Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

OPEN ACCESS

Citation: Cara, M., Amoia, S. S., Sota, V., Merkuri, J., Cara, O., Hoxhallari, K., Papakosta, E., Kongjika, E., & Minafra, A. (2026). Preserving autochthonous Albanian plum germplasm: Plum pox virus-free status and *in vitro* sanitation perspectives for the 'Tropojane' cultivar. *Phytopathologia Mediterranea* 65(1): 93-105. doi: 10.36253/phyto-17066

Accepted: March 4, 2026

Published: May 14, 2026

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Assunta Bertaccini, Alma Mater Studiorum, University of Bologna, Italy.

ORCID:
MC: 0000-0002-1715-8635
SSA: 0000-0002-1661-8417
VS: 0000-0002-5840-0387
JM: 0009-0004-5610-763X
OC: 0009-0000-0682-0796
KH: 0009-0002-7618-7660
EP: 0009-0005-4601-4920
EK: 0000-0002-5328-3919
AM: 0000-0002-0547-7129

Research Papers

Preserving autochthonous Albanian plum germplasm: Plum pox virus-free status and *in vitro* sanitation perspectives for the 'Tropojane' cultivar

Magdalena CARA[1,2], Serafina Serena AMOIA[3,*], Valbona SOTA[4,7], Jordan MERKURI[2], Orges CARA[5,2], Klevis HOXHALLARI[1,2], Elektra PAPAKOSTA[6], Efigjeni KONGJIKA[7], Angelantonio MINAFRA[3]

[1] *Agricultural University of Tirana, Faculty of Agriculture and Environment, Rruga Paisi Vodica 1025, Tirana, Albania*
[2] *NanoBalkan, Academy of Sciences of Albania, Murat Toptani Avenue, 1000 Tirana, Albania*
[3] *Institute for Sustainable Plant Protection (IPSP)—National Research Council, Via Amendola 122/D, Bari 70126, Italy*
[4] *Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania, Bul. Zog. 1, 1001, Tirana, Albania*
[5] *International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM of Bari), Valenzano, Italy*
[6] *Agricultural Technology Transfer Center, Shamogjin, Vlora, Albania*
[7] *Research Center of Biotechnology and Genetics, Academy of Sciences of Albania, Murat Toptani Avenue, 1000, Tirana, Albania*
*Corresponding author. E-mail: serafinaserena.amoia@cnr.it

Summary. The stone fruit industry, particularly plum (*Prunus domestica*) production, is important to the Albanian economy. The *Prunus domestica* 'Tropojane' predominates due to its high and stable productivity, adaptability, and superior organoleptic qualities. Assessing phytosanitary status of this fruit plant is important, to ensure sustainable production and preserve genetic resources. During the spring seasons of 2022, 2023 and 2024, 129 samples of 'Tropojane' plum were collected across the regions of Tropoja, Kukës, Has, Puka, Durrës, Paskuqan, and Kamëz of Albania. All samples were tested by ELISA, PCR, and qPCR to detect plum pox virus (PPV), the most destructive virus infecting plum, and to screen for additional stone fruit viruses including *Prunus* necrotic ringspot virus (PNRSV), prune dwarf virus (PDV), apple mosaic virus (ApMV), and apple chlorotic leafspot virus (ACLSV). ELISA tests demonstrated that PPV was present in 35.5% of the samples, whereas RT-PCR and RT-qPCR assays detected PPV in 45.6% of the samples, confirming greater sensitivity of the PCR assays for virus detection. No infections with other assessed stone fruit viruses were detected. PPV identity was confirmed by sequencing, and phylogenetic analyses showed that the Albanian isolates clustered within the Rec strain group, also indicating their possible regional origin. Meristem culture was employed as a sanitation strategy for PPV-infected explants. Differences in regeneration capacity among plum populations were observed, yet stable *in vitro* cultures were established in all cases, and molecular diagnostics confirmed that regenerated plantlets were PPV-free. *In vitro* shoots were successfully subcultured, rooted, and acclimatized. These results highlight the need to conserve native plum germplasm, and enforce the use of certified planting material, to ensure the long-term preservation of autochthonous cultivars, while preventing further spread of PPV.

Keywords. Plum pox virus, Sharka disease, virus detection, *in vitro* sanitation, meristem tip culture.

INTRODUCTION

Albania has a long tradition in the cultivation of stone fruits (*Prunus* spp.), which have considerable socio-economic importance. The European plum (*Prunus domestica* L.) is the most widely grown *Prunus* species in Albania. Both native and foreign plum varieties are cultivated across all regions, with total production of 40,196 tons over 2,559 ha in 2024 (ISTAT, 2024). Albanian germplasm includes more than 20 autochthonous plum varieties, among which the *Prunus domestica* 'Tropojane' predominates, accounting for more than 50% of all plum trees in the country (Çakalli *et al*., 2007). 'Tropojane', also known as "Kumbulla e Hasit", derives its name from the area where it originated, and is cultivated throughout Albania due to its high adaptability and desirable agronomic and pomological traits. This cultivar is late flowering, and produces large, dark-skinned, elliptic fruits (each approx. 60 g) that ripen in September each year. The fruit flesh is yellow-reddish, juicy, aromatic, and moderately firm (Kokaj, 2025). 'Tropojane' plum is used extensively for fresh consumption and processing (*e.g.*, dried prunes, jams, and traditional alcoholic drinks), underscoring its importance as a valuable genetic resource within Albanian *Prunus* germplasm.

Nevertheless, plums are susceptible to numerous pests and diseases, among which virus infections pose significant threats, leading to yield losses and deterioration of fruit quality (Rubio *et al*., 2017). In particular, *Potyvirus plumpoxi* (plum pox virus, PPV), the etiological agent of Sharka, is considered one of the most harmful virus pathogens that internationally affect plums and other stone fruits, with cumulative losses estimated to value € 2.4 billion over the last 28 years (Cambra *et al*., 2024). For these reasons, PPV is ranked as either a quarantine pest or a regulated non-quarantine pest (RNQP), due to its widespread endemic presence [EPPO PM 7/32 (2)].

