

RESEARCH PAPER

## Molecular detection and characterization of potato virus Y-NTN and cucumber mosaic virus-S in microtubers and G0 tubers of red and Granola potato

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### ABSTRACT

This study aimed to detect and molecularly characterize viruses infecting microtubers and G0 tubers of red and Granola potato varieties produced by PT. G10 Agrotechnology, Medan, Indonesia. A total of 50 microtubers and 50 G0 tubers from each variety were collected and tested using serological methods, including enzyme-linked immunosorbent assay (ELISA) and dot immunobinding assay (DIBA), followed by reverse transcription polymerase chain reaction (RT-PCR) and nucleotide sequence analysis. Serological and molecular assays showed that all microtuber samples were free from potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS), and cucumber mosaic virus (CMV). In contrast, G0 tubers were infected by PVY and CMV, whereas PVX and PVS were not detected. The incidence of PVY and CMV reached 80.0% and 82.0%, respectively, in red potato G0 tubers and 28.0% for both viruses in Granola G0 tubers. RT-PCR successfully amplified the coat protein (CP) genes of PVY and CMV, producing fragments of approximately 801 bp and 657 bp, respectively. Sequence and phylogenetic analyses identified the PVY isolates as belonging to the PVY-NTN strain and the CMV isolates as closely related to the CMV soybean stunt strain (CMV-S). These findings indicate that microtubers produced through tissue culture were virus-free, whereas viral infection occurred during the screen-house multiplication stage. The results provide important baseline information for virus monitoring and management strategies to support the production of high-quality potato seed in Indonesia.

**Keywords:** CMV-S, ELISA, PVY-NTN, RT-PCR, seed potato production, *Solanum tuberosum*

### INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food and horticultural crops worldwide, serving as a major source of carbohydrates and contributing significantly to food security (Aksoy et al., 2021; Motti, 2021). In Indonesia, potato ranks among the most economically important vegetable commodities, with substantial domestic market demand and export potential (Saptana et al., 2022). Potato is also among the vegetable crops prioritized for development due to its strong competitive advantage compared to other vegetables (Devaux et al., 2021). However, potato productivity in Indonesia remains relatively low compared with its production potential due to several constraints, including limited availability

of high-quality seed tubers, restricted areas suitable for cultivation, and high disease pressure under tropical environmental conditions (Saptana et al., 2022; Pronk et al., 2024; Maulidiyah et al., 2024).

The use of healthy and pathogen-free seed tubers is a critical factor determining potato productivity and crop quality (Chaudhary et al., 2024; Lindqvist-Kreuze et al., 2024). Consequently, the development of reliable seed production systems has become a priority for sustainable potato cultivation (Stemerding et al., 2023). In Indonesia, however, the supply of certified, high-quality potato seed remains insufficient, meeting only a small proportion of national demand (Saptana et al., 2022; Pronk et al., 2024; Ru et al., 2024). To overcome this limitation, *in vitro* propagation techniques have been increasingly adopted to produce disease-free planting materials in the form of microtubers. Microtubers offer several advantages, including genetic uniformity, reduced storage and transportation costs, and year-round seed availability (Dinatingrat et al., 2024; Sivakumar et al., 2024; Vishal et al., 2025).

Seed multiplication typically begins with microtubers produced under laboratory conditions, followed by successive generations grown in screen

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houses and open fields. Although protected cultivation reduces disease incidence, viral infections that occur during early stages of seed production may remain undetected and subsequently spread to later generations through vegetative propagation. Therefore, early detection of viruses in microtubers and initial seed generations is essential to maintain seed health and prevent disease dissemination throughout the seed production chain (Abohatem et al., 2024; Kumari et al., 2025).

Among the viruses affecting potato production worldwide, potato virus Y (PVY) and cucumber mosaic virus (CMV) are among the most economically important because significant impacts on tuber yield and quality. In Indonesia, other potato viruses, including potato virus X (PVX) and potato virus S (PVS), have also been reported (Chikh-Ali et al., 2016; Damayanti et al., 2014; Taylor & Dawson, 2021; Wardani et al., 2021; Shabrina et al., 2024; Santosa et al., 2025). PVY, a member of the genus *Potyvirus* (family Potyviridae), is regarded as one of the most destructive potato viruses worldwide. In particular, the PVY-NTN strain is associated with potato tuber necrotic ringspot disease (PTNRD), causing severe reductions in tuber quality and substantial economic losses (Funke et al., 2024). CMV, a member of the genus *Cucumovirus* (family Bromoviridae), has a broad host range and is efficiently transmitted by aphids, resulting in mosaic symptoms, leaf deformation, stunting, and yield reduction in potato and numerous other crops (Baranwal et al., 2021). Because CMV populations exhibit considerable genetic variability and can infect multiple host species, molecular characterization is essential for understanding strain diversity, host associations, and virus epidemiology (Mahfut et al., 2025).

