HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Doctoral School of Biological Sciences

CHARACTERIZATION OF VALUABLE FRUIT TRAITS AND VIROME PROFILE OF SOUR CHERRY (*PRUNUS CERASUS* L.)

The Thesis of the PhD dissertation

Francesco Desiderio

Gödöllő

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Doctoral (PhD) thesis

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1 INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is cultivated globally for fruit production, originating from Caspian Sea and adapting to central European climates. Domestication dates back to 5,000-4,000 B.C.E., but recent breeding efforts have incorporated advanced methods to develop new varieties. *P. cerasus* is significant in the international fruit market, with Hungary being a major producer of sour cherries. Molecular breeding accelerates the development of desirable traits such as size, flavor, and color. These fruits also offer health benefits due to their polyphenol and antioxidant content. Effective breeding requires selecting germplasm and propagation methods to maintain pathogen-tested plants. The Hungarian sour cherry germplasm collection located in Érd offers opportunities for screening, with field testing providing insights into pathogen populations. Stonefruit viruses, prevalent worldwide, pose risks to plant exchange and imports due to regulatory limitations. This research explores using genetic material in breeding, assessing fruit traits, and analyzing viral presence in the germplasm with high-throughput sequencing and RT-PCR validation.

2 OBJECTIVES TO ACHIEVE

1. Comparative analysis of phenotype with markers associated with fruit size, skin and fruit flesh colour among sour cherry accessions.
2. Identify possible candidates for the breeding program
3. Determine the virome of sour cherry gene bank accessions and reference cultivars using small RNA HTS.
4. RT-PCR survey and phylogenetic analysis of the detected viruses.

3 MATERIALS AND METHODS

3.1 Plant material for genetic analysis

Thirty-one sour cherry landraces from the Érd, Elvira major germplasm collection were selected. Fruit phenotyping was conducted from 2021 to 2023, focusing on fruit size, color, and breeding traits. The collection includes 2 to 4 clones per accession, grown on *P. mahaleb* L. rootstock in open fields without irrigation.

3.2 Fruit morphology and phenotyping

Various characteristics of sour cherry fruits were measured over different years to conduct phenotypical analysis. Sour cherry traits selected were such as fruit size (diameter, length, thickness), weight, surface area, skin color, firmness, acidity, sweetness, and stone shape were analyzed.

Measurements followed standard guidelines: UPOV for sour cherry. Around 500g of fresh fruits were collected from 2 to 4 trees in the germplasm collection, with 20 healthy fruits selected for analysis. Data were collected from 2021 to 2023, using digital calipers, scales, and formulas to calculate fruit surface area. Fruit firmness was tested with a durometer, soluble solid content (SSC) with a refractometer, and color with the CIELab system and spectrophotometer. In total, 10 fruits were measured per accession with 50 repeated measurements. Samples were stored at -20°C for further chemical analysis.

Sour cherry total polyphenolic content (TPC) was analyzed using the Folin-Ciocalteu colorimetric method with slight modifications. A 5g sample was mixed with 96% acidified ethanol, and phenolics were extracted using an ultrasonic water bath for 30 minutes. After centrifuging for 20 minutes, the liquid was filtered. The extract, Folin-Ciocalteu reagent, and distilled water were combined, incubated for 5 minutes, and then mixed with sodium carbonate. After 2 hours in a dark room, absorbances were measured at 750 nm using a UV-VIS spectrophotometer. Tests were done in triplicate, with results expressed as mg gallic acid equivalent (GAE) per gram of dried sample.

3.3 DNA extraction and SSR markers selection

DNA from selected sour cherry germplasm material was extracted from fresh young leaves using the Plant Genomic DNA extraction miniprep system. PCR was performed with DreamTaq DNA polymerase in a 25 µL reaction volume. The optimized reaction included 2.5 mM MgCl2, 0.2 mM dNTPs, 0.25 µM primers, and 1 ng genomic DNA. PCR conditions were: denaturation at 95°C for 3 minutes, 35 cycles of 95°C for 30s, annealing at the primer's optimal temperature for 30s, and 72°C for 15s, followed by a final extension at 72°C for 3 minutes. Forward primers were labelled with 6-FAM dye, and the PCR products were analyzed by capillary electrophoresis using an ABI Prism 3100 Genetic Analyzer. Allele size was determined using Peak Scanner software, followed by haplotype and genotype determination.

3.4 Statistical analysis

Data from three consecutive years (2021–2023) on sour cherry fruit characteristics were analyzed. Landraces were grouped by genotype, and ANOVA was used to assess whether genotype differences affected fruit weight or color. Physical traits (weight, firmness), secondary traits (acidity, sweetness, juiciness), UPOV breeding scale values, chemical components, and CIELab values were analyzed using Spearman's correlation ($p < 0.05$). Principal component analysis (PCA) was performed to understand trait variation.