PPV (*Potyvirus*, *Potyviridae*) has flexuous filamentous particles. It possesses a positive-sense single-stranded RNA (ssRNA) genome of approx. 9.7 kb (García *et al*., 2014), which encodes a single large open reading frame (ORF). To date, ten independent strains have been identified, varying in established sequences, geographical distributions, and host ranges: D, M, EA, Rec, T, C, CR, CV, W, and An (García *et al*.,2025). The M (Marcus), D (Dideron), and Rec (Recombinant) strains are considered the most epidemiologically important, with the D and Rec strains exhibiting preference for plum hosts (Sihelská *et al*. 2017). The symptoms caused by this virus variants are strongly influenced by the host cultivar, environmental conditions (*e.g.*, temperature), and age and physiological stage of the infected plants (Clemente-Moreno *et al*., 2015). PPV induces yellowish to olive-green bands, rings, spots and mottling on host leaves, which may be associated with leaf malformation. On plum fruits, PPV infections cause shallow depressions that deepen as fruits ripen, leading to deformations and irregular line patterns on the fruit surfaces. Infected fruits are tasteless and fibrous, making them unmarketable, and, in some cases, all fruits may prematurely drop (Llácer and Cambra, 2006; Pedrelli *et al*., 2024).

PPV was first reported in 1918 in Bulgaria infecting plums (Atanasoff, 1932), and has since spread to other European countries, the Near and Middle East, Asia, North Africa, and America (EPPO, 2025). In Albania, several studies of PPV on stone fruits have been conducted, confirming its presence in plum, apricot, and peach and showing high infection rates in tested plants from orchards, nurseries, and mother plots (Myrta *et al*., 1995; Musa *et al*., 2010). Myrta *et al*. (1998) and Stamo *et al*. (2003) reported that PPV-M and PPV-D were the predominant strains. In contrast, Palmisano *et al*. (2015) confirmed the presence of PPV-Rec and PPV-T, with the latter representing one of the few occurrences of the virus recorded outside Türkiye. PPV transmission pathways explain this widespread distribution. Long-distance dissemination of the virus is primarily facilitated by the movement of infected propagation material, often introduced through insufficiently regulated commercial or illegal exchanges. Approximately 30 aphid species mediate natural non-persistent plant-to-plant transmission of PPV (Rimbaud *et al*., 2015). Among these aphids, *Myzus persicae*, *Aphis spiraecola*, and *Hyalopterus pruni* are the most efficient vectors in the Mediterranean region (Cambra and Vidal, 2016). Effective surveillance and implementation of preventive containment strategies remain essential to mitigate spread and impacts of PPV.

Virus-free host propagation through *in vitro* meristem culture is widely recognized as an effective strategy for eliminating PPV and other graft-transmissible pathogens from infected plant material (Bhat and Rao, 2020; Szabó *et al*., 2024). Shoot apex (0.4 to 0.7 mm) meristems often do not have systemic infections, because viruses typically fail to invade the actively dividing cells within the apical domes and youngest leaf primordia (Mori and Hosokawa, 1977; Mochizuki and Ohki, 2015; Vivek and Modgil, 2018; Singh, 2025). Use of the meristem culture, alone or combined with thermotherapy, is efficient for producing PPV-free plants in many stone fruit species, including plum (Kabylbekova *et al*., 2025), sweet cherry (Naddaf *et al*., 2021), nectarine (Manganaris *et al*., 2003), peach (Dessoky *et al*., 2018), and apricot (Pérez-Caselles *et al*., 2025). Although the regenerative potential after meristem excision can decrease due to the reduced explant size, optimizing the physicochemical culture factors can regenerate high numbers of healthy plantlets during subculturing stages, and these can be used as mother plants for certified propagation programs. The effectiveness of meristem culture can be reliably confirmed through the integration of molecular diagnostics, particularly PCR-based assays, which enable rapid and highly sensitive detection of virus infections in regenerated plantlets (Gong *et al*., 2019; Kang *et al*., 2025).

Given the significant socio-economic value of the autochthonous European plum (*Prunus domestica* 'Tropojane') in Albania, and the potential threats posed by Sharka, the present study aimed to assess prevalence and distribution of PPV in native plum germplasm across this country's major plum-producing regions. The study also aimed to optimize an integrated *in vitro* sanitation and PCR-based protocol for production of healthy material from plum plants that tested PPV-positive, for subsequent conservation and multiplication.

MATERIALS AND METHODS

Sources and locations of plant material

During the spring seasons (April to May) of 2022, 2023 and 2024, ten orchards of *Prunus domestica* 'Tropojane' were inspected for virus symptoms across seven regions of Albania. A total of 129 leaf samples were collected from Tropoja (17 samples), Kukës (12), Pukë (22), Has (18), Paskuqan (from Kukës, 30), Kamëz (from Kukës, 14), or Durrës (from Tropoja, 16 samples). Field sampling included asymptomatic and symptomatic leaves mainly exhibiting chlorotic spots, bands, and/or rings. After collection, the samples were briefly stored at 4°C for further analyses.

Serological and Molecular detection of PPV and other common Prunus *viruses*

DAS-ELISA, RT-PCR, and RT-qPCR assays

All samples were initially screened for PPV presence using a Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) (Clark and Adams, 1977). Monoclonal antibodies from line 5B were used to detect all known serotypes of PPV, using the commercial K-10 PPV-Universal ELISA KIT (AGRITEST) (Cambra *et al*., 1994). Sample assessments were carried out using internal positive and negative controls supplied with the kit, and absorbance was measured at λ = 405 nm using a PR 4100 Absorbance Microplate Reader (Bio-Rad). A sample was considered positive when its signal exceeded at least three times the value of the negative internal control.

