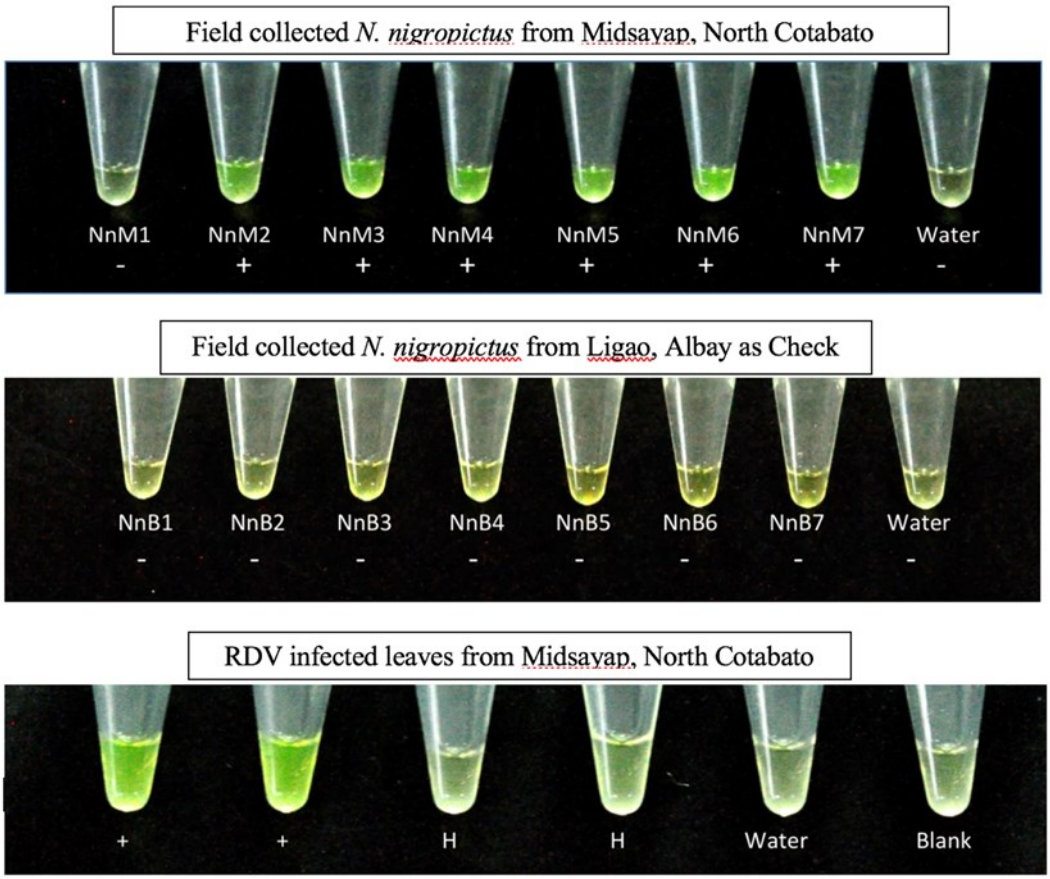

Figure 2. LAMP detection of rice dwarf virus (RDV) in field collected *Nephotettix nigropictus* and rice leaves.

Table 1. Percentage of *Nephotettix nigropictus* detected with rice dwarf virus by LAMP collected from rice and grass fields in PhilRice Bicol and Midsayap branch stations. 2015 DS.

N. nigropictus	**Bicol branch station**			**Midsayap branch station**	
collected[a] in:	**In station**	**In FF[b]**	**Infected (%)**	**In station**	**Infected (%)**
Rice field	6	7	0.00	15[c]	66.67
Grass field	0	0	0.00	2	0.00

[a]by repeated sweeping; [b]farmer's field; [c]from three threshing spots

present in wide areas of the field. In the latter case, the insect had a shorter distance to navigate and access young seedlings, and ,thus have more contact with rice host in short distances that save needed energy to find amiable food. In a tungro study by Ling & Palomar (1966), *N. impicticeps* (now *N. virescens*), a close relative of *N. nigropictus*, infected more 15- and 30-day old rice plants than those at 45, 60, and 90 days old. A comprehensive probe on the effect of employing updated rice threshing machinery on the apparent role of post-harvest re-growths on the spread of insect vector and rice dwarf disease to new plantings is underscored.

None of the 13 *N. nigropictus* individuals collected from the PhilRice BS and farmers' fields in Libon, Albay was infected with RDV. No rice dwarf disease has been reported from these places. This information served to fill the information gaps about the rice dwarf disease epidemiology and vector infectivity in the Philippines and the plausible absence of RDV-viruliferous *N. nigropictus*, at least in Libon, Albay, where the disease is not observed to occur.

