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Characterization of valuable fruit traits and virome profile of sour cherry (*Prunus cerasus* L.)

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CONTENTS

LIST OF LEGENDS AND ABBREVIATIONS

General terms

Ab: Antibody

Ag: Antigen

AGO: Argonaute

CL: Large capsid

CS: Small capsid

DCL: Dicer-like

DNA: Deoxyribo Nucleic Acid

dsRNA: Double-stranded RNA

EFSA: European food safety authority

ELISA: Enzyme-linked immunosorbent assay

EPPO: European plant protection organization

hpRNA: Hairpin of RNA

LAMP: Loop-mediated isothermal amplification

MP: Movement protein

mRNA: Messenger RNA

miRNA: Micro RNA

NTP: Nucleoside triphosphate

PCR: Polymerase chain reaction

PTGS: Post-transcriptional gene silencing

qPCR: Quantitative PCR

QTL: Quantitative Trait Locus

RdRP: RNA-dependent RNA polymerase

RISC: RNA-Induced Silencing Complex

RNA: Ribo Nucleic Acid

RNAi: RNA interference

RNQP: Regulated Non-Quarantine Pest

RPA: Recombinase Polymerase Amplification

RPM: Reads per million

RPM: Revolution per minute

RT-PCR: Reverse transcriptase Polymerase Chain Reaction

siRNA: Small interfering RNA

SNP: Single nucleotide polymorphism

sRNA: Small RNA

SSR: Simple Sequence Repeat

ssRNA: Single strand RNA

TGS: Transcriptional gene silencing

VIGS: Viral induced RNA silencing

VPG: Viral protein genome-link

VSR: Viral suppressor of RNA silencing

Virus names

ACLSV: Apple chlorotic leaf spot virus

ApMV: Apple mosaic virus

ApNMV: Apple necrotic mosaic virus

ArMV: Arabis mosaic virus

ASGV: Apple stem grooving virus

ASPV: Apple stem pitting virus

CGRMV: Cherry green ring mottle virus

ChLVA: Cherry luteovirus A

ChMLV: Cherry mottle leaf virus

ChVT: Cherry virus T

CIRV: Carnation Italian ringspot virus

CLRV: Cherry leaf roll virus

CVA: Cherry virus A

CVF: Cherry virus F

CVTR: Cherry virus Turkey

LChV1: Little cherry virus 1

LChV2: Little cherry virus 2

NSPaV: Nectarine stem pitting associated virus

PDV: Prune dwarf virus

PetAMV: Petunia asteroid mosaic virus

PNRSV: Prunus necrotic ringspot virus

PPV: Plum pox virus

PrVF: Prunus virus F

RpRSV: Raspberry ringspot virus

SLRSV: Strawberry latent ringspot nepovirus

TBRV: Tomato black ring nepovirus

ToRSV: Tomato ringspot virus

1 INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is widely cultivated in the world due to its use for fruit production. The native area of distribution of sour cherry is the Caspian Sea, making the plants of this species acclimatized to the central European climate. The domestication of *Prunus* species can be dated back to 5,000 - 4,000 B.C.E. (Aranzana et al., 2019; Shulaev et al., 2008), but in the last century breeding has been focused more and more on integrating advanced methods to develop new varieties. Sour cherry international market is quite important for fruit production, and Hungary remains a top producer of *P. cerasus*. In the recent years, molecular breeding is being integrated to the traditional breeding as in order to shorten the path to the production and development of new and desirable varieties, for example for their size, flavour, and color profile. Fruit consumption of sour cherry has health benefits for humans, due to the high content of polyphenols and antioxidants in sour cherry fruits. The sour cherry breeding must focus on the selection of germplasm material to identify candidate cultivars and select ideal methods of propagation of healthy plant material, to maintain pathogen-tested plants. The germplasm material available in Hungary represents an opportunity for screening the different materials available, since a living collection of sour cherry cultivar and accessions is available at the Fruit Growing Research Institute, at the Hungarian University of Agriculture and Life Sciences (MATE). In the institute, breeding sour cherry cultivars is traditional, and we have available a germplasm living collection, which shows great variability among traits. Screen houses can be used to test plant material health, but routine testing of open fields might give more information about plant pathogens and their population, since pathogens might move freely from one to another plant. Stone fruit viruses have been described in many different countries, and pose a health risk due to the limitations in national and international regulation, posing a health risk to the plant exchange material process and the importation of infected plant material. In this research, we focus on the use of plant genetic material to integrate possible candidates into the breeding process, analysing the fruit size, color, and secondary characteristics, while analysing the viral presence and distribution in the germplasm collection with high-throughput sequencing method and RT-PCR validation. Among fruit trees, the number of molecular markers associated with important traits described is far lower than among annual plants. In our work, we focused on sour cherry fruit traits where associated markers that have been already described in the literature but not validated and compared to the phenotype of Hungarian cultivars.

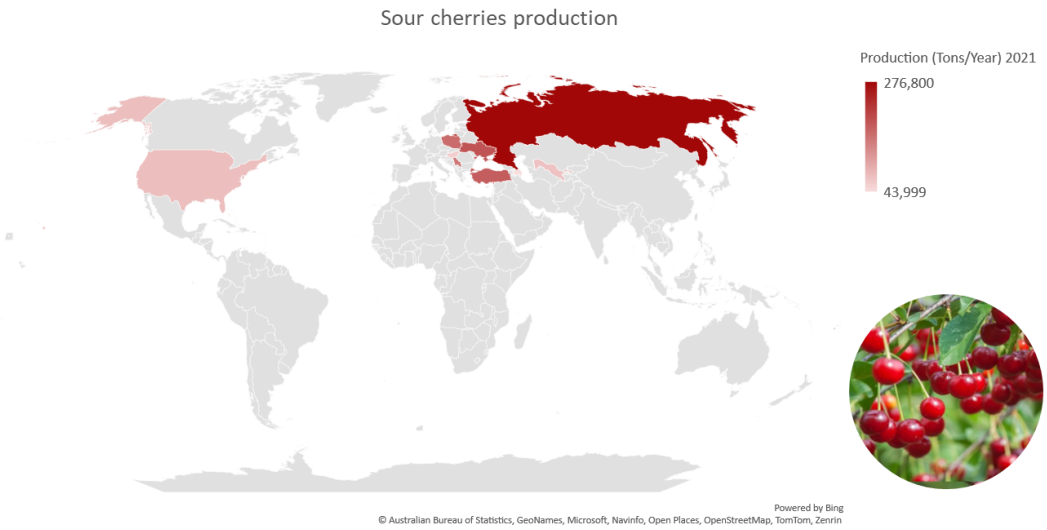
2 OBJECTIVES

1. Comparative analysis of phenotype with markers associated with fruit size, skin and fruit flesh color 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 LITERATURE OVERVIEW