Following serological screening, all samples were subjected to molecular analyses using reverse transcriptase (RT-PCR) and quantitative qPCR assays. Total nucleic acids (TNA) were extracted from 0.2 g of leaves from each sample, which was homogenized in 1 mL of grinding buffer, then isolated using silica particles, as described by Foissac *et al*. (2001). The TNA quality was checked by electrophoresis on agarose gel (1.2%) in Tris-Borate-EDTA buffer, and the extracts were stored at -20°C until used. Starting from 500 ng of TNA, cDNA was synthesized by reverse transcription, using 1 μL of random hexamer primers (0.5 mg mL^{-1}) and M-MLV reverse transcriptase, following the manufacturer's instructions (Thermo Fisher). PCR and qPCR assays were carried out on all samples using specific primers for PPV and to discriminate between M and D strains of PPV (Table 1). Positive (cDNA from plums previously infected by the PPV-M, isolate GR046, GenBank accession number PV955083) and negative (*i.e*., cDNA from a healthy plum) controls were added in the reactions.

In addition to PPV, a subset of ten samples, selected from across the seven regions (above), was also screened for Prunus necrotic ringspot virus (PNRSV), prune dwarf virus (PDV), and apple mosaic virus (ApMV; *Ilarvirus*, *Bromoviridae*) and apple chlorotic leafspot virus (ACLSV; *Trichovirus*, *Betaflexviridae*), because these viruses are often found in mixed infections with PPV in most stone fruit hosts. All the primers used in this study are listed in Table 1.

Each PCR reaction consisted of 12.5 μL of GoTaq® Green Master Mix (Promega), 2 μL of cDNA, 0.5 μL of each primer, and DNase/RNase-free water to 25μL final volume. qPCR assays were carried out using a 2× Fast SYBR™ Green Master Mix (Applied Biosystems™), a ready-to-use reaction mix optimized for dye-based qPCR on a CFX96 real-time thermocycler (BioRad).

Table 1. List of primers used in RT-PCR and RT-qPCR for detecting stone fruit viruses. The sequences (sense and antisense), amplicon sizes, and corresponding references are also reported.

Virus	Primer sequence (5´ to 3´)	PCR amplicon (bp)	Reference
PPV (PCR)	F: CAGACTACAGCCTCGCCAGA R: ACCGAGACCACTACACTCCC	243	Wetzel *et al.*, 1992
PPV-D PPV-M (qPCR)	F: CGTTTATTTGGCTTGGATGGAA F: GATTAACATCACCAGCGGTGTG R: GATTCACGTCACCAGCGGTGTG	76	Olmos *et al.*, 2005
ACLSV (qPCR)	F: GTTCCTGGCCGCAGAAGGCAGACCCCT R: GCTATGTTCGCGAAGATGGACTCC	86	Nickel and Fajardo,2014
ACLSV (PCR)	F: GCAGACCCCTTCATGGAAAG R: TTCGGGTCCGAAGATGTAGTC	218	Diaz-Lara *et al.*, 2020
PNRSV (qPCR)	F: GGTTTGCCGAATTTGCAATC R: GCCCTGAGTGGGACCAGAG	89	Palmisano (personal communication)
PNRSV (PCR)	F: TCACTCTAGATCTCAAGCAG R: GACACTTTTGCGCGTACGCA	200	Rosner *et al.*, 1997
ApMV (PCR)	F: ATCCGAGTGAACAGTCTATCCTCTAA R: GTAACTCACTCGTTATCACGTACAA	262	Menzel *et al.*, 2002
	F: TAGTCGCGAGCGTTTTATTTTCAT R: CTTCGAGCTTCACAGTCCT	784	Valasevich *et al.*, 2015
PDV (PCR)	F: CCGAGTGGATGCTTCACG R: CCTTTAATGAGTCCGTAGAC	220	Jarosova and Kundu,2010

Sequence analyses

Two PCR amplicons, each one amplified from a single accession, were Sanger sequenced in both directions by Macrogen Europe. The resulting sequences were then compared with those available in GenBank, using BLASTN to determine nucleotide similarity (%). Sequence alignments and phylogenetic analyses were carried out using MEGA software v. 12.1.1 to evaluate possible origins of the isolates, and to examine their overall clustering and potential strain-level differences.

Meristem excision and in vitro *regeneration of plum plantlets*

Stabilization of in vitro *cultures*. Apical shoot tips (each approx. 3–5 cm long) were collected from PPV-positive mother plants of 'Tropojane' grown in Durrës (origin Tropoja) and Paskuqan (origin Kukës). A total of five mother plants were used, and 30 apical shoots were collected *per* plant (n = 150 explants in total). The explants were sterilized with 0.01% mercuric chloride for 10 min. After several rinsing with sterile H_2O, the explants were then inserted into test tubes containing MS medium (Murashige and Skoog, 1962) supplemented with 1 mg L^{-1} of 6-benzylaminopurine (BAP), 0.1 mg L^{-1} of 1-naphthaleneacetic acid (NAA), 3% sucrose, and 0.6% agar. The pH value of the medium was set at 5.8 before autoclaving. The tubes containing explants were incubated for 4 weeks in a growth chamber at 25°C under a 16 h light/8 h dark photoperiod, with cool white fluorescent light at 46.25 µmol m^{-2} s^{-1}.

Meristem culture and PPV-testing. Meristem tips (each 0.7 to 0.9 mm) from mother plants of both origins (30 meristem tips from each geographic region) were aseptically excised using a binocular stereomicroscope, transferred to hormone-free MS medium supplemented with 3% sucrose and 0.6% agar, and incubated at 25°C under standard growth-room conditions for regeneration. Meristem-derived shoots were tested for PPV by RT-PCR at the first subculture stage at 42 to 56 d after meristem excision, when regenerated shoots reached 1.5 to 2.0 cm in length and had produced at least two to three expanded leaves.

Subculture stage and shoot regeneration. When the regenerated shoots reached 1.5 to 2 cm, they were transferred to subculture for further micropropagation. MS medium supplemented with 3% sucrose and 0.6% agar was used. Different BAP concentrations (0, 1.0, 1.5, or 2.0 mg L^{-1}) were compared to evaluating their efficiency for promoting lateral shoot growth.

