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Survey of Potyviruses, Carlaviruses, and Begomoviruses in Potato Cultivation Centers of West, Central, and East Java Provinces, Indonesia

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Abstract: Our knowledge of the molecular data on viruses infecting potato (*Solanum tuberosum*) and its weeds in Indonesia still needs to be expanded. Thirteen potato leaves, together with one carrot (*Daucus carota* subsp. *sativus*) and one *Oxalis latifolia* leaves grew adjacent to potato fields were sampled in West, Central, and East Java Provinces of Indonesia to be tested using RT-PCR with universal primers to monitor the presence of potyviruses, carlaviruses, and begomoviruses in the surveyed regions. According to the test results and BLAST nucleotide analysis, four potato samples were found to be infected by potato virus Y (PVY, *Potyvirus*). All samples tested negative for carlaviruses and begomoviruses. Both phylogenetic trees constructed by MEGA11 software based on partial coat protein and partial coat protein + 3′-UTR sequences demonstrated that the four new PVY isolates belong to strain N. Nucleotide and amino acid homology analysis determined that all four new and 12 other strain N isolates registered in NBCI GenBank were identical at the observed genome region, suggested the same origin of Indonesian isolates. This study improved our understanding of the variation in PVY isolates in Indonesia and highlighted the need for tight surveillance since the country imports most of its tubers used as planting material.

Keywords: genetic diversity; molecular test; percentage identity; phylogeny; plant virus disease

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1. Introduction

In tropical Indonesia, potato (*Solanum tuberosum*) is a highly valuable horticultural product that can only be grown optimally in elevated areas with low temperatures, resemble the growing conditions of its origin in South America [1]. Thus, global warming may

further limit productive regions. Different diseases, including those caused by viruses, also continue to pose challenges to the efforts to increase potato production to meet a rising demand in the country [2,3].

Potato virus Y (PVY, genus *Potyvirus*), which has RNA as genetic material, is commonly detected among potato cultivated around the world [4]. The virus is differentiated into O, C, and Z strains according to specific hypersensitive responses to three potato genes: Ny, Nc, and Nz, respectively [5]. PVY has been attributed as one of the most important plant viruses due to its wide host range, distribution, and huge economic impact [6]. A recent estimation predicted losses of EUR 187 million per year due to PVY infection in potato in Europe [7].

In Indonesia, PVY has been identified in several production centers: Lembang Subdistrict in West Java Province [2], Karo Regency in North Sumatera Province [8], and Wonosobo and Banjarnegara Regencies in Central Java Province [9]. Observations in Modoinding Subdistrict in North Sulawesi Province [10] and Kajoran District in Central Java Province [3] detected strains NTN and N, respectively. Therefore, molecular variation among isolates in Indonesia is present, but still insufficiently observed. Besides PVY, potato virus M (PVM, genus *Carlavirus*), potato virus X (PVX, genus *Potexvirus*), and cucumber mosaic virus (CMV, genus *Cucumovirus*) have been shown to infect potato cultivated in Pangalengan as well as nearby Rancabali and Boyongbong Districts in West Java Province [11].

DNA viruses belonging to the genus *Begomovirus* are also major obstacles in the production of solanaceous crops in Indonesia [12,13]. Although none has been reported in potato in the country so far, one begomovirus (potato yellow mosaic virus/PYMV) is considered an emerging pathogen of potato and tomato (*Solanum lycopersicum*) in some South American countries [14,15].

Molecular tests performed in the current survey could monitor potyviruses, carlaviruses, and begomoviruses that may be present and risk the yield losses among potato grown in West, Central, and East Java Provinces which cover most of Java Island. The obtained molecular data also updates our knowledge on the genetic variation among Indonesian isolates that may contribute to accurate country-wide management plans [16].

2. Materials and Methods

2.1. Fields Survey

Leaves of potato plant variety 'Granola', carrot (*Daucus carota* subsp. *sativus*) and *Oxalis latifolia* weed showing viral symptoms in fields in West, Central, and East Java Provinces, Indonesia, were collected in this study. All samples were put in an ice box and then brought to the Plant Pathology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada to be kept at −4 °C until further tests.