3.1 *Prunus cerasus* origin, distribution and uses

More than 230 different species belong to the *Prunus* genus, divided into four subgenera called *Amygdalus, Cerasus, Prunus,* and *Eplectocladus* (Potter, 2012; Shulaev et al., 2008).A well-known domesticated fruit tree, sour cherry (*P. cerasus* L.), has been cultivated since 5,000 - 4,000 B.C.E., where rosaceous fruits were a valuable source of food and were naturally selected for their characteristics (Maria José Aranzana et al., 2019; Shulaev et al., 2008). Domestication event in sour cherry has been described as a slow process starting from the Neolithic period, with a primordial selection of fruit trees (Woldring, 1997). The geographical origin of sour cherry has been identified as the area between the Black Sea and the Caspian Sea, where Theophrastus in 300 B.C described for the first time the cherry as *kerasos* (in Greek: κέρασος) a Greek world coming from the town Kerasun in the Pontus, on the Black Sea, which subsequently became the species name *cerasus*. However, several authors expressed a contrary opinion suggesting that the town received its name from the cherry growing, thus the origin is unclear (Faust & Surány, 1997). Sour cherry production is often considered a fruit species of eastern Europe because of the most important producing countries are all located in this parlt of the world (Bujdosó & Hrotkó, 2017). *P. cerasus* production varies according to climatic requirements worldwide but generally sour cherries produce worldwide 1 million tons/year. Sour cherry top producer is Russia, with 13% of the global production, followed by Ukraine, Turkey, Poland, Serbia, Iran, USA, Uzbekistan, Hungary and Azerbaijan. In particular, sour cherry production is quite important in central and eastern Europe, where Hungary places itself in the top 10 producers worldwide (Figure 1) (FAOSTAT, 2023). In Hungary, the production of sour cherry fruits has remained relatively stable in the last 10 years. Hungarian stone fruit production count for 65.860 tons for sour cherry, with production peaks higher than 90.000 tons in 2013, as shown in Figure 2 (FAOSTAT, 2024).



Species	Country	Production (Tons/Year) 2021
	World	1,514,665
	Russian Federation	276,800
	Ukraine	193,720
	Türkiye	183,757
	Poland	166,600
Sour Cherries	Serbia	155,137
	Iran (Islamic Republic of)	122,702
	United States of America	78,060
	Uzbekistan	73,285
	Hungary	60,530
	Azerbaijan	43,999

Figure 1 Worldwide production of Prunus cerasus and top 10 producers in 2021 (FAOSTAT, 2021)

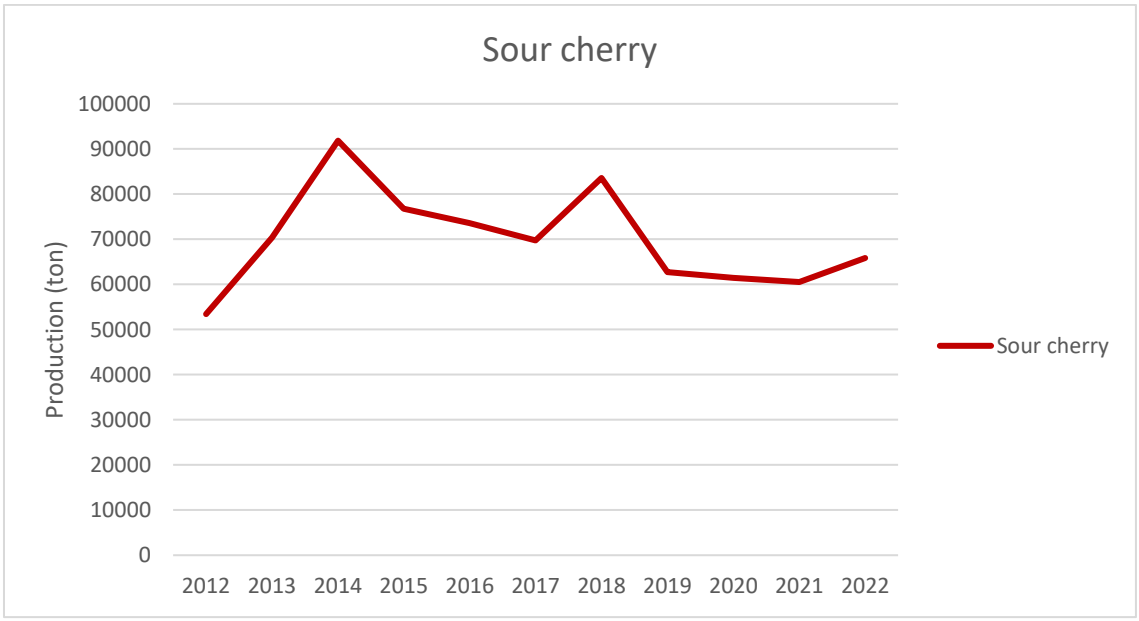


Figure 2 Sour cherry production in Hungary between 2012 and 2022. (FAOSTAT, 2024)

3.2 Sour cherry importance in the world and in Hungary

Sour cherry (*Prunus cerasus*, L.) are deciduous trees originating from West Asia and South-Eastern Europe and belong to the *Rosaceae* family. Sour cherry are economically important fruit trees cultivated in temperate climatic areas, with important health benefits for humans. While sweet cherry fruits are appreciated for their flavour profile, crunch and juice color and are consumed mostly fresh, even if canned products are still a marketable margin, sour cherry fruits are described as more astringent than sweet cherry, and they are generally used for both fresh and canned production (Ferretti et al., 2010, Bujdosó et al., 2020). Sour cherry is an allotetraploid, generally self-incompatible spontaneous hybrid (Hauch et al, 2006). It is cultivated mainly for its sour and succulent flavour fruits, which contain a wide range of antioxidant phytochemicals reported as relatively resistant to processing (Papp et al, 2010). The trees can be cultivated in areas with low winter temperatures as they are resistant to cold, but even if considered generally more tolerant to biotic and abiotic stresses compared to sweet cherry, they are susceptible to a wide range of plant pathogens. In Hungary, there is a long-time tradition of sour cherry breeding aimed to improve quality and productivity since sour cherry is considered in Eastern and Central Europe a valuable horticultural crop. The demand for new and healthy varieties in sour cherry is constant, and Hungarian sour cherry varieties have the possibility not only to be reintroduced in the local market but also for breeding material to expand the gene pool already available and to maintain characteristics desirable for the consumers (Apostol, 2014; Schuster et al., 2017). Commercial varieties are required to be screened to be sold as certified material in and out of the EU zone. However, certified material is tested only for known viruses in the EPPO list, reducing the list of possible infected plants to a fraction (Bragard et al., 2019). This approach is a double-edged sword; it is very useful to eliminate possible harmful pathogens, however, it does not take into consideration pathogens that are not on the quarantine list. Sour cherry breeding in Hungary has been organized since the 1950s (Schuster et al., 2017). Since then, the main focus of Hungarian breeders has been to increase marketability, with the development of better-quality fruits as well as more resistant to biotic and abiotic stresses (Rozsnyay & Apostol, 2005). The traditional breeding was initially carried out as clonal selection of local varieties, where populations derived from ‘Pándy’ and ‘Cigány’ cultivars represent the majority at the end of the 19th century (Baris et al., 2017). Since that time crossbreeding program led to the licensing of new cultivars with desirable traits: ‘Érdi Bőtermő’, ‘Érdi Jubileum’, ‘Újfehértói fürtős’ and ‘Pipacs’. In recent years improved molecular marker-based selection methods have been used and helped to identify desirable traits such as self (in)compatibility or flowering and ripening time in Hungarian germplasm material, assisting the breeders’ decision process (Bedő et al., 2023; Halász et al., 2019).