Yield losses caused by potato virus infections in Indonesia have been reported to range from 5% to 80%, primarily due to PVX, PVY, and PVS (Shabrina et al., 2024; Syamsiyah et al., 2024). These viruses may occur as single or mixed infections and often induce similar symptoms, making visual diagnosis unreliable. Therefore, sensitive and specific diagnostic methods such as enzyme-linked immunosorbent assay (ELISA), dot immunobinding assay (DIBA), and reverse transcription polymerase chain reaction (RT-PCR) are required for accurate virus detection and identification (Baranwal et al., 2021; Wardani et al., 2021).

Despite extensive research on potato viruses, information on the occurrence and molecular characteristics of PVY-NTN and CMV in microtubers

and early seed generations (G0) produced under tropical seed production systems remains limited. This knowledge gap is particularly relevant for commercially important potato varieties such as red potato and Granola, which are widely propagated through certified seed systems in Indonesia. The lack of information on virus incidence at the earliest stages of seed multiplication may compromise seed quality and facilitate virus dissemination to subsequent generations.

Red and Granola are among the most widely cultivated potato varieties in Indonesian seed production systems and therefore represent important targets for virus surveillance and seed health assessment. Red potatoes are preferred for processing because of their relatively high carbohydrate content and good storage quality, whereas Granola is widely cultivated due to its high productivity and relative resistance to PVY. The major characteristics of both varieties are summarized in Table 1.

PT. G10 Agrotechnology is one of the major producers of certified potato seed in North Sumatra, utilizing microtuber-based propagation systems for red and Granola potato varieties. Therefore, this study aimed to detect and molecularly characterize PVY-NTN and CMV in microtubers and G0 seed generations of red and Granola potatoes produced by PT. G10 Agrotechnology using serological (ELISA and DIBA) and molecular (RT-PCR) diagnostic approaches. The findings of this study provide baseline information on virus occurrence in early-stage potato seed production and support the development of effective disease management strategies for the production of high-quality virus-free seed potatoes.

## MATERIALS AND METHODS

**Research Site.** The research was conducted from January to October 2025 at the Tissue Culture Laboratory and Screen House of PT. G10 Agrotechnology, Medan, Indonesia. Molecular analyses was performed at the National Research and Innovation Agency (BRIN), Cibinong, Indonesia.

**Sample Collection and Processing.** Microtubers of red and Granola potato varieties were collected from in vitro cultures maintained at PT. G10 Agrotechnology. A total of 50 microtubers per variety, each approximately 2 cm in diameter, were randomly selected from multiple culture vessels to ensure representative sampling of the production batches. Only healthy microtubers without visible abnormalities or contamination were included

Table 1. Comparison of the characteristics of red and Granola potato varieties

Characteristic	Red potato variety	Granola variety
Tuber skin color	Red	Yellow to light yellow
Flesh color	Yellow	White to pale yellow
Carbohydrate content	Relatively higher	Moderate
Moisture content	Relatively lower	Moderate
Processing quality	Suitable for chips and processed products due to crisp texture	Suitable for fresh consumption and processing
Storage ability	Good postharvest storage durability	Moderate to good
Market potential	Stable price and strong agribusiness potential	Widely cultivated commercial variety
Plant height	Not specifically reported	Approximately 65 cm
Growing period	Similar to commercial potatoes	100–115 days
Yield potential	Commercially promising	Up to 26.5 t ha <sup>-1</sup>
Resistance to PVY	Not well documented	Relatively resistant to PVY
Importance in seed production	Commercially important variety	Major certified seed potato variety

Sources: Diningrat et al. (2024); Putri et al. (2024); Lalpekhlua et al. (2024); Tkaczyńska et al. (2024); Hadiarto & Ambarwati (2024); Maulana et al. (2024); Funke et al. (2024).

in the study.