2.2. Molecular Identification

Total DNA and RNA were, respectively, extracted using the 'Genomic DNA Mini Kit (Plant)' and 'Total RNA Mini Kit (Plant)' (Geneaid Biotech Ltd., New Taipei City, Taiwan). Extraction was performed according to standard procedures provided by the kits' manufacturers. The extracted RNA was applied in cDNA synthesis using a ReverTra Ace kit (Toyobo, Tokyo, Japan), with a reaction volume of 10 µL: 2 µL RNA template, 1 µL dNTP, 0.5 µL (10 pmol μL^{-1}) Oligo(dT) primer, 0.5 µL RNAse inhibitor, 0.5 µL ReverTraAce®, 2 µL 5× RT Buffer, and 3.5 µL nuclease-free water. The reverse transcription program was set at 42 °C for 20 min and then 99 °C for 5 min.

DNA templates were tested against begomoviruses using F-CCNMRDGGHTGTGAR GGNCC/R-SVDGCRTGVGTRCANGCCAT universal primer pair with annealing at

55 °C for 30 s to amplify ± 580 bp of AV1 gene [17], while cDNA templates were tested against potyviruses using F-CCGGAATTCATGRTITGGTGYATIGAIAAYGG/R-GGATCCCGGGTTTTTTTTTTTTTTTV universal primer pair with annealing at 56 °C for 1 min to amplify 600–800 bp of the 3′-tail [18] and against carlaviruses using F-TGCTGCYTTTGATACYTTCGAT/R-GGATCCCGGGTTTTTTTTTTTTTTTV universal primer pair with annealing at 56 °C for 1 min to amplify ± 715 bp of the 3′-tail [19].

PCR mixes were each prepared in a volume of 40 µL: 4 µL of DNA or cDNA templates, 20 µL of MyTaq HS Red Mix (Bioline, Luckenwalde, Germany), 2 µL (10 pmol μL^{-1}) each of reverse primer and forward primer, and 12 µL of double distilled sterile water. The PCR program was set at 95 °C for 3 min as a pre-denaturation, 35 cycles of denaturation at 95 °C for 1 min, annealing according to each primer pair, and elongation at 72 °C for 1 min, ending with 72 °C for 7 min as a final elongation.

Single and specific-size targeted bands were observed using a UV transilluminator (Optima Inc., Tokyo, Japan) following electrophoresis of PCR products on 1% agarose gel stained with Florosafe DNA Staining (1st BASE, Seri Kembangan, Malaysia) for 60 min at 50 V. Successfully amplified PCR products were delivered to 1st BASE biotechnology enterprise (Malaysia) to be bidirectionally Sanger sequenced. Species identification was determined using nucleotide BLAST online software v. 1.4.0 against NCBI GenBank database (https://blast.ncbi.nlm.nih.gov, accessed on 11 May 2025). Nucleotide sequences of the obtained new isolates were registered to obtain NCBI GenBank accession numbers.

2.3. Phylogeny and Percentage Identity Analyses

Sequences of selected isolates of the same species registered in NCBI GenBank were aligned using ClustalW suits in MEGA11 software v.11.0.13 [20] and then trimmed according to the lengths of the isolates obtained in this study. MEGA11 software was also applied in the construction of a phylogenetic tree using the isolates alignment with the maximum likelihood (ML) statistical method and Kimura-2 [21] as the best suited parameter model. The statistical significancy of the tree branching was examined with 1000 bootstrap replicates. The nucleotide (nt) and amino acid (aa) percentage identities of the aligned isolates were determined using Sequence Demarcation Tool (SDT v1.2) software [22].

3. Results

3.1. Fields Survey

Five potato samples were taken from three fields in Pangalengan, Bandung Regency, West Java Province on 19 January 2024; four potato samples were taken from two fields in Ngablak, Magelang Regency, Central Java Province on 10 September 2024; and four potato samples were taken from two fields in Malang Regency, East Java Province on 9 December 2024 (Figure 1). Samples from Central and East Java showed mosaic while samples from West Java besides mosaic also exhibited severe leaf crumple. A single sample showing strong chlorosis was also taken from West Java (Figure 2). In addition, one composite sample of weed *Oxalis latifolia* showed mosaic, chlorosis, and leaf curling, and one composite sample of carrot showing chlorosis was also taken from a carrot field adjacent to the potato fields in West Java Province (Figure 2). In total, 15 samples were collected to be tested for potyviruses, carlaviruses, and begomoviruses.