3.3 Health benefits of consuming *Prunus cerasus*

Sour cherry fruits are consumed in different forms worldwide, depending on the local market requirements and the uses and costumes of each country. While fresh fruit consumption is more common for other stone fruits such as peaches, apricots, sweet cherries, and almonds, conserved or processed form is a major market for sour cherries, where jams, juices or alcoholic derivates represent the main market (Bujdosó et al., 2020; Shulaev et al., 2008). Sour cherry is known to be rich in healthy compounds, particularly polyphenols and antioxidants, which have been associated with the decrease of cardiovascular and cancer diseases as well as the prevention of anti-inflammatory diseases (Acero et al., 2019; Kelley et al., 2018; Shulaev et al., 2008). Polyphenols are chains of phenols, a secondary plant metabolite characterized by one or more aromatic rings with one or more hydroxyl groups attached. Polyphenols occur in plants as glycosylated derivates and conjugated with inorganic acid and malonylate. Phenolics differ in plant tissues and many are synthesized from carbohydrates via shikimate and phenylpropanoid pathways (Ferretti et al., 2010). Phenolic metabolites vary from low molecular weight single-aromatic tannins to large complex tannins (Figure 3). Polyphenols have been studied in small fruits, particularly the anthocyanin and flavonol compounds. Anthocyanin compounds accumulate during the fruit ripening and are involved in the darkening of fruits as well as having a positive effect on human health, reducing the activity of free radicals (Borowiec et al., 2022).

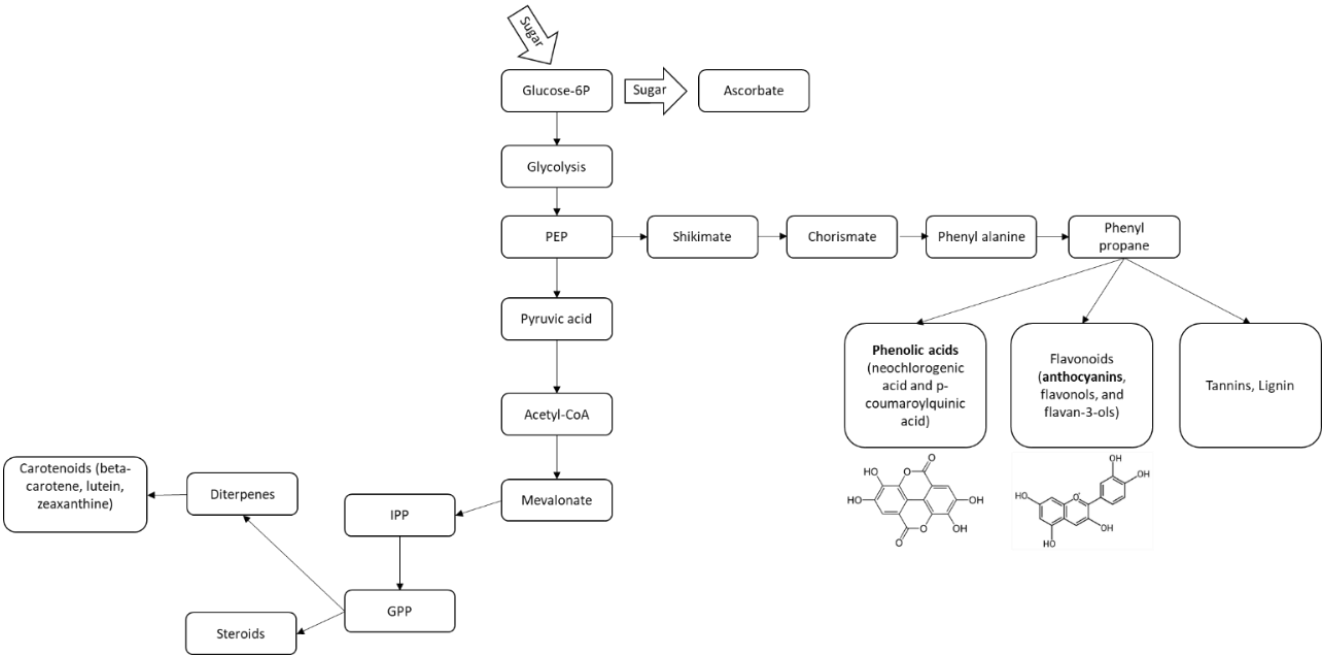


Figure 3 Phenol and anthocyanins pathway. PEP: phosphoenolpyruvate, IPP: isopenthenylpyrophosphate, GPP: geranylpyrophosphate. Modified from Ferretti et al. (2010).

3.4 Sour cherry health benefit, polyphenols, and colorimetry

Sour cherry (*Prunus cerasus* L.) is popular in fruit industry, due to the fact they are rich in anthocyanins and polyphenols. The health benefit of consuming fruit-rich anthocyanins has been related to decreased inflammatory-related diseases, cardiovascular and diabetic-related diseases (Borowiec et al., 2022). Consumption of sour cherry juice from *P. cerasus* cultivar 'Maraska' indicated that the anthocyanin cyanidin 3-glucoside had a strong antioxidative activity, where *in vitro* studies suggested strong anti-inflammatory properties in the liver and blood of mice (Šarić et al., 2009). Furthermore, anthocyanin's quantity in fruit can help researchers to better understand the ripening time and color development in sour cherry fruits (Pedisić et al., 2009). In Poland, researchers investigated the chemical profile of several sour cherry varieties, indicating that investigated sour cherry cultivars had a good source of anthocyanins among fruit species, where some of the analysed cultivars were of Hungarian origin, such as 'Érdi bőtermő', 'Érdi nagygyümölcsű' and 'Pándy' (Wojdyło et al., 2014). High polyphenolic content in fruit has been correlated with antimicrobial activities against foodborne pathogens, which could be applied as preservatives in the food industry (Kołodziejczyk et al., 2013). Polyphenolic compounds are well known free radical scavengers, which may be useful to prevent the development of chronic diseases, such as cancers, obesity and cardiovascular diseases (Głowacka et al., 2020). Moreover, in recent years, the relationship between polyphenol content and fruit color has been investigated in different countries, where in Hungary several autochthonous varieties have been indicated as containing high content of anthocyanin and polyphenols, such as the Hungarian cultivars 'Pipacs1' (Papp et al., 2010), 'Újfehértói fürtös' and 'Tiszabög 50/7' (Desiderio et al., 2023). In these previous studies, the identification of high polyphenolic content suggested the presence of several candidates for utilization both in food products as additives, or alternatives for fresh consumption (Papp et al., 2010).

3.5 Sour cherry breeding and germplasm collection

Due to its importance, sour cherry has been one of the main focuses for breeding in Hungary in the last 70 years. The history of cherry breeding in Hungary started in the 1950s during the Hungarian People's Republic when in Érd (15 km from Budapest) it was decided to focus the attention on selection of bigger fruits and better-flavoured cherry fruits by establishing and collecting local varieties from the Carpathian basin (Rozsnyay & Apostol, 2005; Soltész et al., 2003). Since then, sour cherry breeders developed several new varieties which can still be appreciated in the local and international market, due to the high yield and big fruit sizes. Many varieties of sweet and sour cherries derived from Hungary have been integrated into several breeding programs, such as the well-known 'Újfehértői fürtös' and 'Érdi Bőtermő', respectively known as 'Balaton' and 'Danube' in western Europe and America (Papp et al., 2010; Pedisić et al., 2009). The varieties, or accessions are part of a bigger germplasm collection, which is a live collection of fruit trees of about 20 hectares (ha). The collection is *ex-situ* and *in vivo*, since the collection is assembled and comprehends different varieties of *Prunus* species which are hosted in the institute fields as live trees. The germplasm collection does not only host several varieties of fruit trees. It has the function of maintaining endangered or abandoned varieties, which might not be considered useful anymore as well as providing useful information for germplasm material such as biotic and abiotic resistance for further selection (Bretting & Widrlechner, 1995; Rodrigues et al., 2008). Thus, access to a vast germplasm collection appears to us as a great opportunity to investigate the genetic background of *Prunus cerasus*, where the reintroduction of old accessions into the breeding program may result in the selection of useful

genes for the present and future breeders. We focused our attention on sour cherry accessions and landraces, which were previously collected and are available in the gene bank collection.