Rooting and acclimatization. In vitro rhizogenesis was induced by using ½ MS medium supplemented with 1.0 mg L^{-1} of indole-3-butyric acid (IBA). Plants with well-developed roots were then acclimatized in sterilized peat in alveolate trays in an acclimatization tunnel inside a greenhouse under controlled temperature and light conditions. After acclimatization, the plants were transferred to bags containing fertilized soil mixed with 15 to 20% agriperlite. For each treatment, 30 explants were used *per* replicate, and the experiment was repeated three times, resulting in a total of 90 explants per treatment.

Experimental design and data analyses. The experiment was arranged in a Completely Randomized Design (CRD). After 45 d from excision, the meristematic tips were evaluated for their regeneration potential. During the subculture stage, the effect of BAP concentration on micropropagated 'Tropojane' plum shoots was assessed using physiological and biometric parameters. The lengths of the plantlets and the numbers of new shoots *per* explant were recorded and compared. The results are analyzed with JMP 7.0 and presented as means ± standard errors.

RESULTS

Field observations and symptom expression in 'Tropojane' plum

During field inspections in the native growing areas of 'Tropojane' plum, fewer symptomatic trees were observed compared to areas where this cultivar has been introduced, such as Tiranë and Durrës. At these sites, chlorotic mosaic, interveinal chlorosis, and leaf distortion with yellowing were most frequently recorded, reflecting possible early-season manifestations of PPV (Figure 1).

Figure 1. Symptoms of PPV infections in 'Tropojane' plum trees. (a) Leaves showing mild chlorotic mosaic, light green rings and uneven green zoning (in Tiranë). (b) Leaves exhibiting pronounced mottling along with interveinal chloroses (in Durrës). (c) Leaf distortions associated with yellowing (in Durrës).

Serological and molecular detection of viruses in 'Tropojane' plum

DAS-ELISA conducted on the 'Tropojane' samples yielded 46 PPV-positive reactions out of 129 samples tested, representing an overall PPV infection rate of 35.6%. Sample evaluation was based on absorbance values measured at 405 nm after 2 h of substrate incubation, with duplicate positive controls ranging from 0.315 to 2.315, while negative controls ranged from 0.076 to 0.118. The positivity threshold was defined as three times the mean absorbance value of the negative controls (≥0.23). Accordingly, samples with absorbance values equal to or exceeding this threshold were classified as ELISA-positive, whereas those below this limit were classified as ELISA-negative.

RT-PCR and RT-qPCR screening detected 59 PPV-positive samples (45.6%), exceeding the number of positives detected by ELISA (Figure 2; Table 2), with fully consistent results between the two assays.

The regional distribution of PPV infections was heterogeneous, with the greatest prevalence observed in Durrës (87.5%), Paskuqan (86.6%), and Kamëz (85.7%). In contrast, infection rates in Kukës and Has were lower (16.6%), and only 9% of samples from Pukë tested positive. In Tropoja, the region of origin for the 'Tropojane', PPV infection was not detected in any of the sampled trees. Notable discrepancies were observed between symptom expression and infection status. Several symptomatic trees were found to be RT-PCR-negative for PPV in Tropoja, Pukë, Kukës, and Has, whereas asymptomatic RT-PCR-positive trees were detected in Paskuqan, Kamëz, and Durrës.

In parallel, screening for other stone fruit viruses, including ACLSV, PNRSV, ApMV, and PDV, showed that none of the analyzed samples were infected with these viruses. Therefore, PPV was the only virus detected in the analyzed 'Tropojane' plum samples, with no indication of mixed infections.

Sequence and phylogenetic analyses

Sequence analyses indicated a high level of similarity between the two Albanian PPV isolates partially sequenced in this study (Al_Tropojane_1 and Al_Tropojane_2). The two obtained sequences were, respectively, 213 and 212 nucleotides (nts) in length (excluding the primers sequence). Those two sequences shared 98.4% similarity. When they were compared with reference sequences available in GenBank, through BLASTN analyses, a maximum nucleotide similarity 99.5% was obtained with isolates belonging to the PPV-Rec strain

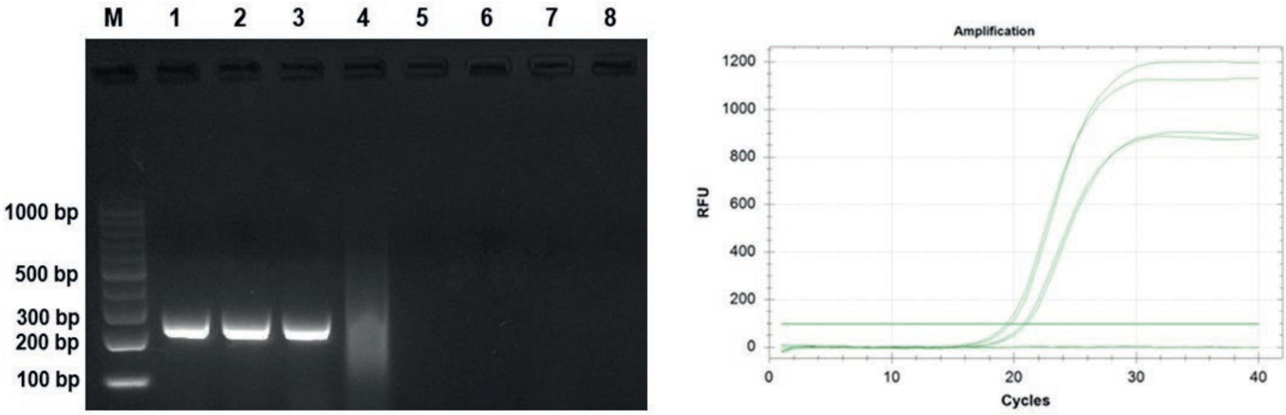

Figure 2. Representative results obtained from an RT-PCR/qPCR assay. Left image: Agarose gel electrophoresis of PCR amplicons from PPV-infected plum samples. Lane M: 100 bp DNA ladder; Lane 1: positive control (243 bp); Lanes 2 and 3: PPV-positive samples; Lanes 4 to 7: PPV-negative samples; Lane 8: sterile water used as negative control reaction. Right image: Amplification curves generated by RT-qPCR assays targeting PPV, using two different primer pairs.