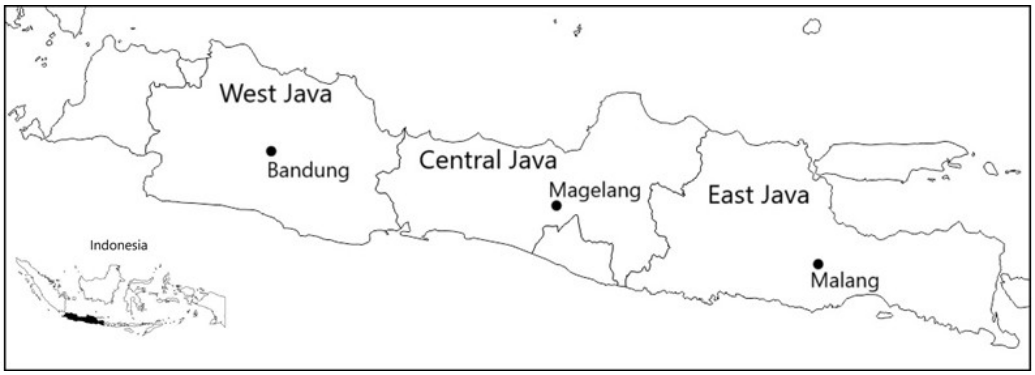


Figure 1. Sampling locations in West, Central, and East Java Provinces, Indonesia were pointed with black dots.



Figure 2. Different symptom types on leaf samples collected from fields on Java Island, Indonesia. (**A**) Four potato samples from Central Java Province showing clear mosaic on potato leaves; (**B**) four potato samples from West Java Province showing leaf crumple and slight mosaic; (**C**) potato samples from West Java Province showing severe chlorosis (yellowing); (**D**) four potato samples from East Java Province showing mosaic and slight leaf crumple; (**E**) mosaic, mild chlorosis, and leaf curling on *Oxalis latifolia* from West Java Province (prepared as a single composite sample); (**F**) mild chlorosis on carrot from a carrot field adjacent to potato fields in West Java Province (prepared as a single composite sample). Mosaic symptoms are shown with black lines.

3.2. Molecular Identification

Two potato samples from West Java, one from Central Java, and one from East Java Province were found by the RT-PCR test to be positive for potyvirus infection. All four isolates were partially sequenced at the 3′-tail region. BLAST analysis on the obtained sequences determined that those isolates were PVY. Nucleotide sequences of both PVY West Java isolates were registered in NCBI GenBank with acc nos. PP596544 and PP596545 while the two isolates from Central and West Java were given acc nos. PV235475 and PV235476, respectively. All 15 samples were tested negative for carlavirus and begomovirus.

3.3. Phylogeny and Percentage Identity Analyses

The four new Indonesian isolates were aligned and tested against other 28 PVY isolates registered in NCBI GenBank database (Table A1). Both constructed phylogenetic trees, based on 417 bp partial CP region and ±751 bp partial CP + 3′-UTR (Figure 3), showed that the isolates obtained in this study were clustered with 12 other strain N isolates, including isolates no. OR180052 and OR180053 from Kajoran, Central Java Province, and formed a group separate to NTN, C, and O groups. Three isolates nos. KT599906–KT599908 from the Indonesian island of Sulawesi were clustered in NTN group.