Statistical analysis was conducted using SPSS (ANOVA with Tukey's b test, $p < 0.05$). SSR fragments were classified as present (1) or absent (0), and linear regression was performed to assess allele-phenotype influence ($p < 0.05$). Pearson's correlation was also used to analyze genotype-phenotype relationships. Data visualization, including boxplots and allele frequency analysis, was performed in R Studio. Additional metrics like heterozygosity (Ho), polymorphism content (PIC), marker index (MI), and discriminating power (DP) were calculated.

3.5 Sour cherry virus analysis

Hungarian sour cherry varieties (*Prunus cerasus* L.) were examined for harmful pathogens. Leaf samples were collected from 31 varieties in 2021 at the Fruit Growing Research stations in Érd, Hungary. In 2021, one tree per variety from new and standard cultivars was sampled and analyzed by HTS. In 2023, the test was repeated using RT-PCR, with 58 samples collected from all surviving trees to screen for viral presence and distribution.

3.5.1 Sample preparation for HTS and RT-PCR

HTS samples were categorized into three groups: 22 new cultivars and 9 standard Hungarian cultivars collected in 2021, and samples collected again in 2023 for RT-PCR further testing. Four leaves per tree were collected twice to ensure comprehensive virus detection and stored at -80°C to prevent RNA degradation.

3.5.2 RNA extraction

RNA was extracted from four leaves per tree using a modified CTAB method. The leaves (150-200 mg each) were frozen in liquid nitrogen, homogenized, and mixed with 900 µL of extraction buffer (EB) with 17 µL of β-mercaptoethanol. After incubation at 65°C and vortexing, chloroform alcohol was added, and the mixture was centrifuged. The supernatant was processed with LiCl, incubated on ice, and centrifuged again. The pellet was resuspended in SSTE buffer, re-centrifuged, and mixed with NaAc and isopropanol before a final centrifugation. The pellet was washed with ethanol, dried, resuspended in sterile water, and quantified using Nanodrop and gel electrophoresis.

3.5.3 small RNA library preparation and sequencing

RNA was extracted from 20 µL samples (500 ng from each RNA) and subsequently pooled into three different libraries for small RNA library preparation using the TruSeq Small RNA Library Preparation Kit containing different cultivars. RNA was purified to small RNA (sRNA) fraction following the protocol from Jaksa-Czotter (2024). The sRNA is purified from extracted RNA, eluted with NaCl and precipitated. The RNA 3’ adapter was ligated, followed by the RNA 5’ adapter, and reverse transcription was performed with RT Primer and Revert Aid H-reverse transcriptase. sRNA library was purified and precipitated. After a quality check, cDNA was synthetized and purified, and sent for sequencing before bioinformatic analysis.

3.5.4 Bioinformatic analysis: pipeline for data evaluation and HTS bioinformatic results

Bioinformatic analysis was performed using CLC Genomic Workbench. Sequenced reads were trimmed and assessed for quality. High-quality reads were assembled de novo into contigs with default settings (word size 20, bubble size 50, min contig length 35 nt). The Fastq files are available on the NCBI GEO database (GSE233558). To identify known viruses, contigs were compared against reference genomes from NCBI GenBank and *Prunus*-infecting viruses without reference genomes. Annotation was done using the BLASTN algorithm. If virus-specific contigs were found, reads were mapped to viral reference genomes to count normalized reads (read/1 million reads: RPM) and calculate genome coverage. Viral presence was confirmed if at least two of the following criteria were met: (1) presence of viral-specific contigs, (2) >200 normalized viral reads, or (3) >60% viral genome coverage.

3.5.5 Confirmation of the obtained results by RT-PCR and Sanger sequencing

To validate bioinformatic results, RT-PCR was conducted using virus-specific primers. cDNA was synthesized from RNA of each library and individual tree with random primers using the RevertAid and Maxima cDNA kits. For amplification and Sanger sequencing of viral genomes, cDNA from Maxima was amplified with Q5 Polymerase and primers previously designed for PrVF RNA1 and RNA2, CVA and PNRSV RNA3 or designed based on the sRNA reads were used. Amplified products were purified, cloned into pJET vectors, and sequenced. Sequences were deposited into NCBI GenBank (accession numbers OR596712-OR596738).

3.5.6 Phylogenetic analysis

Phylogenetic tree analysis was conducted using Geneious Prime to examine the relationships and variance among PNRSV RNA3, CVA, PrVF RNA1, and RNA2. Sequences were aligned with Geneious's global alignment function (free end gaps, 65% similarity cost matrix, gap open penalty 12, gap extension penalty 3, 2 refinement iterations). Reference sequences were used for PNRSV RNA3 (NC004364), CVA (NC_003689), and PrVF RNA1 (NC_039077.1), RNA2 (NC_039078.1) as well as previously identified variants available in NCBI.