For G0 generation seed tubers, 50 tubers per variety were randomly collected from screen house-grown plants. Sampling was performed across different sections of the screen house to account for possible spatial variation in environmental conditions and vector exposure.

All samples were collected using sterile tools, individually labeled, and stored at  $-20^{\circ}\text{C}$  until further analysis. The sampling strategy was designed to assess the occurrence of viral infection at two critical stages of the seed production system, namely microtubers and G0 tubers, thereby enabling identification of potential points of virus introduction during seed multiplication.

**Serological Detection of Potato Viruses.** potato virus Y (PVY), potato virus X (PVX), and potato virus S (PVS) were detected using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) according to the manufacturer's protocols (DSMZ, Germany; Agdia, USA). cucumber mosaic virus (CMV) was detected using the dot immunobinding assay (DIBA) following the protocol provided by Agdia (USA).

ELISA reactions were visualized using *p*-nitrophenyl phosphate (PNP) substrate. Samples were considered positive when the absorbance value at 405 nm was greater than twice that of the healthy control. Absorbance measurements were obtained using an ELISA reader (Bio-Rad 550, USA).

For DIBA, positive reactions were identified by the appearance of purple coloration on nitrocellulose membranes following development with nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (Raza et al. 2025).

Positive and negative controls were included in each assay to ensure diagnostic accuracy. All samples were tested in duplicate, and only samples showing consistent results between replicates were considered valid.

**RNA Extraction and RT-PCR Analysis.** Total RNA was extracted from potato tissue samples using a commercial RNA extraction kit according to the manufacturer's instructions (Phile Korea Technology). RNA concentration and purity were determined spectrophotometrically, and samples with an A260/A280 ratio greater than 1.8 were used for subsequent analyses. First-strand complementary DNA (cDNA) was synthesized from total RNA using a reverse transcription kit (Thermo Fisher Scientific, USA) at  $42^{\circ}\text{C}$  for 60 min following the manufacturer's protocol. The resulting cDNA was used as the template for PCR amplification (Raza et al., 2025; Kumar et al., 2023).

PVY coat protein (CP) genes were amplified using primer pairs PVY-cpF (5'-ATGGSAAATGACACAATYGATGCA-3') and PVY-cpR (5'-ACATGTTACTCCAAGYAG-3'), producing an expected amplicon of approximately 800 bp. PCR conditions consisted of an initial denaturation

at 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min, with a final extension at 72 °C for 7 min.

For PVX detection, primer pairs PVX-cpF (5'-AAGCCTGAGCACAAATTCGC-3') and PVX-cpR (5'-GCTTCAGACGGTGGCCG-3') were used to amplify a 535 bp fragment of the CP gene. Likewise, primer pairs PVS-cpF (5'-AAGTGGTGATCATGTGTGCAAGCG-3') and PVS-cpR (5'-ATTGCAATGATCGAGTCCAAGGGC-3') amplified a 590 bp fragment of the PVS CP gene. Both assays were performed under the same PCR conditions as described for PVY.

CMV coat protein genes were amplified using primer pairs CMV-cpF (5'-ATGGACAAATCTGAATCAACCAGTGCC-3') and CMV-cpR (5'-ACTGGGAGCACTCCAGATGTG-3'), generating an expected amplicon of approximately 650 bp. PCR amplification consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 45 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min.

Each PCR run included positive controls, negative controls, and no-template controls. To minimize contamination, RNA extraction, cDNA synthesis, PCR setup, and post-PCR analyses were conducted in physically separated work areas.

**Agarose Gel Electrophoresis.** PCR products were separated on 1% agarose gels prepared in 0.5× Tris-borate-EDTA (TBE) buffer containing ethidium bromide. Electrophoresis was performed at 100 V for 25 min. Amplified DNA fragments were visualized under UV illumination and documented using a gel documentation system (Kumar et al., 2023; de Oliveira et al., 2025; Kuan et al., 2024).

**Selection of Samples for Sequencing.** Samples that tested positive by RT-PCR and produced strong, clear amplification bands were selected for sequencing.

Representative positive samples from each potato variety and seed generation were chosen to confirm virus identity and obtain sequence data for molecular characterization.

**DNA Sequencing and Phylogenetic Analysis.** PCR products were purified and sequenced commercially by First Base Laboratories (Singapore). The resulting nucleotide sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) available through the National Center for Biotechnology Information (NCBI) database.