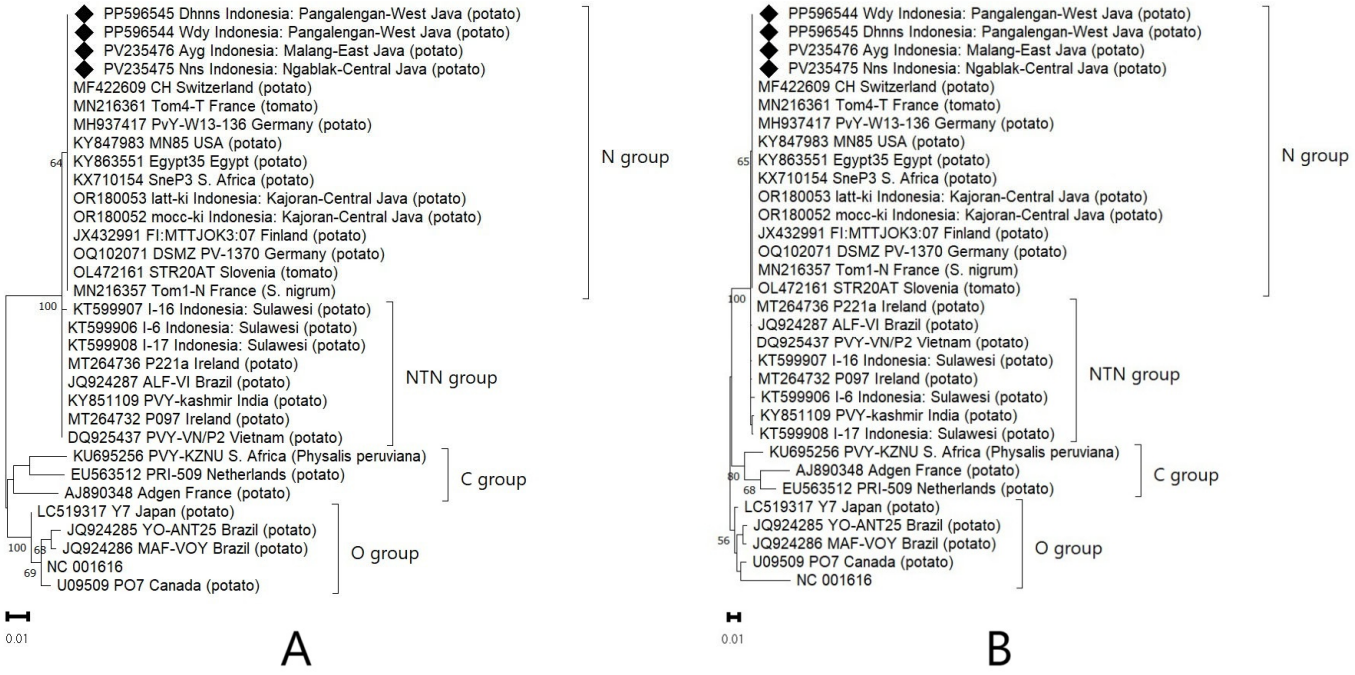


Figure 3. Maximum-likelihood phylogenetic trees were built under Kimura 2-parameter model with 1000 bootstraps using MEGA11 software. Only >50% of bootstrap values were shown. (**A**) The tree based on 417 bp of the partial coat protein region of PVY genome. (**B**) The tree based on ±751 bp of partial coat protein + 3′-UTR of PVY genome. The four new Indonesian isolates reported here are highlighted with black diamond signs.

According to the observation on the 417 bp partial CP, the four new Indonesian PVY isolates shared 100% identities to each other at both nt and aa levels, indicated that they were identical. Further examination revealed that they and 12 other strain N isolates tested in this study were also identical. Strain N isolates had 99.5–99.8% nt and 99.3% aa identities in strain NTN isolates, and 95.2–95.9% nt and 96.4–99.3% had aa identities in strain O isolates at the observed genome region (Figure 4).