Table 2. Summary of plum pox virus (PPV) detection in 'Tropojane' plum samples by ELISA, RT-PCR and RT-qPCR (2022–2024).

Region / Orchard location	Number of samples Total/Symptomatic	No. ELISA Positive	No. RT-PCR Positive	No. RT-qPCR Positive	Infection proportion (%)
Tropoja	17/3	0	0	-	0
Pukë	22/7	1	2	2	9
Kukës	12/5	1	2	2	16.6
Has	18/5	2	3	3	16.6
Kamëz - Tiranë (origin: Kukës)	14/9	10	12	12	85.7
Paskuqan - Tiranë (origin: Kukës)	30/23	20	26	26	86.6
Durrës (origin: Tropoja)	16/11	12	14	14	87.5
Total	129/63	46 (35.6%)	59 (45.6%)	59 (45.6%)	

(*i.e.*, GenBank accession numbers: EU117116, JX013532, AY690609). No recombination signals or strain-specific mutations were detected. These results support the classification of the PPV isolates from 'Tropojane' as stable, non-recombinant members of the PPV-Rec lineage.

The phylogenetic analysis to determine the strain affiliation and infer the possible origin of the Albanian isolates (Figure 3) delineated the isolates by their respective PPV strain affiliations. The two Albanian isolates clustered consistently within the PPV-Rec group, with Al_Tropojane_2 forming a well-supported subcluster (bootstrap value >80%) with isolates from Serbia (Serbia-T; GenBank accession number AY690609) and Russia (Al-Ch; GenBank accession number MN734791). In contrast, although positioned slightly basal within the Rec clade, Al_Tropojane_1 showed closest phylogenetic affinity to isolates from Poland (J4c; GenBank accession number EU117116), Croatia (Valjevka; GenBank accession number JX013532), and Ukraine (sREC; GenBank accession number KF472134), suggesting a regional evolutionary signature, and supporting absence of recent virus introductions or recombination events in this germplasm. The tip closest to the nodes belonging to PPV-M and -Rec progeny leads to the Ancestor strain (PPV-An), which gave the origin through recombination to both the above strains (Palmisano *et al*., 2025).

Meristem culture and in vitro *plantlet regeneration*

Establishment of in vitro *cultures*

Only materials from plum mother plants grown in Durrës (origin Tropoja) and Paskuqan (origin Kukës) were used at this stage, as these tested positive for PPV. In addition to the relevant proportion of explant stabilization achieved (Figure 4a), some differences in asepsis and regeneration rates were observed between the

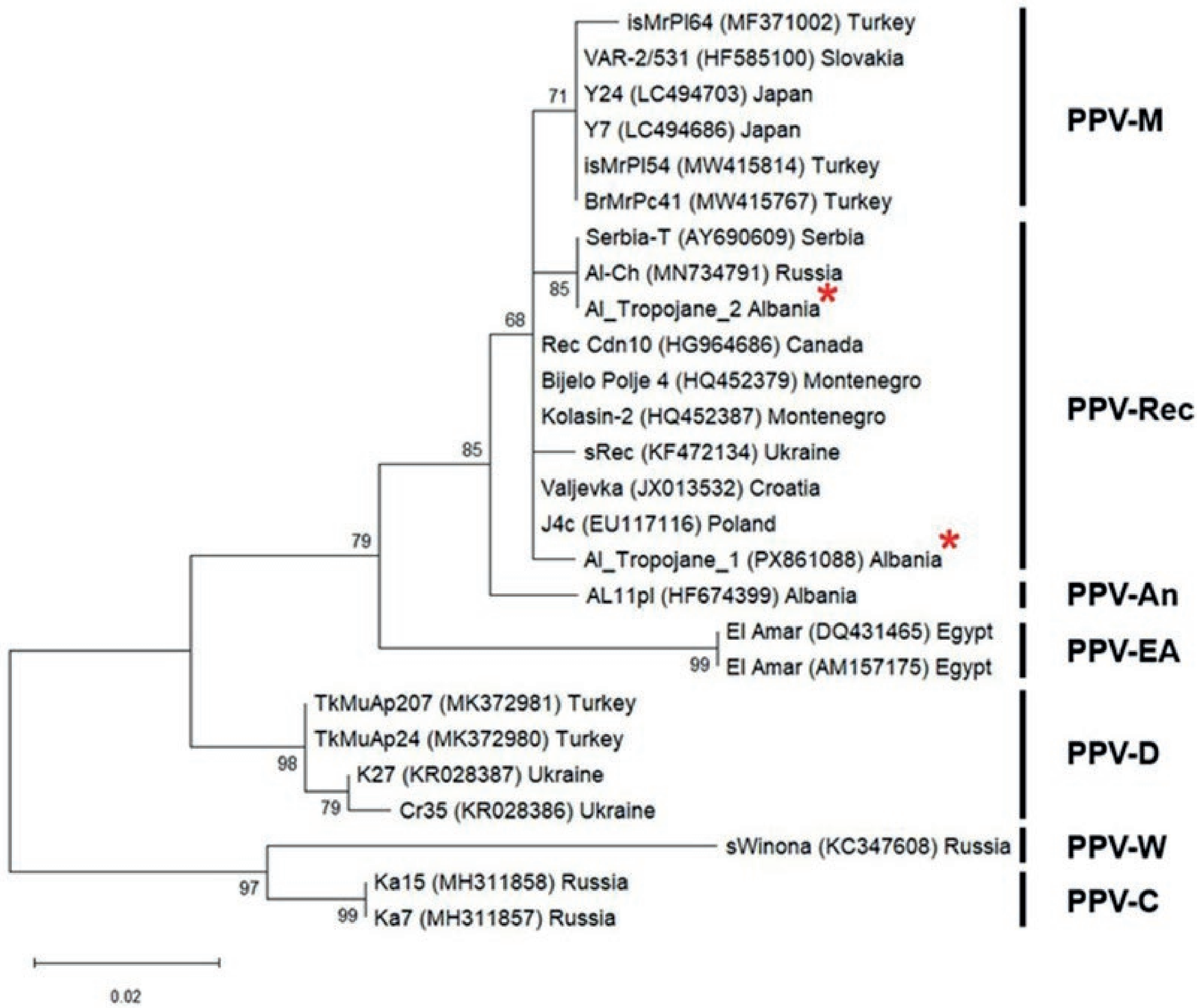

Figure 3. A maximum likelihood phylogenetic tree, constructed using nucleotide sequences of genomic 3′ end (Wetzel *et al*., 1992) PCR amplicons, of PPV isolates from Albania (indicated with red asterisks), together with reference sequences retrieved from GenBank, including their respective accession numbers and geographic origins. Bootstrap values are shown at the branch nodes.