4 RESULTS

4.1.1 Sour cherry size

Sour cherry trees were analyzed over three years (2021-2023). The smallest fruit diameter was for ‘Helyi sötét’ (14.4 mm), and the largest for ‘Mogyoródi kései’ (22.4 mm). The shortest fruit was ‘Helyi sötét’ (12.4 mm), while the longest was Tiszabög 50/7. ‘Helyi sötét’ also had the smallest thickness (12 mm) and ellipsoid area (10.5 cm^3), with ‘Mogyoródi kései’ having the largest (25.15 cm^3). Fruit weight ranged from the lightest in ‘Helyi sötét’ (2.31 g) to the heaviest in ‘Mogyoródi kései’ (6.93 g). Seed weight was lightest in ‘Helyi sötét’ (0.2 g) and heaviest in Pipacs 1 (0.5 g), while pulp weight ranged from 1 g in ‘Helyi sötét’ to 3.2 g in ‘Mogyoródi kései’.

4.1.2 Sour cherry firmness and SSC

Fruit firmness varied from very soft in ‘Bosnyak’ (3) to very hard in ‘Pipacs 1’ (7). Firmness, measured with a durometer, was lowest in ‘Favorit’ (19.8) and highest in ‘Pipacs 1’ (62.8). Soluble solid content (SSC) ranged from 16.3 in ‘Favorit’ to 24.9 in ‘Bagi meggy’. Acidity varied from high in ‘Pipacs 1’ (8) to low in ‘Favorit’ (3). Sweetness ranged from very sweet in ‘Érdi Jubileum’ (7) to non-sweet in ‘Pipacs 1’. Juiciness ranged from very juicy in ‘Szamosi meggy’ (7) to non-juicy in ‘Bagi meggy’ (3). Stone shape ranged from 1.3 in ‘Bosnyak’ to 3 in ‘Cigány késői’. Fruit shape in ventral view ranged from 3.5 in ‘Bosnyak’ to 1 in several accessions, while fruit pistil end varied from 1.6 in ‘Érdi Jubileum’ to 2.7 in ‘Bagi meggy’.

4.1.3 Fruit colour and TPC

Fruit color was measured on several scales. Skin color ranged from lightest in ‘Pipacs 1’ (1) to darkest in ‘Érdi Jubileum’ (6). On the CIELab scale, L* score was highest in ‘Hortenzia királynője’ (33.4) and lowest in ‘Bosnyak’ (24.7). For a*, it was highest in ‘Hortenzia királynője’ (31.9) and lowest in ‘Bosnyak’ (6.6). b* was highest in ‘Hortenzia királynője’ (15.8) and lowest in ‘Bosnyak’ (1.7). Chroma ranged from lowest in ‘Bosnyak’ (6.9) to highest in ‘Hortenzia királynője’ (35.7), while hue ranged from lowest in ‘Bosnyak’ (2.2) to highest in ‘Hortenzia királynője’ (19.3). Ctifl scale varied from very dark in ‘Bosnyak’ (6.5) to very pale in ‘Hortenzia királynője’ (2.5). Flesh color was very dark for ‘Bosnyak’ (4) and very pale for ‘Hortenzia királynője’ (1). Juice color ranged from lightest in ‘Hortenzia királynője’ (1.3) to very dark in ‘Bosnyak’ (5). In

2022, total polyphenolic content (TPC) was highest in ‘Pipacs 1’ (650.57 mgGAE/100g) and lowest in ‘Kantorjanosi 3’ (122.76 mgGAE/100g). ‘Pipacs 1’ also had the highest a* (26.45) and Chroma (28.18). ‘Hortenzia Királynője’ had the highest L*, b*, and hue values, while ‘Bosnyak’ had the lowest values for L*, a*, b*, Chroma, and hue.

4.1.4 Bivariate analysis and principal component analysis (PCA) for fruit characteristics

Bivariate analysis showed that colourimetric data (L*, a*, b*, Chroma, and hue) are positively correlated. TPC positively correlates with SSC, firmness, and acidity but negatively with sweetness and weight-related traits, especially fruit weight. SSC negatively correlates with acidity, juiciness, and weight but positively with colourimetric measures. TPC correlates positively with L*, a*, b*, and Chroma but not with hue. PCA showed that the first component positively correlated L*, a*, b*, Chroma, hue, and juiciness. The second component linked TPC positively with L*, a*, b*, Chroma, and SSC.