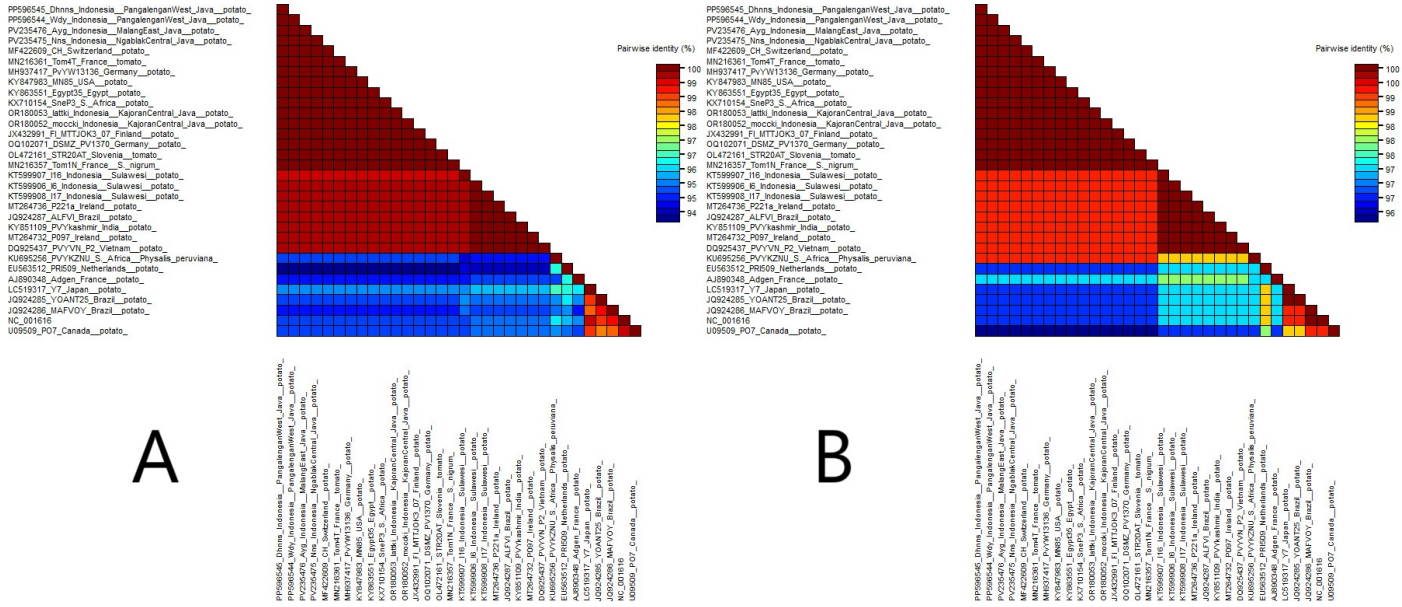


Figure 4. Percentage identities of the four new Indonesian PVY isolates at 417 bp of 3′-end of coat protein region of PVY genome when compared using SDT v1.2 software to the other 28 isolates in NCBI GenBank database. (**A**) Nucleotide identity; (**B**) Amino acid identity.

4. Discussion

PVY was reported to have a very high incidence rate of 80% in potato according to a previous study in Pangalengan District which also confirmed 28% and 82% incidence rates, respectively, in Rancabali and Bayongbong Districts of West Java Province [11]. Similarly, the current survey detected PVY in two of four tested potato samples from West Java as well as one sample in each of Central and East Java. PVY has also been reported to have mixed infection with rehmannia mosaic virus (ReMV, genus *Tobamovirus*) in tobacco (*Nicotiana tabacum*) in Central Java [23].

Although PVY has not been detected in carrot, other potyviruses—carrot thin leaf virus (CTLV) [24], carrot virus Y (CarVY) [25], apium virus Y (ApVY) [26], and watermelon mosaic virus (WMV) [27]—have been described as pathogens of carrot, wild carrot (*Daucus carota*), and plant members of Family Apiaceae. PVY has also not been identified in *O. latifolia* but the weed could harbor tomato severe rugose virus (ToSRV) [28], a species of *Begomovirus* that infects tomato and potato [29]. Primer pairs applied in this study successfully detected different carlaviruses [3,19] and begomoviruses [13,17] commonly found in Indonesia and other countries, respectively.

Severe leaf malformation, chlorosis, and plant dwarfism may be experienced by potato exposed to PVY at the early growing stage but the most often symptoms found on potato leaves are mosaic and mottle [30]. All four isolates reported here also led to clear mosaic on the leaves of infected potato var. Granola. Interestingly, potato samples with severe leaf crumpling from West Java as well as samples with mild leaf crumpling from East Java all tested negative for PVY (Figure 2). Since all samples were also not infected by carlavirus and begomovirus, the leaf crumple symptom was probably caused by other causal agents, including phytoplasma or abiotic factors. This report should incite further research on undiagnosed abnormalities.

The only samples with yellowing symptoms tested negative for the three viruses. Chlorosis followed by the rapid drying of leaf and branch (necrosis) of potato is usually attributed to late blight disease caused by *Phytophthora infestans* [31]. Our field survey in West Java found relatively widespread chlorosis and necrosis only in the third field, while few plants exhibited the symptoms in the first and second fields. Although the

identification and genetic information of Indonesian isolates of *P. infestans* are still very limited, a few studies had already attempted to determine tolerant Indonesian potato clones to the pathogenic fungus [32,33].