3.6 Phenotyping systems used in cherry

A crucial part of data collection in *Prunus cerasus* species is the yearly phenotyping. The phenotyping analysis conducted in this study followed previously established standardized methods for growers and breeders, such as UPOV and Ctifl. The Union of the Protection of new Varieties of Plants (UPOV) is the established guideline for breeders and growers worldwide and provides a thorough system to measure in a consistent manner variety belonging to the same species (UPOV, 2024). For *Prunus* species, several protocols have been developed and implemented through the years, where for example for sour cherry the protocols used is called CPVO-TP/230/1. Each protocol uses known and cultivated registered reference varieties, for sour cherry for example cultivars 'Meteor', 'Montmorency', 'Favorit' are used as standards since their characteristics are well known and documented. In UPOV system the scoring varies according to the different characteristics, for example, tree vigour in sour cherry can be scored from very weak to very strong on a 1-9 scale (UPOV, 2006). Colorimetric data collection is quite challenging due to the short ripening time and color differentiation. For this reason, we relied on the use of established colorimetric scaling system such as the UPOV and the color chart from the Centre Technique Interprofessionnel des Fruits et Légumes (Ctifl). The Ctifl system was developed originally for breeders working with sweet cherry, to help them better identify favourable traits such as skin color in a fast and reliable way (Kappel et al., 1996). In our previously published paper, we argue that the use of Ctifl scale could be applied to sour cherry too, once compared with other scaling systems in use and enforce the consistency of our data (Desiderio et al., 2023).

3.7 Selected genetic SSR markers in sour cherry

Cherry full genome has been published in recent years, allowing to better understand the relationship between tetraploid sour cherry and its parent diploid sweet cherry, where a 6K SNP array was developed by Peace and colleagues (2012). In this study substantially different germplasm were evaluated, to consistently assess polymorphism within sour cherry and sweet cherry germplasm collection, showing one in three polymorphic SNPs in sweet and sour cherry. In Germany, Wöhner and colleagues (2023) focused on sour cherry origin and allotetraploidy, where using advanced sequencing allowed to track the origin of sour cherry to sweet cherry and Mongolian cherry (*Prunus fruticosa*). Furthermore, several loci and markers have been identified and associated with sweet and sour cherry traits. Fruit firmness QTL has been identified for group 4 in sweet cherry for qP-FF4 by Cai and colleagues (2019), fruit cracking has been reported as associated to SSR $BPPCT004_{197}$ (Khadivi-Khub, 2015). For cherry fruit size, two markers have been analysed and successfully were able to identify differences in fruit size, called respectively BPPCT034 and CPSCT038. Those markers were designed flanking the locus group 2 (LG2), called *FW_G2a*, a hotspot previously identified for fruit size and weight gene *PavCNR12*, involved in cell number regulation (Szilágyi et al., 2022), thus were considered valid candidates for analysing the correlation between phenotype and genotype. Fruit color analysis for cherry was focused on previously published primers, analysing the fruit skin and fruit flesh color. The LG3 was previously indicated as a region where fruit flesh and skin color gene might reside and called MYB10 (Calle et al., 2021). Furthermore, in LG3 different markers have been indicated as possible candidates, such as Ma039a, Pav-Rf-SSR and LG13.146 (Cmejla et al., 2021; Sooriyapathirana et al., 2010; Stegmeir et al., 2015). Ma039a

and Pav-Rf-SSR have been indicated as good indicators for fruit skin color, where Ma039a could differentiate between light to red skin color, while Pav-Rf-SSR was used to differentiate between red to dark red skin color (Cmejla et al., 2021; Sooriyapathirana et al., 2010). LG13.146 is the only marker between the one used that was designed specifically for sour cherry, and was used to differentiate flesh color since is an important characteristic for commercial sour cherry (Stegmeir et al., 2015).

3.8 Sour cherry viruses in the world and in Hungary

Cherry viruses have been investigated for several years, using classical virus diagnostic methods (Németh, 1986). Several diagnostic methods help us to identify virus infection in cherry trees. The antibody-based ELISA and lateral flow test are both available for PNRSV (for both three serotypes), PDV, PPV, ACLSV, ApMV, ArMV, CLRV, SLRSV, PetAMV, RpRSV, TBRV and ToRSV and were used for virological survey of sour cherry trees in Bulgaria (Kamenova et al., 2019; Kamenova & Borisova, 2021; Milusheva & Borisova, 2005) Iran (Soltani et al., 2013), Ukraine (Pavliuk et al., 2021). The method is cost-effective; however, the sensitivity is often below the detection level of the low virus titre. Furthermore, when multiple variants are present or when further distance variants are present in a sample, a false negative diagnosis could appear. In recent years a more sensitive method, RT-PCR, nucleic acid detection method has been used for surveying sour cherry orchards alone or in combination with ELISA, to validate both methods. Sour cherry trees surveyed using RT-PCR alone or in combination with ELISA in Turkey (Ulubas & Ertunc, 2004) (Çevik et al., 2011), Serbia (Mandic et al., 2007), in the Iberian Peninsula (Pérez-Sánchez et al., 2017) and Poland (Komorowska et al., 2020) showed promising results to validate the presence of viruses in open fields. Other method based on nucleic acid molecular methods such as LAMP and RPA are also available for detection of several *Prunus* infecting viruses such as ApNMV, ASPV, ASGV, ACLSV, ApmV, PNRSV, LChV2, PPV (Hadersdorfer et al., 2011; Jiao et al., 2021; Mekuria et al., 2014; Wani et al., 2023). Because of their isothermal methodology, they can be used directly at the orchards. While the methodologies detect the presence of a particular pathogen, usually a single and possibly a variant, high-throughput sequencing methods can be used to identify the presence of all pathogens in the investigated sample. HTS is usually used to investigate the viromes in trees showing unusual symptoms to find the causative agent of the disease, rather than surveying purposes, resulting in new virus discoveries. In research conducted in the Czech Republic, sweet and sour cherry germplasms and orchards were surveyed by combining RT-PCR and HTS to provide data about the presence of all viral pathogens in the investigated trees and to more broadly validate the methodology (Přibylová et al., 2020). Using high-throughput sequencing (HTS) for virome characterization led to the discovery of novel viral species infecting fruit trees in an unexpected number (Hou et al., 2020; Maliogka et al., 2018). In 2017, 28 viruses and 3 viroids have been reported to infect sweet cherry, while out of those only 20 viruses and two viroids have been reported from sour cherry (Rubio et al., 2017). Since this last review, additional infections of sour cherry with four newly described viruses such as cherry luteovirus A (ChLVA), cherry virus F (CVF), cherry virus Turkey (CVTR), cherry virus T (ChVT) and six already known virus species nectarine stem pitting associated virus (NSPaV), tomato black ring nepovirus (TBRV), prunus virus F (PrVF), little cherry virus 1 and 1 (LChV1 and 2), cherry virus A (CVA) has been reported, resulting the list of 28 sour cherry infecting viruses and two viroids (Appendix A3). Interestingly, the original description and identification of CVF and ChVT has been reported from sour cherry samples, highlighting the importance of research in

sour cherry (Koloniuk et al., 2018; Marais et al., 2020). On top of that, we will elucidate some of the most important sour cherry viruses that have been found in this study.