4.2 Results of genotyping in sour cherry

Thirty-one sour cherry accessions were analyzed with two SSR markers for fruit size. The BPPCT034 marker showed sizes between 204 and 251 bp, while CPSCT038 ranged from 185 to 204 bp. A total of 21 allelic combinations were identified, with 5 shared by different accessions. Groupings included ‘Bagi meggy’, ‘Korai Cigány’, ‘Cigány kesői’ (208-208-226-230-190-190-190-190), ‘Dunabogdányi’, ‘Májusi hólyag’, ‘Édes pipacs’ (204-204-226-237-185-185-204-204), ‘Késői parasztmeggy’, ‘Velencei kései’ (226-226-237-237-185-185-204-204), ‘Szamosi meggy’, ‘Tiszabög 50/7’ (204-204-226-237-185-185-185-185). Ma039a ranged from 157 bp to 205 bp, showing 6 allelic combinations. Four shared combinations included: ‘Bagi meggy’, ‘Cigány késői’, ‘Cigánymeggy 7’, ‘Késői Cigány’, ‘Korai Cigány’, ‘Pándy Bb. 119’ (157-157-205-205), ‘Érdi Jubileum’, ‘Fűzlevelű kosszemű’, ‘Helyi sötét’, ‘Májusi hólyag’ (157-157-175-175), ‘Bosnyak’, ‘Dunabogdány’, ‘Édes pipacs’, ‘Fehérvári’, ‘Hortenzia királynője’, ‘Kantorjánosi 3’, ‘Későn virágzó’, ‘Korai Pándy’, ‘Mogyoródi kései’, ‘Nagy Gobet’, ‘Pándy 279’, ‘Pándy 43’, ‘Péceli nagy’, ‘Pipacs 1’, ‘Tiszabög 50/7’, ‘Újfehértói fürtös’, ‘Velencei kései’ (157-157-175-205), ‘Favorit’ and ‘Szamosi meggy’ (175-175-205-205) Pav-Rf-SSR ranged from 343 bp to 353 bp, with 10 allelic combinations. Five shared combinations

included: ‘Bagi meggy’, ‘Cigánymeggy 7’, ‘Késői Cigány’ (343-343-351-353), ‘Cigány késői’, ‘Dunabogdány’, ‘Édes pipacs’, ‘Favorit’, ‘Fehérvári’, ‘Fűzlevelű kisszemű’, ‘Helyi sötét’, ‘Mogyoródi kései’, ‘Velencei kései’ (351-351-353-353) Érdi bőtermő’, ‘Érdi Jubileum’, ‘Kantorjánosi 3’, ‘Késői paraszmeggy’, ‘Májusi hólyag’, ‘Pipacs 1’, ‘Szamosi meggy’ (349-349-351-353), ‘Bosnyak’, ‘Hortenzia királynője’, ‘Nagy Gobet’, ‘Pándy Bb. 119’, ‘Pándy 279’ (347-349-351-353), ‘Pándy 43’, ‘Péceli nagy’ (347-347-351-353), LG3_13.146 Observed only at 218 bp in ‘Édes pipacs’, ‘Érdi bőtermő’, ‘Érdi Jubileum’, ‘Késői parasztmeggy’, ‘Pándy Bb. 119’, ‘Pándy 279’, ‘Péceli nagy’, and ‘Újfehértói fürtös’.

4.3 Correlation between phenotype and genotype in sour cherry

Pearson correlation analysis of markers and phenotype revealed the following: Sour cherry fruit size (diameter, length, thickness, SA) positively correlated with $BPPCT034_{204}$, but negatively with $BPPCT034_{208}$. $BPPCT034_{222}$ showed a negative correlation with diameter, while $BPPCT034_{230}$ and $BPPCT034_{237}$ negatively correlated with diameter, length, and SA. $CPSCT038_{185}$ positively correlated with diameter, length, thickness, and SA, whereas $CPSCT038_{190}$ negatively correlated with these traits. $CPSCT038_{204}$ positively correlated with diameter, length, thickness, SA, fruit weight, seed weight, and pulp weight, while negatively correlating with $CPSCT038_{190}$. Linear regression showed that single alleles significantly influenced fruit diameter, length, thickness, SA, and weights (fruit, seed, and pulp).

Sour cherry color analysis revealed that $Ma039a_{157}$ negatively correlated with L*, $Ma039a_{175}$ positively correlated with L* but negatively with Ctifl, flesh color, and juice color. $Ma039a_{205}$ negatively correlated with Ctifl and flesh color. Pav-Rf-SSR_{343} positively correlated with juice color. Linear regression for single alleles showed significant effects on L*, a*, b*, Chroma, hue, Ctifl, flesh color, juice color, and SSC.

4.4 Frequency analysis and marker analysis

In sour cherry fruit frequency analysis, markers BPPCT034 and CPSCT038 were the most polymorphic with 22 alleles each. Ma039a and Pav_Rf_SSR had 3 and 5 unique alleles, respectively, with high heterozygosity (0.94 to 1.00).

4.5 HTS base virological survey of sour cherry accessions

For small RNA HTS three libraries were prepared. 1_PC_E1, 2_PC_E2, contained RNA from 11-11 gene bank accessions, while 3_PC_E3 represented RNA of nine reference cultivars. Illumina sequencing resulted in 17-20 million reads. After trimming the reads, 1-1,6 million non-redundant reads were left. Contig numbers varied from 1,597 (2_PC_E2) to 2,631 reads(3_PC_E3).

The small RNA HTS identified three viruses: CVA, PNRSV, and PrVF. In library 1_PC_E1 CVA, PNRSV RNA1 and RNA2, RNA3 contigs were found. Library 2_PC_E2 had 1 contigs for CVA, PNRSV RNA2, PNRSV RNA3. Library 3_PC_E3 had contigs for CVA, PNRSV RNA2, PNRSV RNA3. Coverage of PrVF RNA1 was above threshold limit in 1_PC_E1 library.