Sequence alignments and homology analyses were performed using ClustalW implemented in BioEdit version 7.2. Phylogenetic relationships were inferred using Molecular Evolutionary Genetics Analysis (MEGA 11) software. Phylogenetic trees were constructed using the Neighbor-Joining method with 1000 bootstrap replications to assess branch support (de Oliveira et al., 2025; Kuan et al., 2024).

**Statistical Analysis.** Virus incidence was calculated as the percentage of positive samples relative to the total number of samples tested for each potato variety and seed generation. Serological and molecular detection results were summarized descriptively. Sequence similarity, nucleotide identity, amino acid identity, and phylogenetic relationships were used to characterize PVY and CMV isolates detected in red and Granola potato seed production systems.

**RESULTS AND DISCUSSION**

**Molecular Detection.**

**Serological Testing.** Serological assays revealed no detection of PVY, PVX, PVS, or CMV in any microtuber samples of either red or Granola potato varieties (Table 2). In contrast, virus infections were detected in G0 tubers. PVY and CMV were the only viruses detected, whereas PVX and PVS were

Table 2. Incidence of potato viruses detected by serological assays in microtubers and G0 tubers of red and Granola potato varieties

Sampel type	Variety	Virus disease incidence (%)			
		PVY	PVX	PVS	CMV
Microtubers	Red potato	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)
	Granola	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)
G0 tubers	Red potato	40/50 (80.0)	0/50 (0.0)	0/50 (0.0)	41/50 (82.0)
	Granola	14/50 (28.0)	0/50 (0.0)	0/50 (0.0)	14/50 (28.0)

PVY = potato virus Y; PVX = potato virus X; PVS = potato virus S; CMV = cucumber mosaic virus. Values represent the number of positive samples/total samples tested, with percentage incidence shown in parentheses.

not detected in any sample. The highest incidence occurred in red potato G0 tubers, where PVY and CMV were detected in 80.0% and 82.0% of samples, respectively. In Granola G0 tubers, both PVY and CMV were detected at a lower incidence of 28.0%. These results indicate that virus infection most likely occurred after microtuber production, during the screen-house multiplication stage. Although PVX and PVS have previously been reported in Indonesian potato production systems, neither virus was detected in the present study, suggesting that these viruses were either absent or present below the detection limits of the serological assays employed.

The absence of detectable viruses in microtubers indicates that the tissue culture propagation system effectively produced virus-free planting materials. In contrast, the occurrence of PVY and CMV in G0 tubers suggests that infection was acquired during screen-house cultivation. Virus introduction at this stage may be associated with aphid-mediated transmission, mechanical contamination, or the presence of alternative host plants surrounding the production facility (Kumar et al., 2023; Kunjan, 2024). In addition, both PVY and CMV are efficiently transmitted by aphid vectors and can spread rapidly even under protected cultivation conditions when vector exclusion measures are insufficient. The lower virus incidence observed in Granola compared with red potato may be related to varietal differences in susceptibility, as Granola has been reported to exhibit relative tolerance to PVY infection (Hadiarto & Ambarwati, 2024). However, further studies under controlled conditions are required to confirm the contribution of host resistance to the differences observed in virus incidence between the two varieties.

**RT-PCR Analysis.** RT-PCR successfully amplified the coat protein (CP) genes of PVY and CMV, producing amplicons of approximately 801 bp and 657 bp, respectively (Figure 1). No amplification was obtained for PVX or PVS.

The molecular results were consistent with the serological assays and confirmed mixed infection by PVY and CMV in G0 tubers of both potato varieties. In contrast, no amplification was detected in microtuber samples, supporting their virus-free status. These findings demonstrate that viral infection occurred after the tissue culture stage and likely during screen house multiplication.

**CP Gene Sequencing Analysis.** Partial sequencing of the amplified CP genes generated 759 bp of PVY sequence encoding 253 amino acids and 528 bp of CMV sequence encoding 176 amino acids. The obtained sequences were subsequently used for homology and phylogenetic analyses. Aphid populations surrounding the screen house may have contributed to virus transmission, as aphids are recognized as the principal vectors of both PVY and CMV (Vishal et al., 2025; Kuan et al., 2024; Singh et al., 2025).