Observation on the partial CP (417 bp) and partial CP + 3′-UTR (±751 bp) sequences formed phylogenetic trees, which suggest that the four new isolates were strain N. The exact topographies shared by both trees indicated that the examined isolates were not recombinants, at least in the observed region (Figure 3). Similarly, a recent study identified two strain N isolates in Kajoran District, Central Java Province [3]. These results could be indications of the dominance of strain N among PVY in Java Island. All Indonesian strain N isolates were confirmed identical to other strain N isolates from different hosts and origins examined in the identity study, at least at the observed region (Figure 4). The high genetic similarity strongly suggests a common origin [34,35] with a pattern of occurrence that raises concerns regarding its potential widespread distribution and epidemiological importance. Aphids, a vector of PVY, were not found on potato plants during field visits, probably due to routine sprays of insecticides. However, the virus could be easily transmissible to the next generation by infected potato tubers [36]. Thus, this scenario may be associated with the dissemination of infected planting material. This highlights a critical issue in the Indonesian potato industry, as the country relies heavily on imported tubers due to the limited capacity for local seed production. The potential introduction and dissemination of PVY through such planting materials requires urgent attention and strict quarantine surveillance. For farmers, this underlined the importance of healthy seeds in the management of PVY.

This quick survey of plant viruses only identified PVY in potato in the West, Central, and East Java Provinces. The finding of PVY strain N could incite future studies to understand the genetic diversity of PVY in Java Island and other regions of Indonesia which may lead to widely applicable preventive measures against the emergence of new PVY recombinants that could threaten potato production in the country.

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Data Availability Statement: Partial genome sequences of four Indonesian PVY isolates reported in this study have been made available in NCBI GenBank, with reference numbers PP596544, PP596545, PV235475, and PV235476.

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Abbreviations

The following abbreviations are used in this manuscript:

CP	coat protein
nt	nucleotide
aa	amino acid
MEGA	Molecular Evolutionary Genetics Analysis
SDT	Sequence Demarcation Tool

Appendix A

Table A1. Isolates included in phylogenetic and homology analyses.

No.	Accession No.	Isolate Name	Origin	Host	Strain
1.	PP596544	Wdy	Indonesia: West Java	potato	N
2.	PP596545	Dhnns	Indonesia: West Java	potato	N
3.	PV235476	Ayg	Indonesia: East Java	potato	N
4.	PV235475	Nns	Indonesia: Central Java	potato	N
5.	MF422609	CH	Switzerland	potato	N
6.	MN216361	Tom4-T	France	tomato	N
7.	MH937417	PvY-W13-136	Germany	potato	N
8.	KY847983	MN85	USA	potato	N
9.	KY863551	Egypt35	Egypt	potato	N
10.	KX710154	SneP3	South Africa	potato	N
11.	OR180053	latt-ki	Indonesia: Central Java	potato	N
12.	OR180052	mocc-ki	Indonesia: Central Java	potato	N
13.	JX432991	FI:MTTJOK3:07	Finland	potato	N
14.	OQ102071	DSMZ PV-1370	Germany	potato	N
15.	MN216357	Tom1-N	France	*Solanum nigrum*	N
16.	OL472161	STR20AT	Slovenia	tomato	N
17.	KY851109	PVY-kashmir	India	potato	NTN
18.	MT264736	P221a	Ireland	potato	NTN
19.	JQ924287	ALF-VI	Brazil	potato	NTN
20.	DQ925437	PVY-VN/P2	Vietnam	potato	NTN
21.	MT264732	P097	Ireland	potato	NTN
22.	KT599906	I-6	Indonesia: Sulawesi	potato	NTN
23.	KT599907	I-16	Indonesia: Sulawesi	potato	NTN
24.	KT599908	I-17	Indonesia: Sulawesi	potato	NTN
25.	JQ924285	YO-ANT25	Brazil	potato	O
26.	U09509	PO7	Canada	potato	O
27.	JQ924286	MAF-VOY	Brazil	potato	O
28.	NC 001616	-	-	-	O
29.	LC519317	Y7	Japan	potato	O
30.	KU695256	PVY-KZNU	South Africa	*Physalis peruviana*	C
31.	AJ890348	Adgen	France	potato	C
32.	EU563512	PRI-509	Netherlands	potato	C

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