Detecting RDV in the insect vector *N. nigropictus* by LAMP was not performed by Le et al. (2010). In this study, the detection of RDV in the insect vector was a significant achievement in the Philippines because of the RDV's limited studies in the country. RDV is the first plant virus shown to be insect transmitted and it is retained in its insect vector for several generations. RDV is also transmitted to the young through the insect eggs (Fukushi, 1939; Ling et al., 1983). These virus-vector relationships make the detection of the virus in the insect vector an important advantage in addressing the RDV disease epidemiology and insect vector management. Detecting RDV in the insect vector was an easy task because insects were easily collected by net sweeping and crushed effortlessly with a rod compared with rice leaves as experienced by the authors in this study. With an efficient virus detection tool as the LAMP, the detection of RDV in the insect vector will provide another forecasting tool that would pre-empt the imminent threat of spread of RDV infection. It can provide an advance warning of 2-3 weeks, the latent period of the viruses in the plant and in the vector insect (Ling, 1972). Such time would be enough to mobilize the disease and insect control systems and employ the needed protection to the standing crop before the vector and virus disease become fully establish, thus, making the insect and disease control process simpler, if not much less complicated.

RDV Incidence in NCT entries

2014 dry season assessment. A total of 2,260 rice plants in the NCT test fields, including TN1 as check, were methodically assessed visually for rice dwarf

disease symptoms during the 2014 DS trial. Rice lines developed for the upland ecosystem obtained the highest infection rate of 13.89%; nearly as much as the TN1 check plants with 15.21% (Table 2). On the other hand, the MAT lines obtained the lowest infection rate of 0.59%. It is important to note that the MAT lines that passed the rigorous NCT field evaluation tests will be recommended for approval as a variety for release to rice farmers. Therefore, the preliminary results of low rice dwarf disease incidence on MAT lines that might have been released to farmers is encouraging. In addition, the lines for Transplanted and DWSR ecosystems have also low infection rates of 1.94% and 2.50%, respectively. Table 3 lists each elite line selection that was infected with rice dwarf during the 2014 DS trial.

2015 wet season assessment. A total of 4,078 plants were scored for rice dwarf disease infection in the 2015 WS NCT trial. Test entries in the Traditional rice group recorded the highest percentage of RDV infected plant at 4.81% followed by those in the DWSR category at 3.47% (Table 2). On the other hand, rice entries in MAT and Transplanted categories obtained the two lowest scores of 1.20% and 1.25%, respectively. TN1 check plants obtained 2.54% infection. Table 4 lists NCT test entries that became infected with rice dwarf disease in the 2015 WS trial.

When compared with the NCT 2014 DS results, the 2015 WS trial had lower percentage of rice dwarf infected NCT test entries among the different test entry categories, including the TNI check variety (Table 2). In addition, three 10-sweep strokes of an insect net in the NCT test fields collected an average of 2.33 *N nigropictus* individuals, nearly twice as low as the 4.33 recorded during the 2014 DS test. The reason for the difference is unclear except that they were conducted during different cropping seasons when environmental conditions differ and may have affected the population dynamics of the insect vector.

Table 2. Rice dwarf disease incidence in the National Cooperative Trial (NCT) by category. PhilRice Midsayap branch station, North Cotabato. 2014 DS and 2015 WS trials.

Category	2014 DS		2015 WS	
	Infected plant/ Total plant examined (No.)	RDV Infected (%)	Infected plant/ Total plant examined (No.)	RDV Infected (%)
Transplanted	7/360	1.94	10/798	1.25
Direct-wet Seeded	4/160	2.50	11/317	3.47
Multi-Adaptation Trial	1/170	0.59	4/333	1.20
Special Purpose	37/400	9.25	16/794	2.02
Hybrid	18/500	3.60	14/876	1.60
Upland	25/180	13.89	4/176	2.27
Rainfed	17/260	6.54	5/242	2.07
Traditional	-*	-	9/187	4.81
TN1 (check)	35/230	15.21	9/355	2.54

* = not included in the 2014 dry season trial

It is noteworthy that since the rice dwarf disease was reported in 1994 in the Philippines, no major disease outbreak has been reported. Cabauatan et al. (1994) reported that about 10% of *N. nigropictus* were infective while all *N. virescens* and *R. dorsalis* tested were non-infective. They also reported that about 10% of the *N. nigropictus* nymphs that emerged from eggs of females that had access to infected plants were infective. RDV is persistent in the insect vector that would allow them to infect more than one plant during their lifespan. However, Shinkai (1960) reported the reduced adult survival and increased mortality of transovarially infected progeny of RDV-viruliferous leafhoppers. This may have a major effect in limiting the viruliferous vector population. The situation may also be confounded by the resistant rice varieties planted in the area, although this study only started to address this view in a limited scale.