3.8.1 Cherry virus A (CVA)

CVA was described for the first time in Germany by Jelkmann (1995), from symptomatic cherry leaf material. The virus is a member of the family *Betaflexiviridae* genus *Capillovirus*. It is a linear monopartite virus ssRNA+, translating aRNA-dependent RNA polymerase (RdRP), capsid and movement protein. Symptomatology of CVA is still quite obscure and recent studies have highlighted how CVA appear in both single and mixed infections however without observing significant correlation between the presence of virus and symptoms (Přibylová et al., 2020). At the moment CVA is not considered a quarantine pest in Europe and is in the EPPO quarantine list only in Israel (Quarantine pest) and Iran (A1 list), leaving some concerns regarding its status in Europe and possibility to be exported elsewhere (EPPO, 2024a).

3.8.2 Prunus necrotic ringspot virus (PNRSV)

Among the most important viruses causing economic loss in sour cherry plantations is prunus necrotic ringspot virus (PNRSV). PNRSV belong to the family of *Bromoviridae*, genus *Ilarvirus* non-enveloped, with a quasi-spherical virion containing positive single-stranded RNA (ssRNA+), a tripartite virus segmented in RNA1, RNA2 and RNA3. The RNA1 and RNA2 encode for a protein 1a and 2a respectively, involved in replication and internal transcription of sgRNA4 from the minus-strand copy of RNA3. RNA3 and sgRNA4 instead encode for the movement and capsid protein. Furthermore, ORF2b located on RNA2 terminal 3' encodes for a viral suppressor RNA silencing (VSR) and is expressed as subgenomic RNA (sgRNA4A) (ViralZone, 2024). PNRSV has been considered for a long time a problematic virus since it is widely spread in the EU. It is possible to spread PNRSV with seed and pollen-mediated transmission mechanisms as well as with vegetatively propagated plants for planting, however, it is not included in the quarantine list due to the widespread in Europe as well as the limited impact (EFSA, 2014). Together with prune dwarf virus (PDV), it is considered as a regulated non-quarantine pest (RNQP), where RNQPs regulations are limited to reduce the contamination of new areas with a certain tolerance for the presence of the pest (EPPO, 2024b).

3.8.3 Prunus virus F (PrVF)

Prunus virus F was first described in Canada by Villamor and colleagues (2017) when sweet cherry *P. avium* Mazzard seedlings were analysed for HTS. It is a relatively new *Fabavirus*, with a bipartite ssRNA (+) genome encapsidated, RNA1 and RNA2 encoding a polyprotein 1 and polyprotein 2 respectively (Koloniuk et al., 2018). RNA1 encodes for a co-factor protease, NTP-binding protein peptide, VPg protease and RNA-dependent RNA polymerase (RdRP) while RNA2 encodes for movement protein (MP), large capsid (CL) and small capsid (CS) (Koloniuk et al., 2018). Since its discovery has been found in several other countries, such as the USA, Belgium and Czech Republic (James et al., 2019; Šafářová et al., 2017; Tahzima et al., 2019). PrVF appears to be a highly variable virus, as previously indicated by several authors. Already during the first identification of PrVF, Villamor et al., (2017) suggested the presence of two different variants in the sweet cherry (symptomatic *Prunus avium* grafted on *P. avium* cv. 'Mazzard') tested. Safarova et al., (2017) indicated in their first report that three different sour cherries (*Prunus cerasus*) were infected with PrVF (KY817222-24 PrVF RNA1; (KY817225-27 PrVF RNA2), and they were also able to infect *Prunus avium* cv. 'Bing' with chip budding. In Canada, James et al., (2019) reported symptomatic *P. avium* cv. Staccato showing dieback and leaf deformity was infected with PrVF as well as a non-symptomatic P.

avium cv. ‘Staccato’, suggesting the presence of two isolates (MH998208-20 PrVF RNA1 and RNA2). Tahzima et al., (2019) found one PrVF variant in a routine screening of different sweet cherries grafted on ‘Gisela 5’ in Belgium. Five of those were positive for PrVF. So far, the most complete variability study on PrVF was conducted by Koloniuk et al., (2018) where different sweet and sour cherries were analysed in Czech Republic. Six samples were collected at Holovousy Research Institute, where there is an important germplasm collection, two samples were from Hroznejovice and Hluboká nad Vltavou, and one sample was collected in Aridaia, Greece. Interestingly, most of the trees were infected with 2 or 3 variants of PrVF, which is in line with the first identification of PrVF (Villamor et al., 2017). The aforementioned studies have not observed obvious symptoms, and most of the infected samples showed mixed infection with different PrVF strains as well as mixed infection with other fruit tree viruses (Koloniuk et al., 2018). The little information available regarding this, and other viruses, requires us to investigate the regulation of cherry-infecting viruses to better understand the risks of infected material.

3.9 Viral diagnostic overview: ELISA, LAMP, PCR and HTS

Eradication of viruses from fields is a long and costly process in stone fruit management. For this reason, control and early detection of viruses in fruit trees or in propagation material is a prerequisite for the identification of pathogens and to guarantee sustainable agriculture. Diagnosis, therefore, is the most important aspect of control in stone fruit plant viruses (Barba et al., 2015). Detection techniques can be divided into two main categories; serological and molecular. Serological diagnostic is based on enzyme-linked immunosorbent assay (ELISA), where an antigen (Ag) and an antibody (Ab) react with a colorimetric reaction and the visualization indicates the presence of a virus (Barba et al., 2015). This method was established by Clark and Adams in 1977 and brought plant virology towards a new period in diagnostics. Before this, the recognition of the description of virus symptoms on hosts as well as studies in bio-assay, indicator plants or electron microscopy were the only available techniques (Boonham et al., 2014). However, ELISA relies on the development and availability of specific antibodies, which can be scarcely available in fruit tree viruses, and cannot satisfy the request for mass-scale diagnosis (Maliogka et al., 2018). To satisfy this growing demand, we must look at the molecular alternative of polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP). PCR methods for viral detection have been used since the ‘90s, offering a superior level of specificity and sensitivity in viral diagnosis (Boonham et al., 2014). More in detail, because most of the fruit tree viruses are RNA viruses, the PCR must be performed after a retrotranscription of RNA into cDNA (Barba et al., 2015). Small amount of cDNA is required to perform PCR, and the development of two pairs of primers is relatively simple. However, due to this step and the long preparation of reagent mixture, an alternative isothermal amplification has been developed as loop-mediated isothermal amplification (LAMP). Three primers (internal, external and loop primers) are used to amplify a product which contains single-stranded loop regions to which primers can bind without a template at a constant temperature (of around 65°C) and hence can also be used in field detection, with a simple block heater (Boonham et al., 2014). Quantitative PCR (qPCR) is often used in fruit tree viral detection, allowing not only the amplification of small fragments of the viral genome but also the detection of multiple viruses in the same host. Nevertheless, this method as well as the LAMP have the disadvantage of relying on previous knowledge of a particular characteristic of the virus to be detected (Rubio et al., 2017). The present trend in virology is then to use new technologies such as high throughput sequencing (HTS), which has changed the field of plant

virology in the last 20 years, allowing the detection and identification of known and unknown agents without previous knowledge (Hou et al., 2020). HTS sample preparation requires nucleic acid extraction, size selection, enrichment of RNA viral sequences, preparation of libraries and bioinformatic analysis. Even if the procedure requires significant time, skills and manipulation, the final results are impressive, making HTS an ideal choice for virus discovery and identification in fruit trees (Maliogka et al., 2018).

