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Building Process of Potato Virus Y (PVY) Management on Tobacco Plants in the Northern Vietnam

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

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ABSTRACT

In recent years, some viral diseases have been increasing in the tobacco-growing regions of Vietnam and causing serious damage to the tobacco yield and quality. If it has no effective control, the viral diseases will injure serious harm in the tobacco field next time. Based on the research results in the prevention of viral diseases from 2016 to 2021, the Vietnam Tobacco Institute has built a process to control Potato Virus Y that harms the tobacco plants in Vietnam. During the experiment and application in tobacco cultivation practice, the process was effective against PVY disease. The application of the process, the disease rate and disease index of PVY were significantly reduced in the tobacco field, contributing to increasing yield, quality, and income for tobacco farmers. The insecticides and antivirus drugs made according to the process have increased their effectiveness, do not harm the growth and development of tobacco plants, little have residues in tobacco production. This process can control many other viral diseases on tobacco plants.

Keywords: Virus; antivirus agent; process of cotrolling PVY; tobacco plant.

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1. INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the most important crops in northern Vietnam. Viral diseases are an important group of plant pathogens that cause severe damage to tobacco yield and quality. Viral diseases often threaten the sustainable development of agricultural production in Vietnam and the world. They infect many crop species and estimated annual losses worldwide of about $600 billion [1]. Viral diseases are the second most common pest after fungal diseases and cause annual losses of about $4 billion [2].

Potato Virus Y (PVY) is the type species of the Potyviridae family, has a wide host range and is distributed worldwide, causes serious crop losses in solanaceous crops such as potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*), and tomato (*Lycoper-sicon esculentum*). PVY is transmitted by more than 50 aphid species in a nonpersistent manner. Aphid species differ in their ability to transmit the virus; however, the consensus is that *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is the most efficient vector. In this mode of transmission, acquisition and inoculation access periods are very short (seconds to minutes) and the aphids remain viruliferous for a very short period of time (minutes to a few hours) [3,4]. PVY is one of the most diverse viruses of genetics and pathogenicity. PVY has three main strains, including PVY^{o}, PVY^{N}, and PVY^{C}. In tobacco plants, the strain PVY^{o} is distributed worldwide and causes leaf vein necrosis [5,6].

Potato Virus Y (PVY) is one of the most important pathogens of solanaceous plants, such as potato, tobacco, pepper, tomato, and ornamental plants. PVY causes significant yield losses for tobacco [3,7]. In recent years, PVY has tended to develop severely in tobacco growing regions in the world. PVY causes symptoms ranging from mild mottling to severe leaf vein necrosis. Those symptoms depend on virus strains [8,9]. The disease rate with Potato Virus Y of 19 tobacco varieties in the Nelson district at the end of the 1964-65 season varied from 0 - 72%, an average of 26%. Virus Y markedly reduced the weight and leaf area of tobacco plants. The quality of the cured leaf was also adversely affected. The nor-nicotine content was higher, with an average increase of 83.3%. The sugar content was lower in infected leaves, with an average decrease of 9.8%. Infection of tobacco plants with PVY did not affect the germination of seed [10]. In 1997, PVY caused damage of about 640 tons, of which Tobacco mosaic virus (TMV) + Cucumber mosaic virus (CMV) caused damage of about 150 tons. In 2001, PVY damaged 100 tons, but TMV + CMV only damaged 6 tons [3]. PVY can cause tobacco yield losses reaching 100% [11] and from 39% to 75% [12]. When infected tobacco plants after 30 days of transplanting, tobacco yield reduced ranging from 71.2 - 74.8%, rate of sugar/nicotine: 3 – 4; plants that were infected after 44 - 60 days after transplanting little effected on the yield, quality but only affected on plant height, rate of sugar/nicotine from 7.2 – 9.0. Compared to healthy plants, this rate was 9.5 [13]. To limit the spread of the virus, using insecticides to control aphids is necessary. But using incorrect insecticides and techniques will affect the effective control and agricultural environment.