4.6 RT-PCR validation of HTS analysis

RT-PCR validation showed that all three libraries were infected with PNRSV and PrVF, while one library was infected with CVA. After confirming the presence of PNRSV, CVA, and PrVF, individual samples were analyzed. In library 1_PC_E1, 6/11 samples had CVA, 7/11 had PNRSV, and 7/11 had PrVF. Library 2_PC_E2 had 3/11 with CVA, 7/11 with PNRSV, and 4/11 with PrVF. Library 3_PC_E3 showed 1/9 with CVA, 1/9 with PNRSV, and 5/9 with PrVF. Single infections were most common (14 samples), with fewer mixed infections: CVA and PrVF (2 samples), PNRSV and PrVF (4 samples), and CVA and PNRSV (2 samples). Triple infections occurred in 4 cases in one library.

4.7 RT-PCR results for 2023 sour cherry collection

In 2023, 58 trees from the same accessions collected in 2021 were tested to understand the distribution of CVA, PNRSV, and PrVF. Results showed 31% positive for CVA, 45% for PNRSV, and 38% for PrVF. Some previously collected trees were found dead and excluded from the analysis.

4.8 Phylogenetic analysis

To assess CVA population size and variants presence, a phylogenetic analysis of the movement protein was conducted. Hungarian CVA variants clustered mainly in Group I with those from India, Czech Republic, and Canada, while Group III included Hungarian variants from sweet cherry alongside those from China and Canada. For PNRSV, Hungarian RNA3 variants all clustered within group PV96. PrVF analysis of the 5’ and 3’ UTRs showed similarity in the protein coding region but higher diversification in the 5’and 3’ UTR. RNA1 phylogenetic analysis revealed close clustering of Hungarian variants, with those from sweet cherry related to Canadian variants. RNA2 phylogenetic analysis showed Hungarian variants clustering with Czech samples, both from sweet and sour cherry, and the polyprotein analysis further confirmed the close relation with Czech variants.

5 DISCUSSIONS

5.1 Discussion on sour cherry genotyping and phenotyping analysis

Total polyphenolic content (TPC) varied across cultivars, with ‘Pipacs1’ having the highest TPC and ‘Kantorjanosi 3’ the lowest. Darkest fruit color was observed in ‘Bosnyak’ and lightest in ‘Hortenzia Királynője’. Firmness and color data were consistent with prior studies, and PCA showed expected correlations between color metrics and scaling systems. Fruit weight was highest in Mogyoródi kései, and this variety could be investigated deeper for fruit size selection and integration in the breeding program.

Fruit size was analyzed using SSR markers CPSCT038 and BPPCT034. CPSCT038 revealed a range of alleles with larger fruits associated with alleles 185 and 204 bp, and smaller fruits with 190 bp. BPPCT034 showed a broader range of alleles, with $BPPCT034_{204}$, $BPPCT034_{208}$, and $BPPCT034_{230}$ correlating with smaller fruits, while $BPPCT034_{237}$ correlated with larger fruits. These findings align with previous studies on sweet cherry. Both markers significantly influenced fruit characteristics such as diameter, weight, and color.

For fruit color, $Pav\text{-}Rf\text{-}SSR_{343}$ positively correlated with darker fruits, while Ma039a markers showed mixed correlations with color traits. $LG3\_13.146_{218}$ was associated with darker fruit colors. Overall, the findings suggest that these SSR markers and TPC analyses can help in selecting and breeding sour cherry cultivars for desired traits.

5.2 Discussion on sour cherry virus analysis

5.2.1 High throughput sequencing and bioinformatic analysis

Three sour cherry libraries were analysed, in which we could observe a size distribution of 21 to 24 nt long sRNA. This is the result of DICER enzyme activity in the host, which enzyme can slice substrate dsRNA into specific-sized products. The peak of size distribution was observed at 22 nt. Many contigs matched cherry virus A, prunus necrotic ringspot virus, and prunus virus F. Results of the bioinformatic analysis were validated with RT-PCR.

5.2.2 RT-PCR validation strategy

To validate the presence of PNRSV, CVA, and PrVF, primers were designed from the reference genomes of CVA (NC_003689), PNRSV RNA3 (NC_004364), PrVF RNA1 (NC_039077), and PrVF RNA2 (NC_039078). Diagnostic primers included CVAmp-Fm/Rm for CVA, PNcip F/R for PNRSV, and two sets for PrVF due to its high variability: PVF1-CAF/CAR and Fab-R2_1808_F/_2546_R. Consensus sequences were used for PrVF to address its variance. Fifteen primers were tested for PrVF, including the previously effective PrVF2_5_F/_R. This strategy enabled analysis of both pooled and individual samples. The sRNA HTS indicated the presence of PNRSV, but not of CVA and PrVF in all the cases. RT-PCR validation confirmed the spread of the viruses. This inaccuracy could be due to the latency of CVA and PrVF viruses.