Comparative sequence analysis of the PVY CP gene revealed that the Indonesian isolates shared nucleotide identities ranging from 89.5% to 99.7% and amino acid identities ranging from 92.0% to 100.0% with reference isolates available in GenBank (Table 3). The highest similarity was observed with PVY-NTN isolates from China (HQ631374.1) and Japan (AB702952), showing 99.7% nucleotide identity and 100% amino acid identity. These results indicate that the Indonesian isolates are closely related to the PVY-NTN strain.

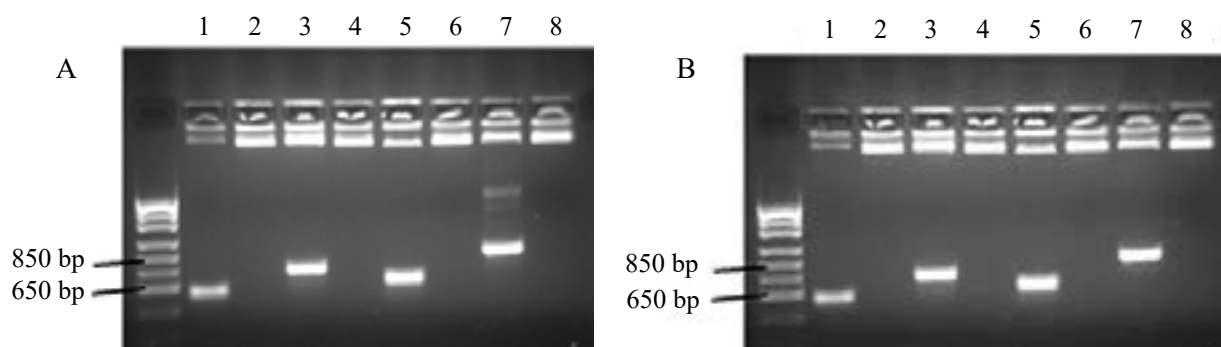


Figure 1. RT-PCR amplification of coat protein (CP) genes of potato virus Y (PVY) and cucumber mosaic virus (CMV) from microtuber and G0 tuber samples. A. Red potato variety; B. Granola variety. 1 = Microtuber red potato tested for CMV (MTCMV); 2 = Microtuber red potato tested for PVX (MTPVX); 3 = Microtuber red potato tested for PVY (MTPVY); 4 = Microtuber red potato tested for PVS (MTPVS); 5 = G0 red potato tested for CMV (G0CMV); 6 = G0 red potato tested for PVX (G0PVX); 7 = G0 red potato tested for PVY (G0PVY); 8 = G0 red potato tested for PVS (G0PVS).

Analysis of the CMV CP gene showed nucleotide identities ranging from 87.6% to 96.9% and amino acid identities ranging from 88.0% to 93.7% compared with reference CMV isolates from GenBank (Table 4). The highest similarity was observed with the CMV soybean stunt strain (CMV-S) from Indonesia (FJ177303.1), with 96.9% nucleotide identity and 93.7% amino acid identity. In contrast, substantially lower identity values were obtained with the outgroup

reference, peanut stunt virus (PSV-Mi), confirming its taxonomic separation from CMV. The high nucleotide and amino acid identities observed in both viruses indicate that the Indonesian potato isolates belong to previously described PVY-NTN and CMV-S lineages. The close relationship of the CMV isolates with the Indonesian CMV-S isolate further suggests that local virus populations may serve as important inoculum sources in potato seed production systems.

Table 3. Nucleotide and amino acid sequence identities of the coat protein (CP) gene of Indonesian PVY isolates from potato compared with reference PVY isolates available in GenBank

Origin	Strain	Coat protein		GenBank accession No.
		Nucleotide identity (%)	Amino acid identity (%)	
Brazil	PVY-NTN	94.4	96.4	AY840082.1
China	PVY-NTN	99.7	100.0	HQ631374.1
England	PVY-NTN	99.2	99.2	EF027869.1
Germany	PVY-NTN	99.3	99.2	AJ890345.1
India	PVY-O	89.5	92.0	AY061994.1
Iran	PVY-NTN	99.3	99.2	HM243480.1
Japan	PVY-NTN	99.7	100.0	AB702952
Spain	PVY-C	89.9	93.6	AF012027.1
Switzerland	PVY-N	97.3	98.8	X97895
USA	PVY-NTN	99.4	99.6	AY884982.1
USA	PeMoV*	68.7	73.9	NC_001517.1

Sequence identities were calculated using BioEdit version 7.2. PVY = potato virus Y; PVY-NTN = Necrotic tuber necrosis strain; PVY-N = Necrotic strain; PVY-O = Ordinary strain; PVY-C = Common strain. \*pepper mottle virus (PeMoV) was included as an outgroup reference. Nucleotide (nt) and amino acid (aa) identities were calculated from partial coat protein (CP) gene sequences.