To effectively manage viral diseases, it is necessary to control well transmission insects in the tobacco field. Control of Potato Virus Y follows main measures: (1) use disease-free seedlings; (2) investigate and destroy diseased plants early; (3) control disease transmission insects (aphids); (4) destroy host plants and weeds in the field before and after planting; (5) use disease resistant tobacco varieties [14]. The viral disease can be prevented by growing resistant cultivars, avoiding tobacco cultivation in the neighbourhood of other solanaceous crops and removing weeds that may be a source of infection into the field [15]. Resistant cultivars are considered the most economic and environmentally acceptable way of controlling viral diseases. Vector control plays an important role in PVY management [12].

The prevention of viral diseases that harm crops with chemical agents is impossible because they are a parasite in the cell. Using chemicals only have the effect of inhibiting the development of virus leads to reduce disease symptoms in the plants. There are no control measures to inhibit plant viruses after plants are infected. Currently, some chemicals can prevent the development of viruses, such as Ningnanmycin (NNM), Cytosinpeptidemycin, Salicylic acid (SA), Flavonoid, Terpenes. In there, Ningnanmycin, Cytosinpeptidemycin and Salicylic acid have been used widely in China to control some viral diseases on the tobacco plants and others. Ningnanmycin, the most successful registered antiplant viral agent, displayed 56,0% in vivo curative effect at 500 µg/mL. For TMV, the

effective control of NNM is over 56% when spraying at a dose of 500 µg mL-1 [1]. Salicylic acid (SA) induces resistance to Cucumber mosaic virus in tobacco (*Nicotiana tabacum*) by inhibition of systemic virus movement and is induced via a signal transduction pathway that also can be triggered by antimycin A, an inducer of the mitochondrial enzyme alternative oxidase (AOX). Cytosinpeptidemycin (CytPM) is a microbial pesticide that displayed broad-spectrum antiviral activity against various plant viruses. CytPM could effectively delay the systemic infection of Tobacco mosaic virus in *Nicotiana benthamiana* and significantly inhibit the viral accumulation in tobacco BY-2 protoplasts. Results of RNAseq indicates that 210 and 120 differential expressed genes (DEGs) are significantly up and down-regulated after CytPM treatment in BY-2 protoplasts, respectively [16].

In Vietnam, observation result of Tobacco Institute showed that PVY is one of the most important pathogens on the tobacco plants in the northern provinces with incidence ranging from 0 - 100%, with an average ranging from 9.3 - 36% in 2015 - 2017, especially had been spreading quickly in the tobacco-growing regions. The disease severely harms the growing tobacco in Bac Son and Chi Lang District - Lang Son province in recent years, and all tobacco line types and varieties are not resistant to PVY. To prevent the damaging of PVY on the tobacco field and increase tobacco yield and quality, building process of Potato Virus Y management is very important role for producing tobacco in northern Vietnam.

2. THE STUDY RESULTS FOR BUILDING PROCESS OF POTATO VIRUS Y MANAGEMENT

Genetic diversity of potato virus Y in Northern Vietnam: Potato virus Y is the type member of genus Potyvirus and one of the most common species and most pernicious of Potyvirus. In Vietnam, PVY causes severe disease in almost tobacco growing areas, especially severely damage in Bac Son, Lang Son province in 2015 and 2016. Therefore, the study of PVY's genetic diversity is essential for disease prevention. In this study, tobacco leaves with rot vein symptoms from major growing-tobacco fields in Northern Vietnam were collected. CP gene fragments encoding the capsid protein of PVY were amplified by Reverse Transcript PCR (RT-PCR) and sequenced by ABI 3500 system. The obtaining sequences were analyzed and built classification trees using MEGA6 software. From the results, we classified four groups of PVY, including PVY^{o}, PVY^{C}, PVY^{NTN}, and a new PVY group. In which, PVY^{o} damaged the most popularity in all tobacco-growing regions. A new PVY strain is not present in the genetic bank of NCBI and similar to strain damaging potato in China. In tobacco-growing regions, Bac Son – Lang Son provinces have the most genetic diversity with 4 strains (PVY^{o}, PVY^{C}, PVY^{NTN}, a new PVY strain), Chi Lang – Lang Son provines: 2 strains (PVY^{o}, PVY^{C}), Bac Giang provines: 2 strains (PVY^{o}, new PVY), Cao Bang and Bac Kan: PVY^{o} [17].