two shoot populations. Contamination was mainly of fungal origin, characterized by fast-growing mycelium emerging from explants within the first week of culture, whereas bacterial contamination was observed less frequently, and typically appeared as turbid exudates around the explant bases. In many cases, even when explants did not show visible contamination, their survival and subsequent regeneration rates were affected by excessive polyphenol production and the release of oxidized compounds into the culture medium, which negatively affected tissue viability. Among the studied plant populations, the explants from Paskuqan (origin Kukës) exhibited the greatest degree of stabilization for both monitored parameters (Figure 5, a and b). Nevertheless, aseptic cultures were obtained from both plant populations, enabling successful regeneration of shoots and progression to the subsequent experimental stages.

Meristem survival rates after three and six weeks of culture

For both plant populations, the meristem tips were excised (Figure 4, b and c). Due to their small size (0.7 to 0.9 mm), the regeneration process was slow, and the first shoots were obtained after 6 weeks. Regeneration rates were evaluated at 3 and 6 weeks. Explants were considered regenerated when they maintained a healthy green colouration, and showed visible increases in size,

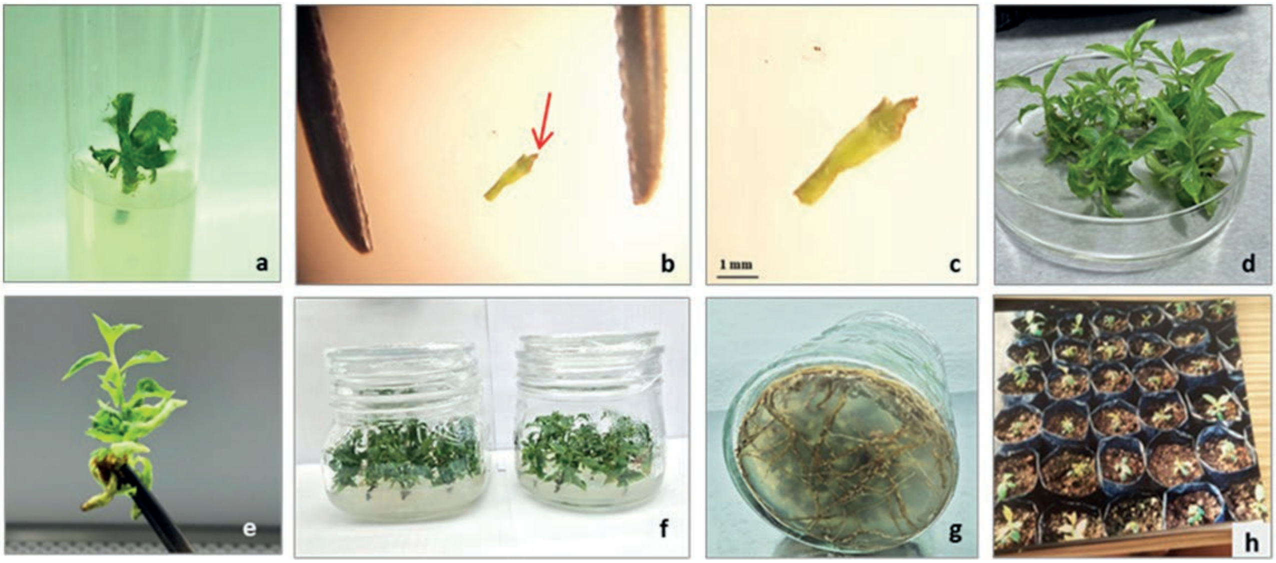

Figure 4. *In vitro* culture of *Prunus domestica* L. a) apical shoots used as primary explants. b) and c) meristematic tip isolation, d), e), and f) multiplication during the subculture stage. g) rooted plantlets. h) acclimated plants.

even if minor. Differences between the two plant populations were observed, following trends similar to those observed in the previous stage. The Paskuqan population (origin Kukës) exhibited greater regeneration rates at both evaluation points, reaching 48.6% after 3 weeks and 53.6% after 6 weeks. In contrast, the Durrës population reached 43.8% after 3 weeks and 49.1% after 6 weeks (Figure 5, c and d).

Regenerated shoots were further propagated through multiple subcultures. The *in vitro* regenerated shoots tested negative for PPV by RT-PCR at the first subculture stage, corresponding to 87% sanitation efficiency for both plant populations. These results indicated that meristem culture was effective for producing PPV-free 'Tropojane' shoots at the early regeneration stage.

Mass production of plants via *subcultures and* ex vitro *transferring*

For both plant populations, leaf numbers increased progressively with BAP concentrations up to 1.5 mg L^{-1} (Figure 6 a), indicating that cytokinins stimulated leaf development in the plum shoots during *in vitro* culture (Figure 4, d, e, and f). Across all concentrations, the Durrës/Tropoja plant material developed greater mean leaf numbers than material from Paskuqan/Kukës. Also, the shoot numbers increased progressively with increasing BAP concentrations (Figure 6 b). At 0 mg L^{-1}, both populations have low shoot production (1.3 to 1.5 shoots). Beginning at 1 mg L^{-1}, shoot proliferation increased. The greatest shoot numbers occurred at 2 mg L^{-1} BAP (Figure 4, d, e, and f). At all BAP concentration, the Durrës/Tropoja population had slightly greater shoot numbers than the Paskuqan/Kukës population. Once a sufficient number of shoots was obtained after several subcultures, they were transferred to the rooting medium. After 4 weeks, *in vitro* roots developed (Figure 4 g), and the plantlets were transferred to *ex vitro* acclimatization (Figure 4 h).