3.10 Regulation of sour cherry infecting viruses

Virus infection of sour cherry can lead to symptom development and can affect the economic value of fruits. Symptoms can be developed not only on the fruits and leaves but can interest the trunk, where it can interfere with the vascular system causing disturbance of the water and nutrients transport, leading to decline or in serious cases to the death of the tree. Orchards have been established for a long-time using grafts of rootstocks and desirable cultivars as propagating material. This vegetative propagation method ensures the maintenance of the desirable traits but poses a constant risk of pathogen infection. To minimize the risk of immediate infection and early decline of the orchard it is well suggested to use pathogen-tested propagation material. As there is no possible plant protection method available against viruses and viroids, their presence should be avoided in the grafts. Certification programs provide a regulated network to establish, maintain and propagate virus-tested rootstock and cultivars. Their strict regulation contains a list of viruses with economic importance whose presence should be checked and ruled out at certified stock collections (Barba et al., 2015). Stone fruit viruses such as plum pox virus (PPV), prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) are causing great losses since the fruit yield decreases in some cases up to 100% for PPV (Jelkmann et al., 2018) and up to 60% for PNRSV and for 50% PDV (Pallas et al., 2012). Regulation of sour cherry and sweet cherry certification is guided together by EPPO PM 4/29, including 15 viruses. Among them only 11 - apple chlorotic leaf spot virus (ACLSV), apple mosaic virus (ApMV), carnation Italian ringspot virus (CIRV), cherry green ring mottle virus (CGRMV), cherry leaf roll virus (CLRV), little cherry virus 1 and 2 (LChV1-2), petunia asteroid mosaic virus (PeAMV), prune dwarf virus (PDV), prunus necrotic ringspot virus (PNRSV) and tomato black ring virus (TBRV) - have been proven to infect sour cherry, while although they are on the regulated virus list no infection with arabis mosaic virus (ArMV), cherry mottle leaf virus (ChMLV) raspberry ringspot virus (RpRSV) and strawberry latent ringspot virus (SLRSV) has been reported by EPPO or have available sequence record available in the NCBI GenBank from this host (Appendix A3). PNRSV it is currently indicated as a Regulated Non-Quarantine Pest (RNQP) in Europe, while it is considered a quarantine pest in America and Asia. In Africa (Egypt) it is indicated in the A2 EPPO list, meaning that PNRSV is considered as a pest recommended for regulation as a quarantine pest (EPPO, 2023b). PNRSV can be spread through pollen and seed, as well as with vegetative propagation. It is known to be hosted not only by *Prunus* species, but it has been found to infect hop, rose, and raspberry. In Europe, the current voluntary certification scheme is used to reduce the risk of the spread of PNRSV, as well as thermotherapy and chemotherapy, in vitro meristem tip culture are considered all valid methods to allow production of healthy plants (EFSA, 2014). CVA was inserted in the list of quarantine pests in Israel in 2009 (EPPO, 2023a). Due to lack of symptoms, however, it is not considered a quarantine pest in Europe nor USA According to a 2020 document from the Californian department of food and agriculture, the categorization state of CVA is considered as low risk, due to several factors such as limited host range, graft-transmission and absence of symptoms (CDFA, 2020). PrVF is currently not considered a quarantine pest. The legislation does not

consider it a risk, due to a lack of evidence that PrVF may be harmful because little is still known about its symptomatology, pathogenicity, and economic impact. EFSA stated that due to limited information on PrVF distribution, host range and transmission, it is not possible to conclude whether the presence of PrVF would negatively impact the plantation of Prunus host (Bragard et al., 2019). Nowadays there is more information available regarding fruit tree viruses due to the increased use of high throughput sequencing, however as described by (Hou et al., 2020) the validation through RT-PCR does not always follow the bioinformatic preliminary analysis. We decided to analyse sweet and sour cherry material from Hungary to be able to identify the health state of those varieties. To do so, we must first explain the way the defence mechanism of plants works and how the virus can escape the plant's immune response strategy.

3.11 RNA interference in fruit tree

The function of RNA interference (RNAi) is to regulate endogenous genes, as well as recognize exogenous double stranded RNA (dsRNA), thus stopping the proliferation of external and harmful pathogens (Pooggin, 2018). RNA silencing is the defence mechanism which may have been one of the original functions in primitive eukaryotes (Baulcombe, 2004). In this section, we will discuss the plant sRNA mechanism and how viruses evolved to escape regulation and detection.

3.11.1 RNAi mechanism

The plant defence mechanism is regulated by the interaction between host and pathogen (Balcan & Erzan, 2004). Plants use several mechanisms to fight virus infection, including RNAi, systemic acquired resistance, hypersensitivity response and DNA methylation (Singh et al., 2019). Here we will focus on the RNAi machinery against viruses. The recent discovery of large and small non-protein-coding RNAs with specific regulatory roles have opened a new window in understanding plant regulatory mechanisms. In particular, small RNA belong to two classes, microRNA (miRNAs) and short interfering RNAs (siRNAs), which are known to play essential role in gene regulation and expression, as well as adaptive responses to biotic and abiotic stresses(Vaucheret, 2006). Gene silencing results from transcriptional gene silencing (TGS) or from post-transcriptional gene silencing (PTGS), in which the transcription is inhibited and RNA is degraded, respectively.(Fang & Qi, 2015; Lindbo, 2012; Vaucheret, 2006). Production of virus-derived siRNAs have been observed in response to viral infection in plants (Hamilton & Baulcombe, 1975).When RNA viruses enter the host through wounds or by the feeding action of insects, they start to replicate in host cells, move from cell to cell and spread to long distances via phloem (Singh et al., 2019). Viral replication starts with the formation of a new strand of RNA, forming a double strand (dsRNA). The host defence mechanism recognizes foreign double strands of RNA through the Dicer-like (DCL) enzyme. DCL cleaves the double-strand RNA into small fragments of RNA of 21-24 nucleotides, also known as small interfering RNA (siRNA). The siRNA is then recognized by the host RNA-Induced Silencing complex (RISC), formed by protein complex including Argonaute (AGO), which bind one strand of the siRNAs. The “loaded” RISC can recognize the foreign RNA based on sequence complementarity to the loaded small RNA and use it to identify foreign messenger RNA (mRNA) (Johnson et al., 2021). Then, the complex acts to inhibit protein translation or cleave the target mRNA (Figure 4).