Distroy infective plant: Sanitary and destruction of diseased plants in the field play an important role in reducing the source of disease and spreading PVY in the tobacco field. Study results of Vietnam Tobacco Institute in Bac Giang province in 2017 showed that destroying infective plants had the disease rate from 9.9 - 12.3% and disease index from 6.7% to 8.9%, meanwhile, the control formula that did not destroy the diseased plants had the disease rate of 20.4 - 32.1% and disease index from 15.9% to 26.3%.

Effect of PVY on growth, yield, and chemical compostion: The effect of potato virus Y on the growth, yield and chemical composition of C9-1 and GL7 cultivars was conducted in Bac Giang province in 2017. The experiment was determined by time inoculation at 15, 25, 35, 50, and 60 days after transplanting. The result of the study showed that early infected tobacco plants with Potato Virus Y at 15, 25, and 35 days after planting affected the growth, yield, and quality. Early infective plants were sensitive to disease, with rates of dead plants ranging from 13.3 - 16.6% on both experimental varieties. For C9-1, early inoculating plants (15, 25 and 35 days after transplanting) caused height reduction of plants from 47.4 - 62.0% and yield reduction of 45.9% - 58.6%. Similar to C9-1, GL7 caused height reduction of plants from 57.0 – 73.8% and yield reductions of 49.4 - 58.9% when the inoculating at 15 - 35 days after transplanting. PVY also modified the chemical composition of flue-cured leaves. Nicotine content was lower in cured leaves from diseased plants than in cured leaves from healthy plants, especially when inoculating from 15 - 35 days transplanting with nicotine content reduced from 21.8% to 42.9% and sugar from 6.1% to 56.5% [18].

Evaluations of tobacco lines/cultivars resistance to Potato Virus Y: Potato virus Y (PVY) is a destructive plant virus that causes significant economic losses for tobacco. Using tobacco resistant line/cultivar is one of the most important strategies in agriculture. In 2017, all 21 tobacco lines and cultivars were evaluated in the greenhouse and field conditions by mechanical inoculation in Bac Giang province. In which, NC55 cultivar is resistant to PVY was used as the resistant check, and K326 cultivar is susceptible to PVY was used as the susceptible check. The results showed that the D61 line and GL6 cultivar were moderately susceptible to moderate resistance (MS-MR); other lines/cultivars were susceptible to susceptible high (S - HS) to PVY. All tobacco lines/cultivars were not resistant (R) to high resistance (HR) to PVY [19].

Effect of other growing seasons on Potato Virus Y: The diseased plants with PVY will affect the growth, yield and quality of flue-cured tobacco. The use of pesticides lacks efficacy against virus transmission in the field. To determine the effective control measure in preventing PVY, we tried other tobacco growing seasons in Bac Giang province in Spring season 2017 and 2018. The result of the experiment showed that the early season was the most severe infected in both experimental years with the rate of disease ranging from 66.1 – 100% and disease severity index from 40.7 – 90.1% on April 11, 2017, and April 30, 2018. For the late season, the rate of disease from 19.5 – 21.5% and disease severity index: 5.9 -13.5%. The fresh yield of the early season of 2017 and 2018 was 7.1 – 9.8 tons/ha and lower than the late season (19.6 – 22.3 tons/ha). The grade of flue-cured tobacco of the late season was 1.8 times higher than the early season in 2018 [20].