DISCUSSION AND CONCLUSIONS

Serological and molecular analyses detected PPV in 'Tropojane' plum samples with an average prevalences of 35.6% and 45.6%. These results are consistent with previous studies reporting the superior performance of PCR-based techniques over ELISA for PPV detection (Myrta *et al*. 2003; Schneider *et al*., 2004). All infected samples in the present study originated from Tiranë and Durrës, whereas all trees in the native northern region of Tropoja tested negative. One factor that may have progressively reduced the PPV inoculum in Tropoja is the systematic eradication of infected plum trees immediately after symptom appearance (Armand Haxhia, personal communication), a practice widely implemented in the region. The high altitude environment of this region, characterized by cold, prolonged winter conditions, markedly suppresses the aphid vector overwintering success rate, population growth, and early-season activity, thereby limiting virus transmission (BBRO, 2021; MSU Extension, 2020; Labonne *et al*., 1999). Addition-

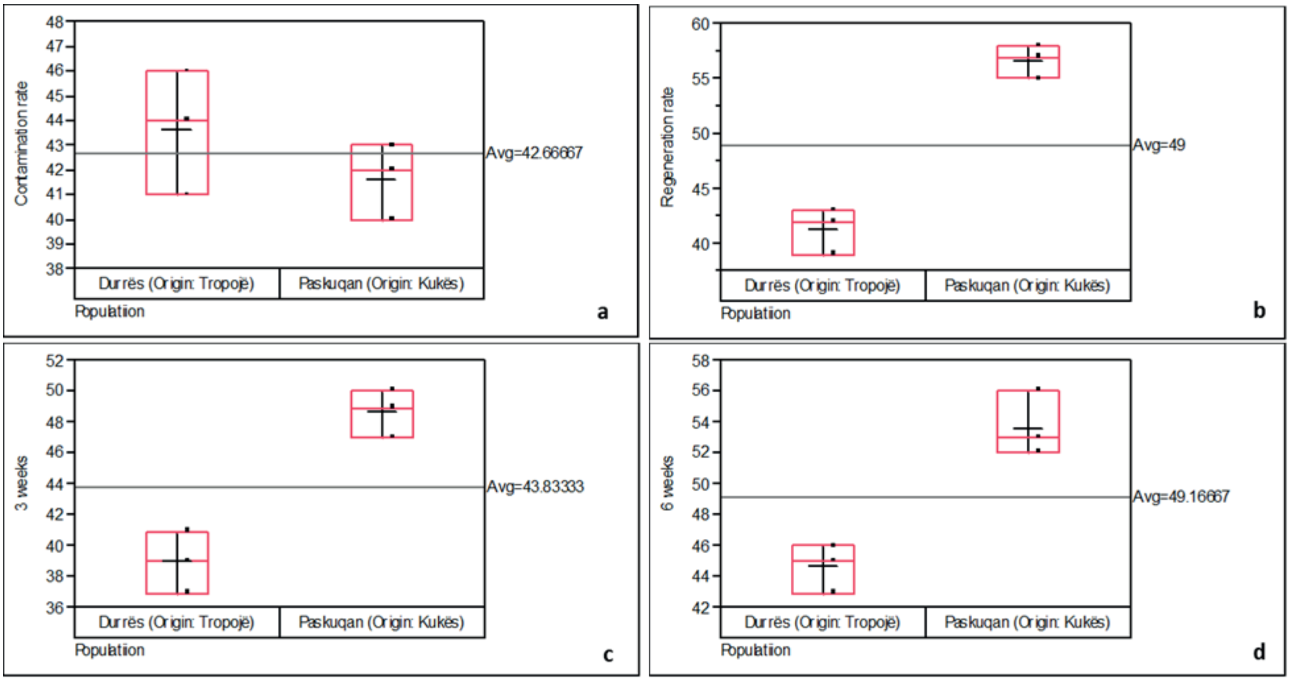

Figure 5. Parameters of *Prunus domestica* explants from two plant populations. a) contamination (%), b) regeneration (%), and survival rates of excised meristems after c) 3 or d) 6 weeks.

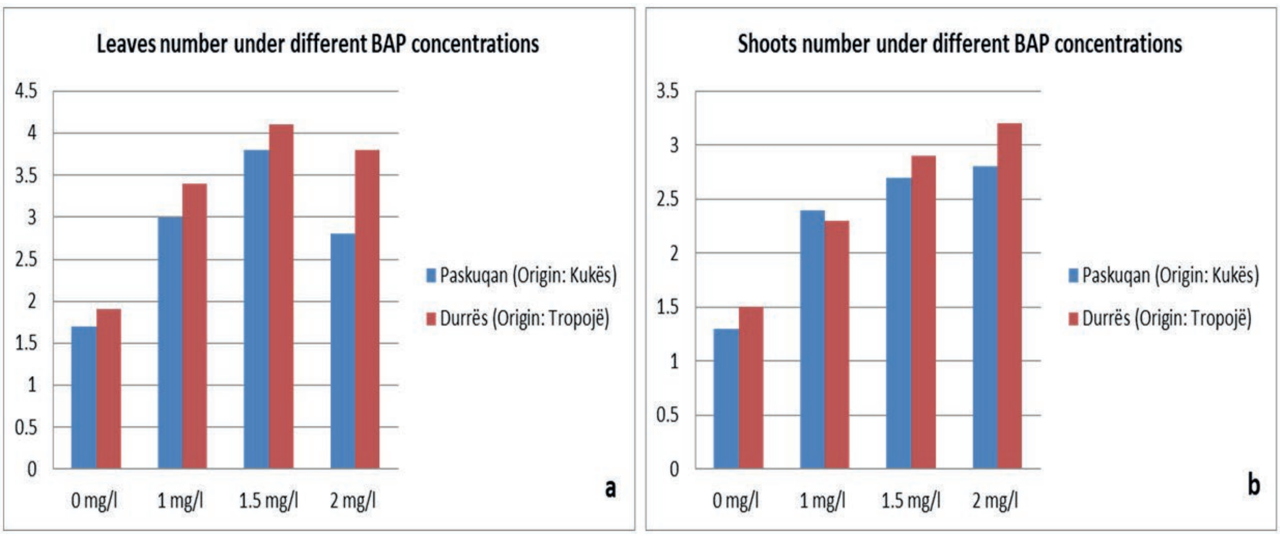

Figure 6. a) Mean leaf, and b) shoot numbers for two *Prunus domestica* types exposed to different BAP concentrations.

ally, plum production in these areas generally follows a traditional, low-input system, where trees are rarely treated with insecticides. Under such conditions, naturally occurring aphid biological control agents, including predators and parasitoids, may help regulate aphid vector populations, contributing to an ecological equilibrium that further restricts PPV dissemination.