5.2.3 RT-PCR validation of three libraries and individuals

All libraries were tested for CVA, PNRSV, and PrVF. PNRSV was present in all three libraries, as expected due to its pollen transmission. CVA was absent in 3_PC_E3, with grafting suggested as a possible spread mechanism. Surprisingly, PrVF was also widespread, marking its first detection in Hungary. CVA and PNRSV were found in over 30% and 40% of samples, respectively, indicating potential issues with propagation material. PrVF was found in 81% of samples, showing it is common in sour cherry. Mixed infections with all three viruses or CVA and PrVF occurred in four cases, but single infections were more common, suggesting that while mixed infections are less frequent, they can occur.

5.2.4 Comparison between 2021 and 2023 sour cherry collection

In 2023, cherry samples from libraries 1_PC_E1, 2_PC_E2, and 3_PC_E3 were reanalysed, including all plants of the same cultivar. CVA infection rates remained consistent with 2021, while PNRSV affected nearly half of the plants. PrVF was less common in 2023, potentially due to its high variability, but it may be more widespread than detected. Additionally, 19 trees were found dead, but the impact of the viruses on tree mortality is unclear, as the trees were exposed to biotic and abiotic stresses in an unirrigated field.

5.2.5 Phylogenetic analysis of CVA, PNRSV and PrVF

Phylogenetic analysis showed that CVA had high identity in the MP coding region, placing all sour cherries in Group I. PNRSV RNA3 variants were grouped in PV96, closely related to Slovakian and Spanish variants, suggesting a local infection source. PNRSV, being pollen-transmitted, likely spread from infected trees in the same field. For PrVF, the 5' and 3' UTR regions showed high variability, with Hungarian and Czech variants clustering closely. PrVF RNA2 displayed even greater divergence for 5’ and 3’ UTR regions. The high variability of PrVF complicates its identification, requiring multiple primer sets for better detection. Its latent nature suggests minimal immediate host response but potential for high mutation rates. Strict containment and further testing are recommended.

6 CONCLUSIONS AND RECOMMENDATIONS

Sour cherry is valuable in Hungary's horticultural market, so the analysis focused on fruit size and color, key factors for breeders, farmers, and consumers. Compared to previous studies, correlations were lower, likely due to differences between diploid and tetraploid samples. MRA analysis showed lower beta coefficients for color, suggesting low correlation between phenotype and genotype. TPC's role in fruit development is notable, and future studies will include antioxidant activity and volatile compounds. The study found that TPC and color are positively correlated, as are TPC with acidity and firmness. Further research will explore the impacts of soil, climate, and genetic factors on chemical composition. This study aids Hungarian breeders by providing tools for early screening and developing new varieties with improved traits and stress resistance.

No cherry breeding program currently targets virus-resistant varieties, and only few natural resistances to stone fruit viruses have been identified, such as PPV resistance. With advances in diagnostic tools like high throughput sequencing, PrVF was detected for the first time in Hungary. The presence of multiple strains on a single tree may affect symptom development, with variable intensity and complexity. Sour cherry plantations showed high PrVF incidence, and even mother plants that tested negative were found positive later. Routine screening and methods like meristematic propagation with thermotherapy and chemotherapy are recommended to ensure virus-free material. *Fabaviruses* can be transmitted by aphids and infected grafting material, but we still don’t know if PrVF can be transmitted by insects. To maintain virus-free areas, insect-proof nets and sterilized tools are necessary. While PNRSV is often associated with symptoms and is transmitted by pollen, CVA is generally latent and may be harder to manage. Early screening and elimination of infected material can help control CVA spread. High throughput sequencing might be more useful in the future to screen massive population as its costs decrease, but RT-PCR remains essential for current virus identification and distribution.

7 NEW SCIENTIFIC RESULTS

1. $BPPCT034_{204}$, $CPSCT038_{204}$, and $CPSCT038_{185}$ showed positive correlation with fruit size among sour cherry genetic resources. $BPPCT034_{230}$ can be used as a negative selection allele for breeding purposes.

2. Colorimetric and chemical analysis indicated ‘Bosnyák’ with its dark colour and ‘Pipacs1’ with its high TPC content as good candidates for future breeding selection.

3. The virus infection status of sour cherry trees in germplasm material and reference cultivars was determined using small RNA HTS and validated by RT-PCR.

4. Three viruses CVA, PNRSV and PrVF have been detected.

5. The presence of PrVF has been described for the first time in Hungary.

6. Frequent and increasing presence of PNRSV in the germplasm was found, raising concerns about infected plants in open fields.