Control vector insect on PVY: Control vector plays an important role in transmission virus and affects the spread of the virus in the tobacco field. To control the spread of the virus in the tobacco field, we used imidacloprid (Confidor 100SL) to spray for tobacco. Study results of Vietnam Tobacco Institute in Bac Giang province in 2017 showed that controlling wing adults and young aphids (non-wing) in the tobacco field had the highest PVY control efficiency of 46.2%. Praying at wingless aphids or periodically every 7 days/times from the transplanting to cut-tops does not mean preventing PVY, opposite to make the disease more harmful. Spraying to prevent aphids from transplanting to 6 - 8 leaves stage had the highest PVY control efficiency of reaching 6.3% and higher than periodic spraying from transplanting stage to 22 - 24 leaves/plant ranging from -20.8 to -14.5% or to the cut-tops stage: -23.1%.

Study prevention measures of PVY in the field: The experiment was conducted in Bac Giang province in 2018 with some technical measures such as the growing season, sanitization and destruction of diseased plants, diseased host plants before a new season and after harvesting. Destroy infective plants on the tobacco field and spray insecticides from the transplanting stage to 12-16 leaves/plant stage. Other techniques were similar between the experiment and the control experiment. The result of the study showed that on the experimental formula, PVY caused mild harm with a disease rate of 3.5 - 16.3% and disease index of 0.9 - 7.2%, meanwhile, the control formula was severely infected with disease rates of 19.5 - 66.1% and disease index 5.5 - 40.7%.

Effective control of antiviruses on Potato Virus Y: In recent years, some viruses have been developing quickly and causing significant damage to the tobacco yield and quality in Northern Vietnam, such as TMV, CMV, and PVY. The object of this study was to evaluate the effective control of some antivirus agents and technical controls for the virus diseases damaging tobacco plants. Antivirus agents were Ningnanmycin - NNM (Ditacin 8SL) and Cytosinpeptidemycin (Sat 4SL). The experiment was performed in the net-house and field conditions in Bac Giang province in 2019. The field experiment was arranged in a randomized complete block design with three replications, and the net-house was by inoculation method. Results of the study showed that in net-house, a result of treating Ditacin 8SL from 3 to 6 times before infected plants had effective control of PVY with ranging from 24 - 33%; TMV: 28.9 - 50%; and CMV: 42.6 - 60% after 21 days of infection. For Sat 4SL, effective control of TMV ranged from 23.7 - 31.6%, CMV: 20,0 - 24.4%, and PVY: 28.9 - 35.6% after 21 days of inoculation. In the field condition, the effective control of NNM was 100% for TMV, PVY: 93.5%, CMV: 60.5%, and TNRV: 52.1%. Similar to Ditacin 8SL, treating Sat 4SL, the effective control of TMV reached 70.9%, CMV: 58.3%, PVY: 96.4%, and TNRV: 36.9%. Spraying Ditacin 8SL and Sat 4SL from transplanting (3 - 4 leaves/plant) to 16 - 18 leaves/plant stage was the best control of viral diseases, increased the

yield, grade index, and less impact on the property of smoke. So, spraying Ditacin 8SL and Sat 4SL controlled the effect of the TMV, CMV, PVY, and TNRV damaging on tobacco plants. To effectively manage virus diseases of CMV, PVY and TNRV have to spray a mixture of virucide, insecticide, and foliar fertilizer. For TMV, only using the virucide, foliar fertilizer, and destroying disease plants is good [21].

Technical use of anti-virus in prevent potato virus Y: In 2021, Vietnam Tobacco Institute experimented with the effective control of Ditacin 8SL, Sat 4SL, and Exin 4.5SC for the TMV, CMV, PVY, and TNRV on the tobacco field in Bac Giang province. Tobacco seeds of the C9-1 cultivar were sown on a free pathogen nursery. The seedlings reached 4 - 6 leaves and were transplant in the experimental field with a distance from the plant to another plant about 0.5m and space between rows 1.1m, rate of fertilizers at 70N: $140P_2O_5$: $210K_2O$ kg/ha with a form of N: NH_4NO_3 and K: K_2SO_4. Around the experiment is planted 3 - 4 rows of tobacco to protect the experimental field. The experiment was designed with a randomized complete block, three replications with two experiments: Spray virucides at a concentration from 1.0 - 2.5 times compared to the guide of the producer; Spray a mixture of virucide and insecticide. Spray pesticides every 6 days/times from the transplanting stage to 16 - 18 leaves/plant and not destroy infective plants. Results of the study show the antiviral agents are effective in the control of the virus disease on tobacco. When only spray Ditacin 8SL, effective prevention for viral diseases ranges from 22.4 - 30.3%; Sat 4SL: 5.5 - 30.2%, and Exin 4.5SC: 8.2 - 24.1%. Spraying a mixture of antiviral agents and insecticides gives higher efficiency than spraying a kind. For example, spray Ditacin 8SL + Confodor 100SL, effective control reaches from 34.3 to 100%; Sat 4SL + Confidor 100SL: 32.6 - 92.3, and 38.5 - 100% with Exin 4.5SC + Confidor 100SL. The spray treatments have a higher yield and quality than the control without spraying [22].