More studies on the vector population dynamics and the epidemiology of the rice dwarf disease in the Philippines is encouraged to fully understand the insect vector and disease situation and prevent a surge in number of infected plants in the future.

Disease field survey

Very low rice dwarf disease incidence (0.4%) was recorded in the rice production area planted to NSIC Rc 222 in the PhilRice Midsayap BS during the 2015 DS cropping. The reaction of NSIC Rc 222 to *N. nigropictus* is not known, although it had an intermediate reaction to its close relative *N. virescens* (National Cooperative Testing Project, 2018). At present, the reaction of NSIC Rc 222 and the other popular varieties to RDV is not known. This is another important study to consider to identify variety(-ies) best suited in the area where the disease is known to occur.

The number of *N. nigropictus* individuals caught in 10 sweeps of a 30-cm insect net ranged from 0-2 per paddy field. This may be due to the periodic spray applications of insecticides in the rice production area. The green leafhopper is generally more sensitive to insecticides than the brown planthopper and is easily controlled by insecticides (Heinrichs, 1979). More *N. virescens* are generally present during the early to mid-growing stages of the rice plant than *N. nigropictus* (Heinrichs, 1979) but *N. virescens* was not reported to be a vector of rice dwarf virus in the Philippines (Cabauatan et al., 1994). On the other hand, the number of *N. nigropictus* increased at the late rice growth stage including at stubble stage. However, this does not provide substantial information or explain the low percentage of infected plants recorded in the study. Along this line, there should be more studies on the population dynamics of *N. nigropictus* and epidemiological studies of rice dwarf disease in the Philippines.

LAMP is a specific, sensitive, and reliable virus detection assay that uses a relatively inexpensive isothermal hot bath that is easy to operate for a short amplification time of 60 min and the results are visualized by the coloration of the reaction mixture. LAMP assay is much faster than the polymerase chain reaction (PCR) technique that requires an expensive thermal cycler. The target sequence of viruses is available from the National Center for Biotechnology Information (NCBI) GenBank that can be accessed for free to develop the primers. Alili et al. (2017) reported that LAMP differentiated the infection of either or both rice tungro bacilliform virus (RTBV) and rice tungro spherical virus

Table 3. National Cooperative Testing (NCT) test entries infected with rice dwarf disease by visual scoring. PhilRice branch station, Midsayap, North Cotabato. 2014 DS.

Category	Elite Lines Selection*	Infected Plant (%)	Category	Elite Lines Selection*	Infected Plant (%)
Transplanted	PR37951-3B-37-1-2	10	Hybrid	PR40638H	20
	IR09A220	10		LP401	20
	IR09A136	20		LP534	10
	IRO5N419	10		IR90876H	30
	PR37921-B-3-4-2-1-2	20		LPP937	10
	PR40422-13-2-1-3-B-B	10		NSIC Rc 216	20
DWSR	PR40762-43-3-2-B-B	10		Mestiso 7	20
	GSR IR1-5-D7-Y3-S1	10		SLT-44	20
	PR40285-44-2-1-1-B-B	10		LPP947	10
MAT	PR37241-3-1-2-1-1	10		Mestiso 1	10
	GSR IR1-4-S5-Y2-Y1	20		NSIC Rc 222	10
	PR38949-B-29-2	10		NSIC Rc 9	10
	IR914041-24-1-2-3	30		GSR IR1-2-Y3-D1-SU1 -L1	20
	PR40073-3B-2	10	Upland	IR83929-B-B-92-1	20
	Hangangchal 1	10		PR31132-B-1-1-1-3-3	20
	PR34859-B-4-1-1-2-1	10		UPL R17	30
	PR37042-B-2-1-1-1-2-2-1-3	20		HHZ19-SAL14-Y1	30
	IR91981-18-1-1-2-2	40		PR40858-NSIC Rc9-M4R-435	20
	MS 11	10		GSR IR2-5-L10-U1-R2	40
Special Purpose	IR89709-1-1-3-3	10		PR40858-NSIC Rc9-M4R-370	60
	Nipponbare-AC-2-1-10-1-1	20		PR38583-IR64/ AC97WP-135-36-1-6	20
	PR34131-B-20-1	20		PSB Rc 14	10
	NSIC Rc 220	30		GSR IR1-6-Y2-Y1-DT1	20
	Nipponbare-(6 EMS)-29-7	20		HHZ8-SAL6-SAL3-Y2	30
	1R10M210	10	Rainfed	RAELINE 10	30
	IR84749-R1L 47-1-1-1-1	10		NSIC Rc 192	30
	MS 13	30		PR37416-18-1	10
	IR83317-54-1-2-3	40		C9301-B-B-12-1-1	10
	IR84841-17-3-1-2	20		PR34363-1-Pokkali/ AC-45-M5R-DrS94	10