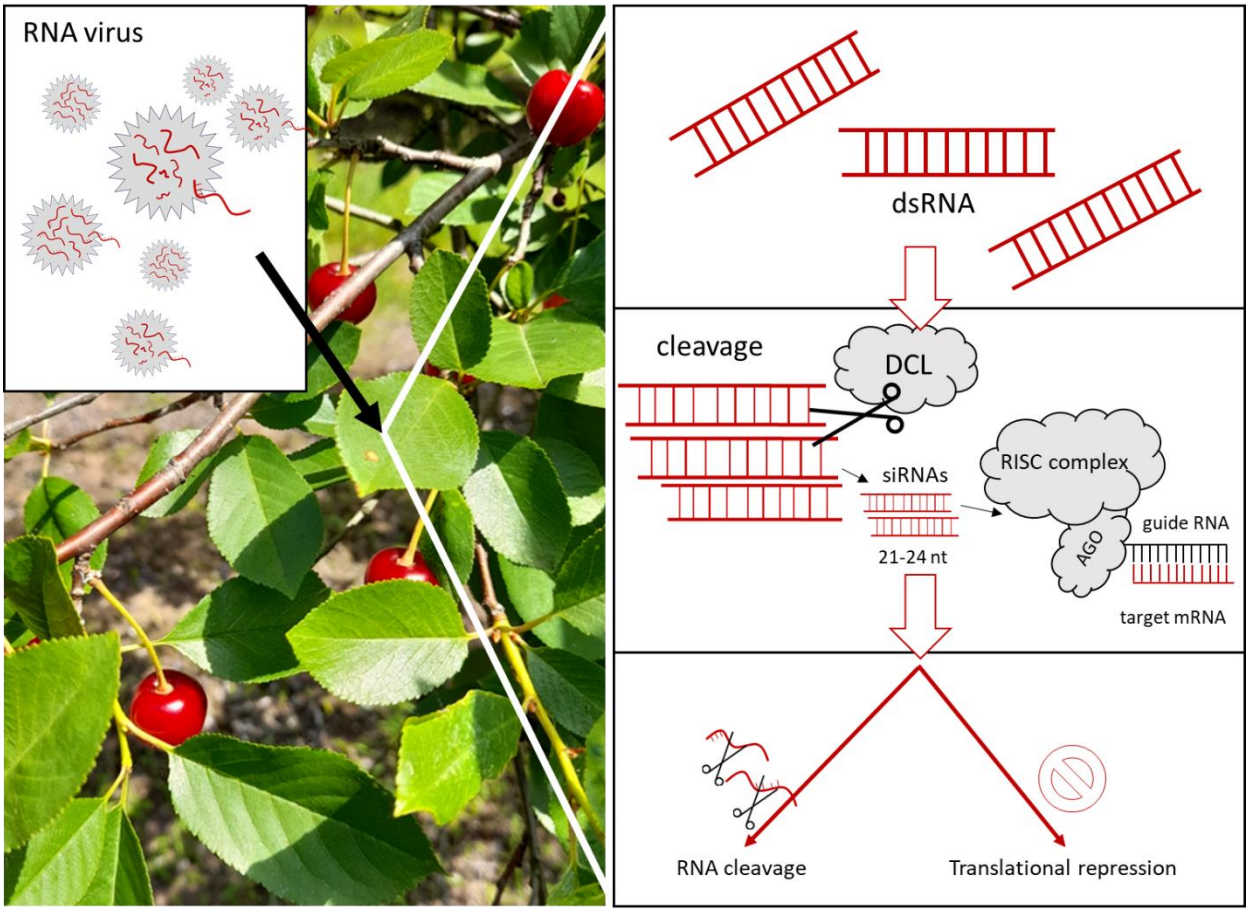


Figure 4 RNAi mechanism in fruit stone tree, modified from Singh et al. (2019).

3.11.2 Escaping detection: viral response to siRNA

RNA silencing in plants target viral proliferation and accumulation, allowing the host to recover from an infection (Baulcombe, 2004). However, viruses have evolved different strategies to counteract the host defence mechanism such as viral suppressor of RNA silencing (VSRs). Besides their function as RNA-silencing suppressors, they have been proven essential in viral survival, functioning as coat protein, replicase, movement protein, helper component or protease and transcriptional regulators (Burgyán & Havelda, 2011). The primary counter defence measure involves the suppressor of proteins involved in silencing, encoded by many viruses. The evolution of such protein is supposedly a common strategy since there are no common sequence motifs in different groups (Baulcombe, 2004). VSRs function against RNAi immunity is to alter the functionality of RISC to cleavage viral transcript. Several viral encoded proteins have been described such as p19 in *Tombusvirus*, p21 in *Closterovirus*, P0 in *Luteovirus* and have been observed working at different stages to suppress RNAi activity (Deng et al., 2022; Johnson et al., 2021).

3.11.3 RNAi based resistance breeding of stone fruits

Particularly in fruit trees, different strategies have been observed. In *Prunus* species, the most well-known study was performed in transgenic plums, conferring resistance to plum pox virus (PPV). The study, conducted by Hily and colleagues (2005) indicated that posttranscriptional gene silencing in plum trees confers resistance to plum pox virus. PPV resistance has been successfully proven in peach as well, with the development of a prunus necrotic ringspot virus (PNRSV) based viral induced RNA silencing (VIGS) vector containing a fragment of the

eukaryotic translation initiation factor 4E isoform, known as *eIF(iso)4E* gene, a host factor of many potyviruses, including PPV (Cui & Wang, 2017). PNRSV was successfully eliminated in cherry rootstock with the development of transgenic cherry through PNRSV-hpRNA, which is based on the development of a construct with a part of the PNRSV RNA3 genome sequence (Singh et al., 2019). Furthermore, it was observed that non-transgenic scion benefit from the rootstock resistance to PNRSV on grafted transgenic cherry, thus suggesting resistance transfer (Zhao & Song, 2014). However, in Europe regulation of GMOs is still challenging and legal problems in obtaining permission to grow and commercialise transgenic fruit still arise (Rubio et al., 2017).

3.11.4 small RNA and HTS in virus identification

In the last years, RNAi mechanism has been used directly to identify viruses. The natural antiviral defence system targets exogenous RNA and process double stranded RNA into 21-24 nucleotide of small RNA, where those can be extracted from plant tissues and sequenced. Sequences can then be reassembled into contigs, as partial or full viral genomes *in silico*, which allows the identification by comparing prepared contigs to known viral sequences available in the database (Santala & Valkonen, 2018). High throughput sequencing (HTS) has been applied in plants for the first time by Kreuze and colleagues (2009), where it was described the use of small RNA-based detection for plant viruses. This method has been developed with the objective of identifying viruses present in a plant host in extremely low titre, and gave the opportunity to open a new entire field of virology, combining bioinformatic and laboratory analysis with the application for diagnostic, discovery and sequencing of viruses. The method has been widely adopted and used for viral surveys in cultivated and wild plants, which allows the detection of different and unrelated viruses simultaneously in a single assay, without the use of probes or other additional elements (Bi et al., 2012; Kashif et al., 2012; Massart et al., 2014). In recent years, the cost of sequencing has decreased significantly, enabling more laboratories to use HTS as a powerful tool in the laboratory (Massart et al., 2014; Olmos et al., 2018). The standardization of bioinformatic protocols and comparison between pipelines made possible to develop a reliable and easy to share system between different laboratories (Massart et al., 2017; Tamisier et al., 2021). Furthermore, HTS offers good opportunities in routine screening, such as recognizing the pest status in a certain region through monitoring programs, certifying plant propagation material and nuclear stock, perform routine quarantine testing to prevent the introduction of unwanted pests into a new country and monitoring imported plant material for new potential risks (Olmos et al., 2018).