3. BUILDING PROCESS OF THE POTATO VIRUS Y MANAGEMENT

Effective management of PVY requires changing from the tobacco nursery to the tobacco field so after completing harvest. The best management method for PVY is to avoid the attack of the virus on the tobacco fields. It is important for controlling PVY to have been detected early and prevent spreading them, the virus and vector, especially the appearance of the virus in the field. The infective plant can not be cured. Once the plant is infected, it stays infected for life, and the virus is easily transmitted to other plants by aphids, especially is wing-aphid. Therefore, the best defence against virus disease is minimizing exposure or being resistant to infection. To manage PVY in tobacco, using integrated pest management (IPM) is for the best results.

Some technical measures to manage Potato Virus Y on tobacco plants in the Northern Vietnam:

- Sanitary and destruction of tobacco plants, host plants of the disease and disease-transmitting insect (Aphids) in the field before the new planting season and after the end of the harvest is a necessary role in controlling potato virus Y. Because infected plants stay on the tobacco field as inoculative sources so should be removed regularly.

- Rotate regularly tobacco with plants of other families such as rice, maize, etc. Do not plant tobacco in the fields that the previous crops have the host plants of the disease and vector, such as Tomato, potato, chilli, eggplant, etc.

- Do not plant tobacco near Solanaceous crops. These crops include potato, tomato, eggplant, pepper, tomatillo, groundcherry, petunia, etc. If tobaccos are planted near the host crops lead to management of PVY is not effective and increase costs of production.

- The growing season has an important role in affecting the growth of vectors and PVY in the tobacco field. In the area where the disease is less harmful is possible to plant all seasons. The areas with the severe annual disease are transplanted in the late season, from mid-February to early March in Northern Vietnam. When the temperature is over 22^oC, the growth of PVY decreases sharply in the field, especially is above 25^oC [23], and the wing-adult aphid emergence period is short lead to reduce the transmission of the virus in the field.

- Using D61 and GL6 cultivars to transplant in the infectve areas.

- Do not conduct tobacco nurseries and fields near the host plants of the disease and insect vectors. The nursery area is free of host plants of the PVY and insect vector; Investigate vector

periodically and spray insecticides when they appear. The tobacco nursery is covered by insect nets to prevent insects into seedlings. Investigate vector periodically and do not use seedlings on infected nurseries. The tobacco nursery that is infected, is destroyed early to prevent PVY to the field by seedling and insect vector. To protect seedlings in the first 2 weeks after transplanting, the tobacco nursery is sprayed with insecticides (kill sucking insects) and antivirus agents before transplanting. Spray from 1-2 times, per spray time is 5 days.

- Investigate the field periodically and destroy the infected plants early in the tobacco field. This practice eliminates potential sources of inoculum before it can be spread. Infected plants stay in the field as sources of inoculum and should be removed regularly.

- Spraying for colonizing aphids may reduce the spread of PVY within the field. It only sprays when scouting indicates an aphid population above threshold levels. Critical factors affecting efficacy are timing, aphid species, choice of chemical and coverage. The aphids only colonize and reproduce on tobacco and are efficient vectors of PVY. Systemic insecticides that are applied to control aphids at a quick growth stage of tobacco plants are effective in reducing the spread of PVY in the field. To limit the harmful effects of the agents on beneficial insects, it is recommended to spray from the transplanting stage until the plant has 12-16 leaves/plant. The specific spray methods are as follows:

+ Use special agents follow the 4 right principles, including: right pesticide, right dose and concentration, right time, and right method.