8 PUBLICATION LIST

List of Publications

•**Desiderio Francesco**; Szilágyi Sámuel; Békefi Zsuzsanna; Boronkay Gábor; Usenik Valentina; Milic Biserka; Mihali Cristina; Giurgiulescu Liviu "Polyphenolic and Fruit Colorimetric Analysis of Hungarian Sour Cherry Genebank Accessions" Agriculture (2077-0472): 13 (7) p. 1287 (2023) Language: English

•Szilágyi Sámuel; Horváth-Kupi Tünde; **Desiderio Francesco**; Kovácsné Békefi Zsuzsanna „Evaluation of sweet cherry (*Prunus avium* L.) cultivars for fruit size by FW_G2a QTL analysis and phenotypic characterization" SCIENTIA HORTICULTURAE (0304-4238 1879-1018): 292 Paper 110656. 5 p. (2022). Language: English

•**Desiderio Francesco**, Nagyné Galbács Zsuzsanna, Demian Emese, Fákó Vivien, Czako David, Varga Tünde, Barath Daniel, Jaska-Czotter Nikoletta, Koloniuk Igor, Várallyay Éva "Cherry and sour cherry trees growing at new cultivar testing orchard and certified stock collection in Hungary are highly infected with CVA and PrVF", SCIENTIA HORTICULTURAE, Under Review

List of Presentations and Posters

•**Desiderio Francesco**; Szilágyi Sámuel; Boronkay Gábor; Lákatos Tamás; Békefi Zsuzsanna "Hidden treasures: Phenotyping and genotyping of Hungarian and Carpathian cherry landraces", 2024 May 12-16, European Horticultural Congress (EHC 2024), S08 p. 51

•Szilágyi Sámuel; **Desiderio Francesco**; Békefi Zsuzsanna 'Cseresznye gyümölcsméretének vizsgálata génbanki tételeken hagyományos és molekuláris genetikai módszerrel" 2023. évi Lippay János – Ormos Imre – Vas Károly (LOV) Tudományos Üllésszak összefoglalói Abstracts of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting, 2023 Conference: Bp, Hungary 2023.11.16. (MATE Buda Campus) p. 37. (2024)

•**Desiderio Francesco**; Sámuel Szilágyi; Gábor Boronkay; Zsuzsanna Békefi "Germplasm Hunt: Sour cherry colour analysis with SSR markers" 2023. évi Lippay János – Ormos Imre – Vas Károly (LOV) Tudományos Üllésszak összefoglalói Abstracts of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting, 2023 Conference: Bp, Hungary 2023.11.16. (MATE Buda Campus) p. 37. (2024)

•**Desiderio Francesco**; Szilágyi Sámuel; Kovácsné Békefi Zsuzsanna; Usenik Valentina; Milic Biserka; Giurgiulescu Liviu "Re-evaluation of traditional Hungarian stone fruits for integration in breeding programs" Conference: Danube Rectors' Conference (DRC) Timisoara, Romania, 2023.10.19-20 Language: English

•**Desiderio Francesco**; Szilágyi Sámuel; Boronkay Gábor; Kovácsné Békei Zsuzsanna "Germplasm Hunt: Characterization of sour cherry collection for fruit size and colour" Conference: XVI Eucarpia Symposium on Fruit Breeding and Geentics, Dresden, Germany, 2023.09.11-16 Language: English

•**Desiderio Francesco**; Nagyné Galbács Zsuszanna; Várallyay Éva "Investigating the presence and distribution of PrVF in Hungarian sour and sweet cherry" Conference: "25th International Conference on Virus and other graft transmissible diseases of Fruit crops", Wageningen, The Netherlands, 2023.07. 9-13 Language: English

•**Desiderio Francesco**; Nagyné Galbács Zsuszanna; Várallyay Éva "The detection of Prunus virus F and its distribution in Hungarian sour cherry orchards" Conference: I. Magyar Agrártudományi Doktoranduszok Szimpózium, Debrecen, Hungary, 2023.02.24-25 Language: English

•**Desiderio Francesco**; Szilágyi Sámuel; Boronkay Gábor; Kovácsné Békefi Zsuzsanna "Germplasm hunt: Hungarian sour cherry analysis of fruit size and colour through phenotyping and SSR markers" Conference: XXIX Növenynemesítési Tudományos Napok, Martonvásár, Hungary, 2023.04.26 Language: English

•**Desiderio Francesco**; Nagyné Galbács Zsuzsanna; Várallyay Éva "New player in the field: Prunus virus F is present and spread in Hungarian cherry orchards" Conference: 32nd Plant Protection Forum Keszthely, Keszthely, Hungary, 2023.01.19-20 Language: English

•**Desiderio Francesco**, Szilagyi Samuel, Békefi Zsuzsanna, Usenik Valentina, Milić Biserka, Giurgiulescu Luviu "Re-evaluation of traditional Hungarian stone fruits for integration in breeding programs" Conference: Danube Rectorate Conference (DRC) 2022, Maribor, Slovenia, 2022.11.09-11 Language: English

•**Desiderio Francesco**, Galbacs Nagyné Zsuzsanna, Varallyay Éva "Prunus virus F is widespread in Hungarian sour cherry orchards" Conference: INTERNATIONAL ADVANCES IN PLANT VIROLOGY 2022, Ljubljana, Slovenia, 2022.10.05-07 Language: English

•**Desiderio Francesco**, Galbacs Nagyné Zsuzsanna, Varallyay Éva "New Putative Viruses in Hungarian Sour Cherry Genebank Collection" Conference:

5th National Conference of Young Biotechnologists (FIBOK 2022) Gödöllő, Hungary 2022.04.11-12 (2022). Language: English