As a consequence of a demographic shift from northern Albania toward the Tiranë and Durrës regions for economic reasons, autochthonous plum cultivars, such as 'Tropojane', were also introduced into these new areas, mainly in home gardens or small private orchards, probably favouring the spread of virus in these regions. Beyond uncontrolled movement of uncertified planting material from the northern regions, another factor that promotes PPV spreads is the adaptability of aphid vectors to the mild climatic conditions of Tiranë and Durrës.

Symptom expression varied widely between orchards, and within individual trees, reflecting the combined influence of environmental conditions, plant phenology, virus concentration and strain, duration of infection, and fertilization regime on symptom manifestation (García *et al*., 2025). Inconsistencies between symptom expression and diagnostic results were also noted in the present study, which may indicate presence of divergent PPV strains that were not fully detected by the assays used, due to their limitations and the high genomic variability of PPV (García *et al*., 2014). However, visual detection is not always reliable, since PPV symptoms may resemble those caused by other disorders, and latent infections can occur, further complicating diagnosis. These points highlight the need for laboratory-based diagnostics using sensitive and comprehensive approaches to improve detection accuracy and support robust and reliable certification of planting material for plum orchards.

Results obtained using *in vitro* techniques demonstrated that meristem culture is effective for producing PPV-free plants. Although a large majority of regenerated plantlets tested negative for PPV at the first subculture stage, virus concentration may remain below the detection threshold during early regeneration. Therefore, confirmation of the long-term PPV-free status ideally requires re-testing after prolonged *in vitro* maintenance and/or after acclimatization (*e.g.*, ≥6 months), preferably using RT-qPCR detection, which provides greater sensitivity than RT-PCR (Kanapiya *et al*., 2024). This additional step will be included in future certification-oriented sanitation workflows. Explant size also affects regenerative potential and efficiency of virus eradication: when explants are too small, regenerative potential is lost (Vivek and Modgil, 2018; Zarghami *et al*., 2023). The efficiency of BAP as a cytokinin for the rapid biomass production of *P. domestica* aligned with reports from previous studies (Yuan *et al*., 2009; Wolella, 2017; Baziuk and Kobyletska, 2025), reinforcing its suitability for mass multiplication of the 'Tropojane' plantlets, successful establishment of *in vitro* cultures, consistent shoot proliferation, and production of PPV-free regenerated shoots. This confirms the efficiency of meristem culture techniques for this cultivar, which could serve as a routine protocol for other plum varieties.

While comparison of cytokinin concentrations is not novel, its inclusion in the present research served a practical purpose within a sanitation-to-multiplication pipeline for an autochthonous Albanian plum cultivar. Establishing stable *in vitro* cultures from field-grown 'Tropojane' material was challenging due to variable asepsis, strong phenolic oxidation, and population-dependent regeneration performance. Therefore, the present dataset demonstrates that: (i) PPV-infected local germplasm can be successfully sanitized through meristem culture; and (ii) the resulting virus-free tested lines can be rapidly multiplied using a simple and reproducible protocol suitable for implementation in national certification and conservation programs. The present study provides an operational framework for producing candidate "basic" category material of 'Tropojane' plum, which is currently lacking in Albania.

The present study underscores the need for systematic conservation and certification strategies for native plum germplasm, especially for cultivars threatened by genetic erosion. Heritage landraces such as 'Tropojane' should be preserved, either in *in situ* orchards or within organized germplasm repositories, to maintain their unique genetic makeup and agronomic traits (Priyanka, 2021; Dumont *et al*., 2025). At the same time, propagation of plum material must rely strictly on certified, virus-free nursery stock to avoid the spread of pathogens such as PPV. Implementing a national certification and traceability scheme for planting material would help safeguard local biodiversity, support future breeding programs, and ensure sustainable use of native cultivars, without risking genetic loss or disease introductions.

ACKNOWLEDGEMENTS

This study was carried out within the framework of the projects *"Use of* In Vitro *Biotechnological Methods and Molecular Biology in the Effort to Obtain 'Basic' Category Planting Material of Autochthonous Plum Cultivars"* (2022–2023), supported by NASRI (https://nasri.gov.al/), and *"Selection of Virus-Controlled Plum Plant Material for the Establishment of a Pilot Plant through Sanitation and Micropropagation (VITROCERT)"* (2022–2024), supported by the Academy of Sciences of Albania (ASA: https://akad.gov.al/). The authors also acknowledge financial support from the CryoFruit project, coordinated and financed by ASA.

AUTHOR CONTRIBUTIONS

Magdalena Cara: Sample collection, serological and molecular analyses, manuscript writing. Serafina Serena Amoia: Molecular analyses, manuscript writing. Valbona Sota: *In vitro* cultures establishment, sanitation procedures, manuscript writing. Jordan Merkuri: Sample collection, serological and molecular analyses, manuscript writing. Orges Cara: Molecular and bioinformatic analyses, manuscript writing. Klevis Hoxhallari: Serological and molecular analyses, manuscript writing. Elektra Papakosta: *In vitro* procedures. Efigjeni Kongjika: *In vitro* establishment, sanitation procedures, manuscript writing. Angelantonio Minafra: Molecular analyses, manuscript writing.

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