Table 4. Nucleotide and amino acid sequence identities of the coat protein (CP) gene of Indonesian CMV isolates from potato compared with reference CMV isolates available in GenBank

Origin	Strain	Coat protein		GenBank accession No.
		Nucleotide identity (%)	Amino acid identity (%)	
China	SXFQ	89.7	88.0	JX993914.1
China*	PSV-Mi	52.0	39.7	AY775057.1
Netherlands	Lily	89.0	88.6	AJ131615.1
India	Ts	90.1	89.2	EF153734.2
Indonesia	CMV-S	96.9	93.7	FJ177303.1
Japan	HL	89.2	88.6	AB049568
Korea	RP20	89.0	88.0	KC527749.1
Taiwan	NT9	89.9	88.6	D28780.1
Thailand	TR15	87.6	88.9	AJ810264.1
USA	113	90.1	89.2	AF523340.1

Sequence identities were calculated using BioEdit version 7.2. CMV = cucumber mosaic virus; CMV-S = soybean stunt strain. The CMV isolates originated from different host plants, including soybean, tomato, pumpkin, lily, chili pepper, and cucumber. \*peanut stunt virus (PSV-Mi) was included as an outgroup reference. Nucleotide (nt) and amino acid (aa) identities were calculated from partial coat protein (CP) gene sequences.

**Phylogenetic Analysis.** Phylogenetic analysis based on coat protein (CP) gene sequences showed that PVY isolates obtained from G0 tubers of red and Granola potatoes clustered with PVY-NTN isolates from China and Japan (Figure 2A). This grouping was supported by high bootstrap values, indicating a close genetic relationship with previously reported PVY-NTN isolates. The results are consistent with sequence identity analysis, which demonstrated high nucleotide and amino acid similarities between the Indonesian isolates and reference PVY-NTN strains.

PVY-NTN is regarded as one of the most economically important PVY strains because it is associated with potato tuber necrotic ringspot disease (PTNRD), a disorder that significantly reduces tuber quality and marketability (Baranwal et al., 2021). Since its first description in Hungary by Beczner in 1984, the virus has been reported in major potato-growing regions worldwide and has become a serious constraint to potato production (Mishra et al., 2025). Infection may cause substantial economic losses in susceptible cultivars, and disease symptoms often continue to develop during post-harvest storage (Kuan et al., 2024; Singh et al., 2025). Owing to its phytosanitary importance, PVY-NTN is regulated as a quarantine pest in several countries, including the United States and Canada (Kumar et al., 2023).

Similarly, CMV isolates from red and Granola potatoes clustered with the CMV soybean stunt strain (CMV-S) previously reported from Indonesia (Figure 2B). The high sequence similarity between potato and soybean isolates suggests that local CMV populations circulating among different host species may serve as potential sources of inoculum. CMV-S has been

reported infecting soybean in several Asian countries, including Japan and Korea (Singh et al., 2025), and has also been associated with potato diseases characterized by mosaic, chlorosis, leaf deformation, stunting, and tuber damage (Wu et al., 2024). The close relationship between the potato isolates identified in this study and Indonesian CMV-S isolates highlights the potential role of alternative host plants as virus reservoirs in potato seed production environments.

Molecular characterization and phylogenetic analysis are valuable tools for understanding virus diversity, evolutionary relationships, and potential dissemination pathways among agricultural production systems (Listihani et al., 2024). Overall, the phylogenetic analyses confirmed that the viruses detected in G0 potato tubers belonged to the PVY-NTN and CMV-S strains. These findings provide molecular evidence that economically important virus strains are present in potato seed production systems and emphasize the need for continuous virus surveillance and strict phytosanitary management to maintain seed health and quality.