*In each line selection, 10 rice plants were visually scored for rice dwarf disease infection

Table 4. National Cooperative Testing (NCT) test entries infected with rice dwarf disease by visual assessment. PhilRice branch station, Midsayap, North Cotabato, 2015 WS.

Ecosystem*	Line Designation	Infected (No.)	Scored (No.)	Infected (%)
Transplanted	IR09A220	1	20	5.00
	PR38729-B-B-1-1	1	20	5.00
	PR35769-B-1-1-2-3-4	1	20	5.00
	NSIC Rc216	1	20	5.00
	C9386-B-7-2-3	1	20	5.00
	12DS-GMET 5	3	20	15.00
	IR05N419	1	20	5.00
	NSIC Rc 302	1	20	5.00
DWSR	IR07A179	1	20	5.00
	PR40432-1-1-1-2-B-B	3	20	15.00
	PR40762-43-3-2-B-B	5	20	25.00
	PR40285-44-2-1-B-B	1	17	5.88
	IR87530-105-2-3-3	1	20	5.00
MAT	NSIC Rc 240	2	20	10.00
	HHZ-2-Y13-DT1-DT1	1	20	5.00
	PR37251-5-4-1-1-2	1	20	5.00
Hybrid	PR40638H	2	12	16.67
	LP 534	1	20	5.00
	INH10008H	2	20	10.00
	IR 64	1	18	5.56
	LOCAL CHECK	1	20	5.00
	SL-18	1	20	5.00
	JY-4H	1	12	8.33
	PR44214H	1	19	5.26
	BIGANTE	2	20	10.00
	BIGANTE plus	2	20	10.00
Special Purpose				
Aromatic	HHZ6-SAL3-Y1-SUB2	3	19	15.79
Glutinous	NSIC Rc 13	1	20	5.00
	PR41035-B-B-17-2-3-1	2	20	10.00
	PR38991-B-37-1-1	2	20	10.00
	PR40073-3B-2G	1	20	5.00
Japonica	PR38697-11-80-2	1	20	5.00
	IR94804-31-2-1-3-1	1	20	5.00
Micronutrient	PR38732-B-B-1	1	20	5.00
Group	IR84842-87-3-1-2-2	3	20	15.00
	IR83317-54-1-2-3	1	20	5.00
Upland	IR12L352	2	10	20.00
	PR40858-NSIC Rc9-M4R-309	1	9	11.11
	NSIC Rc 192	1	10	10.00
	Azucena	1	10	10.00
	Kalinayan	1	10	10.00
	Dinorado	2	9	22.22
	Galo (NEW)	2	9	22.22
	Dumalengan	2	9	22.22
	Chayong	1	10	10.00
RLDS	IR91648-B-20-B-3-1	1	10	10.00
	C9636-B-9-1-3	1	10	10.00
	IR102860-5-7-B-B	1	10	10.00
	IR101465-8-45	1	10	10.00
	PSB Rc 14	1	10	10.00

*DWSR = direct wet seeded rice; MAT = Multi-adaptation trial; RLDS = rainfed lowland dry-seeded

(RTSV) in rice plants sampled from the field with variant symptoms of tungro infection. Since the rice tungro viruses are not persistent in the insect vector (Ling, 1966), virus detection of infected plants is its best option as a support diagnostic tool for the rice tungro disease. With the successful detection of RDV in the insect vector and rice plant by LAMP in this study, it will not only provide information on the presence of the disease in the field but also offer an innovative way to determine the impending threat of virus spread.

The occurrence of RDV in the Philippines is presently confined to the PhilRice Midsayap BS, North Cotabato. This situation should be sustained by prohibiting the transfer of live RDV infected plants and *N. nigropictus* insects from the station without quarantine permit. A methodical disease survey should be conducted in the neighboring farmers' fields to gain insights on the extent of its occurrence outside the branch station. These preventive actions may maintain the present status of the disease for the time being and will provide future studies to gather additional information about the disease and life activities of the insect vector.

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