•**Desiderio Francesco**; Agyemang Duah Evans; Takács András Péter; Várallyay Éva “Prunus virus F is present in Hungarian sour cherries” Conference: HUNLIFE 2021, Eger, Hungary 2021.11.05-07 (2021). Language: English

Additional Publications

•Szabó Luca Krisztina, **Desiderio Francesco**, Kirilla Zoltán,Hegedűs Attila, Várallyay Éva, Preininger Éva "Elimination of cherry virus A from Prunus domestica 'Besztercei Bt. 2' using in vitro techniques" PLANT CELL TISSUE AND ORGAN CULTURE (0167-6857 1573-5044): 157 Paper 45. (2024) https://doi.org/10.1007/s11240-024-02770-0

•Szabó Luca Krisztina; **Desiderio Francesco**; Kirilla Zoltán; Hegedűs Attila; Várallyay Éva; Preininger Éva "A mini-review on in vitro methods for virus elimination from Prunus sp. fruit trees" PLANT CELL TISSUE AND ORGAN CULTURE (0167-6857 1573-5044): 156 2 Paper 42. (2023) https://doi.org/10.1007/s11240-023-02670-9

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•Varjas Virág; Izsépi Ferenc; Tóth Tímea; Szilágyi Sámuel; **Desiderio Francesco**; Vajna László "A manduala új kórokozója (Diaporthe amygdali) az őszibarackot is károsítja – fungicide hatásvizsgálat az eredményes védekezésért" Növenyvédelem (0133-0829): 83 (N.S. 58) (7) pp 289-269 (2022) Language: Hungarian

•Kálmar Klementina; **Desiderio Francesco**; Varjas Virág „First report of Erysiphe corylacearum causing powdery mildew on hazelnut in Hungary" Plant Disease (0191-2917 1943-7692) (2022). Language: English https://doi.org/10.1094/PDIS-12-21-2737-PDN

•Bujdosó Géza; Nagy Ferenc; **Desiderio Francesco**; Kovácsné Békefi Zsuzsanna „Le cultivar di ciliegio dolce derivate dal miglioramento genetico ungherese: Hungarian bred sweet cherry cultivars." FRUTTICOLTURA (0016-2310): 4 Speciale ciliegio pp 26-30 (2021). Language: Italian

Additional Presentations and Posters

•Békefi Zsuzsanna; Keleta Belay Tewelmehedin; **Desiderio Francesco**; Szilágyi Sámuel; Szalay Lazslo "Characterisation of chilling and heat requirement of accessions from the Hungarian almond Genebank", 2024 May 12-16, European Horticultural Congress (EHC 2024), S08 p. 77

•**Desiderio Francesco**; Szilágyi Sámuel; Kovácsné Békefi Zsuzsanna "Blind test for sweet and sour cherry preference in Érd, Elvira major" Conference: XXIX Ifjúsági Tudományos Fórum Keszthely, Hungary, 2023.06.08 Language: English

•Kalmár Klementina; **Desiderio Francesco**; Németh Z. Márk; Varjas Virág "A mogyorót fertőző új lisztharmatgomba (Erysiphe corylacearum) előfortdulása hazánkban" Conference: Hungarian plant protection society, Növenyvedélmi Tudományos Napok, Budapest, Hungary, 2023.02.21 Language: Hungarian

•**Desiderio Francesco**; Agyemang Duah Evans; Demián Emese; Takács András Péter; Pal Salamon; Várallyay Éva "Symptoms on Clematis vitalba can be a reason of Prunus virus I infection." Conference: GBI Napok, Gödöllő, Hungary, 2021.12.14. (2021). Language: English

•Szabó Luca Krisztina; **Desiderio Francesco**; Preininger Éva; Várallyay Éva "In Vitro Kemoterápia És Hőkezelés Hatása Csonthéjasok Vírusmentesítésére" Conference: 5th National Conference of Young Biotechnologists (FIBOK 2022) Gödöllő, Hungary 2022.04.11-12 (2022). Language: English

•Agyemang Duah Evans; **Desiderio Francesco**; Demián Emese; Takács András Péter; Pal Salamon; Várallyay Éva „Symptom on Clematis vitalba could be a reason for an infection with Prunus virus" Conference: HUNLIFE 2021, Eger, Hungary 2021.11.05-07 (2021). Language: English

•Agyemang Duah Evans; **Desiderio Francesco**; Emese Demian; Takács András Péter; Pal Salamon; Várallyay Éva "Putative Ilarvirus found in Clematis vitalba showing virus-like symptoms" Conference: XXIV. Tavaszi Szél Konferencia (DOSZ) Miskolc-Egyetemváros, Hungary 2021.05.28-30. (2021). Language: English

•Agyemang Duah Evans; **Desiderio Francesco**; Emese Demian; Takács András Péter; Pal Salamon; Várallyay Éva "Searching for the causative agent of a viral-like symptom in Clematis vitalba". Conference: Fiatal RNS Kutatók Fóruma 2021 - online konferencia. Forum for Young RNA Investigators 2021 - online conference. Budapest, Hungary 2021.03.22. pp 2-2 (2021)