+ Spray agents with the correct technique. Spraying insecticides covers the leaves, especially spray the undersides of leaves where aphids reside and cause damage.

+ The control of winged aphids and young aphids, especially is winged aphids begin to appear from the planting stage (3 - 4 leaves/plant) to 12-14 leaves/plant has the highest effect. After the 16 leaves stage/plant, spraying will be not effective in the control of PVY.

+ In some areas with annual low disease incidence, spray insecticide when the disease begins to appear in the tobacco field. Spray 2 - 3 times, per times 6 days with the spray dosage according to the manufacturer's instructions, and destroy infective plants after 3 days of treating.

+ For areas with the annual hight disease incidence, spray insecticides to kill vectors after tobacco plants' 3 - 4 leaves/plants and winged aphids appear in the tobacco field. Spray 4 - 6 times from transplanting to 14 – 16 leaves/plants, each time six days apart with an increased dose of spray 1.5 - 2.0 times according to the manufacturer's instructions, and destroy diseased plants after three days of spraying.

+ To have the effective prevention of PVY, it should spray a mixture of insecticides and antivirus agents. When tobacco plants grow slowly, it is possible to spray the mixture with foliar fertilizer. To increas es effective control of antivirus agents should spray them when the tobacco plants are not infected or symptoms have not yet appeared clearly on the young leaves.

- Infected tobacco plants must destroy the diseased plants early and use healthy, disease-free seedlings to replant in the period of 20 days after planting.

- Pesticides: insecticides: use of rotation agents with active ingredients imidacloprid (confidor 100SL) and Thiacloprid (Actara 25WG) to reduce the level of resistance of vector insects. Anti-viral agents: Ningnanmycin (Ditacin 8SL) and Cytosinpeptidemycin (Sat 4SL).

4. APPLYING PVY PREVENTION PROCESS IN TOBACCO PRODUCTION IN NORTHERN VIETNAM

The prevention process of PVY has been issued by the Tobacco Institute under Decision No. 141/QD-VTL, dated November 27, 2018, and has been applied to prevent PVY in the tobacco field. In 2021, the process of using chemical pesticides to control insects and viral diseases on tobacco is issued according to Decision No. 233/QD-VTL, dated December 9, 2021, and applied to production practice in 2021 in the Northern tobacco growing areas. After applying the control process of PVY and the process of using pesticides for insects and viral diseases, the rate of PVY diseases has decreased markedly over the years. In 2014 - 2015, PVY caused heavy damage in Bac Son - Lang Son province with a disease rate of 20 - 36%, and many tobacco fields were severely damaged (60 - 100%). In 2016 - 2017, the PVY disease continued to

cause severe damage in Bac Son and Chi Lang - Lang Son province, with a disease rate of 9.7 - 13.5%, especially causing too heavy damage over 5 hectares in Bac Son district and 10 hectares in Chi Lang - Lang Son province. In 2019 - 2020, the PVY disease causes mild damage in most of the tobacco-growing provinces of the North, with a disease rate below 2.3%. In 2021, PVY causes mild damage in tobacco growing areas of Northern Vietnam.

Beside, increasing the effective prevention of PVY, experimental drugs do not affect the tobacco quality and have little residue in tobacco leaves. Applying the control process not only reduces the incidence of diseases and severe disease index in the tobacco plants, but also improves yield, quality of tobacco, and income for tobacco growing farmers in recent years and the coming years.

5. CONCLUSION

The process is effective for preventing the growth of PVY in the tobacco field. It contributes to increasing yield, quality and income for tobacco growing farmers in the northern mountainous provinces of Vietnam.

The process has been highly appreciated by tobacco production companies in Northern Vietnam for controlling PVY on tobacco plants in 2021.

The process can be applied to prevent some viral diseases on tobacco plants, such as Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV), and Tomato necrotic ringspot virus (TNRV).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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