**Implications for Seed Potato Production.** The absence of viruses in microtubers and their subsequent detection in G0 tubers indicate that the screen house stage represents a critical point for virus introduction in the seed production chain. These findings highlight the importance of strengthening screen house biosecurity, controlling aphid vectors, eliminating alternative host plants around production facilities, and implementing routine molecular testing of G0 seed stocks.

Early detection of PVY-NTN and CMV-S is particularly important because infected seed tubers may

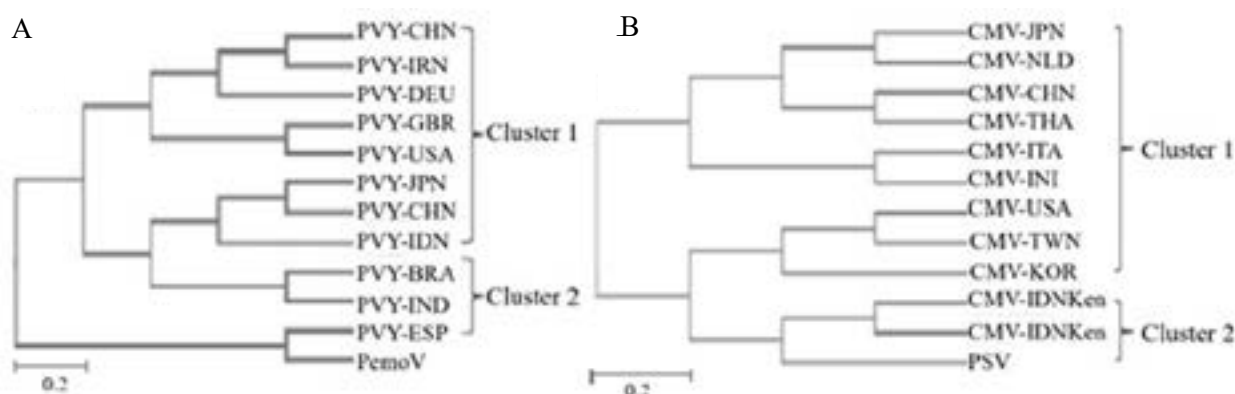


Figure 2. Maximum Likelihood phylogenetic trees based on nucleotide sequences of the coat protein (CP) gene. A. potato virus Y (PVY); B. cucumber mosaic virus (CMV). Isolates from G0 red potato and Granola potato are highlighted in gray. Numbers at branch nodes indicate bootstrap support values (1000 replicates). Scale bars represent nucleotide substitutions per site. pepper mottle virus (PeMoV) and peanut stunt virus (PSV) were used as outgroup references.

serve as a primary source of inoculum for subsequent seed generations, leading to progressive virus accumulation and reduced seed quality. Therefore, integrating molecular diagnostics into potato seed certification programs would contribute substantially to the production of high-quality virus-free seed in Indonesia.

## CONCLUSION

This study successfully detected and molecularly characterized potato virus Y strain NTN (PVY-NTN) and cucumber mosaic virus soybean strain (CMV-S) infecting G0 tubers of red and Granola potato varieties produced by PT. G10 Agrotechnology. No virus infection was detected in microtubers, indicating that the tissue culture system effectively produced virus-free planting materials. In contrast, PVY-NTN and CMV-S were detected in G0 tubers, with a higher incidence in red potato (80–82%) than in Granola (28%), suggesting differences in varietal susceptibility. Sequence identity and phylogenetic analyses revealed that the Indonesian PVY isolates were closely related to PVY-NTN isolates from China and Japan, whereas the CMV isolates clustered with the CMV soybean stunt strain (CMV-S) previously reported from Indonesia. These findings indicate that virus infection most likely occurred during the screen-house multiplication stage and highlight the importance of early virus monitoring in potato seed production systems. Further studies are needed to investigate the epidemiology, vector transmission, and genetic diversity of PVY-NTN and CMV-S in Indonesian potato production areas to support the development of effective disease management and seed certification programs.

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## AUTHORS' CONTRIBUTIONS

DSD was responsible for conceptualization, methodology, investigation, formal analysis, data curation, visualization, and writing of the original draft. LS and AF contributed to methodology, molecular analysis, and review and editing of the manuscript. AH and LAU contributed to resources, supervision, funding acquisition, and manuscript review and editing. HSP and SDP contributed to validation, field investigation, and manuscript review and editing. All authors have read and approved the final manuscript.

## COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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