4 MATERIALS AND METHODS

4.1 Plant material for genetic analysis

A total of 31 sour cherry landraces from the germplasm collection located in Érd, Elvira major in the gene bank collection have been selected. Fruit phenotyping was carried out for three consecutive years from 2021 to 2023 for the main characteristics related to fruit size, color and breeding characteristics (Figure 5 and Figure 6). The living collection presents from 2 to 4 clones of the same accession, the plants are generally grown on a rootstock (*P. mahaleb* L.) and lie in the open field without irrigation (Table 1).

Table 1 Sour cherry accessions selected in the Fruit Growing Research Institute, gene bank collection. Species are indicated as well as known accession name, origin, and parental information. Asterisk indicated commercially grown cultivars.

Species	***Accession***	***Origin***	***Parents/Pedigree***
Sour cherry	Bagi Meggy	Carpathian Basin	Landrace
Sour cherry	Bosnyák	Carpathian Basin	Landrace
Sour cherry	Cigány Késői	Carpathian Basin	Landrace
Sour cherry	Cigánymeggy 7*	Carpathian Basin	Landrace
Sour cherry	Dunabogdányi	Carpathian Basin	Landrace
Sour cherry	Édes Pipacs	Carpathian Basin	Landrace
Sour cherry	Érdi Bőtermő*	Hungary	Pándy x Nagy angol
Sour cherry	Érdi Jubileum*	Hungary	Pándy x Eugenia
Sour cherry	Fehérvári	Carpathian Basin	Landrace
Sour cherry	Fűzlevelű Kisszemű	Carpathian Basin	Landrace
Sour cherry	Helyi Sötét	Carpathian Basin	Landrace
Sour cherry	Hortenzia Királynője	France	Landrace (also called Königin Hortense)
Sour cherry	Kántorjánosi 3*	Hungary	Landrace
Sour cherry	Késői Cigány	Carpathian Basin	Landrace
Sour cherry	Késői parasztmeggy	Carpathian Basin	Landrace
Sour cherry	Későn virágzó	Carpathian Basin	Landrace
Sour cherry	Korai Cigány	Carpathian Basin	Landrace
Sour cherry	Korai Pándy	Carpathian Basin	Landrace
Sour cherry	Májusi hólyag	Carpathian Basin	Landrace
Sour cherry	Mogyoródi kései	Carpathian Basin	Landrace
Sour cherry	Nagy Gobet	France	Landrace (also called Grosse Gobet)
Sour cherry	Pándy 279*	Carpathian Basin	Landrace
Sour cherry	Pándy 43*	Carpathian Basin	Landrace
Sour cherry	Pándy Bb. 119*	Carpathian Basin	Landrace
Sour cherry	Péceli nagy	Carpathian Basin	Landrace
Sour cherry	Pipacs1*	Carpathian Basin	Landrace
Sour cherry	Szamosi meggy	Carpathian Basin	Landrace
Sour cherry	Tiszabög 50/7	Carpathian Basin	Landrace
Sour cherry	Újfehértói fürtös*	Újfehértó, Hungary	Landrace
Sour cherry	Velencei kései	Carpathian Basin	Landrace

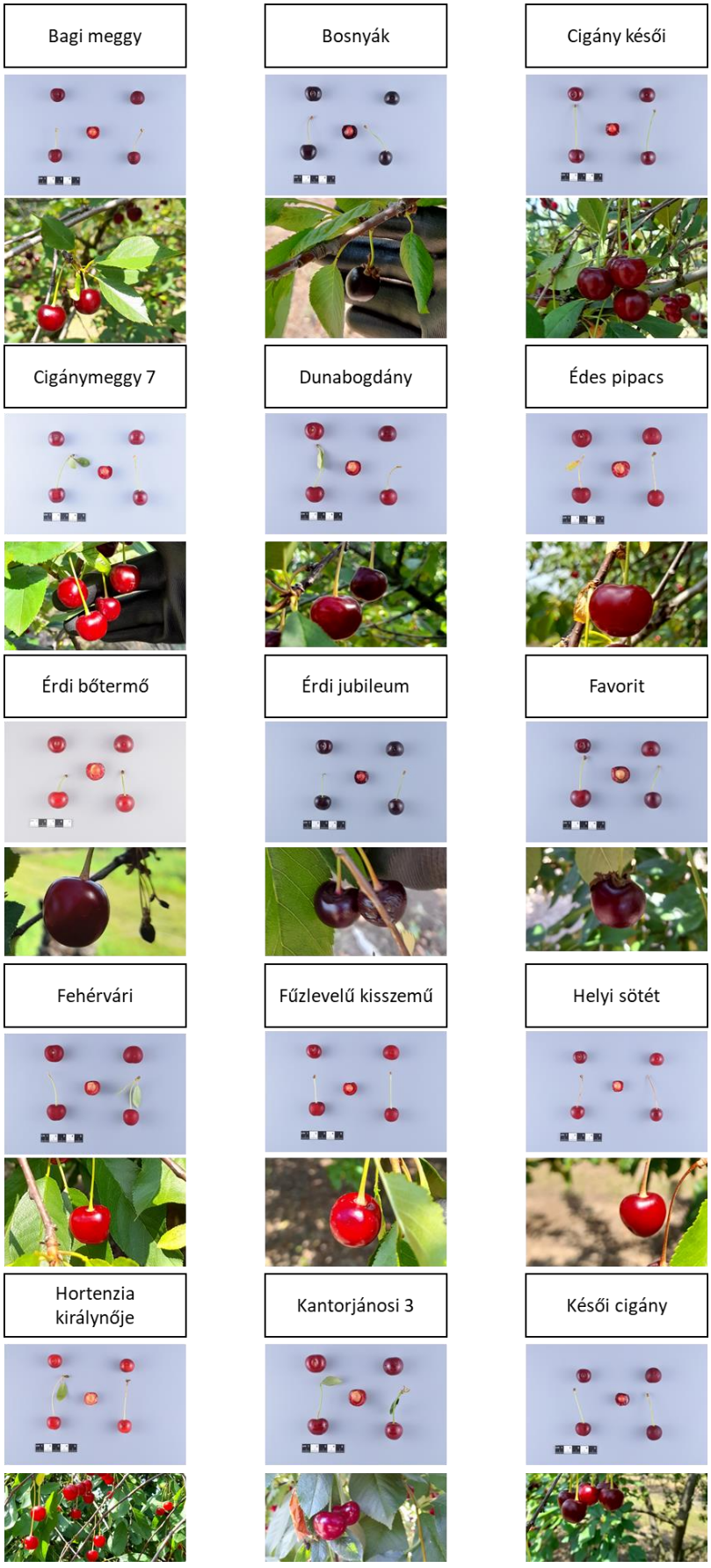


Figure 5 The 31 sour cherry selected accessions from the germplasm and reference cultivars analysed in this research. Picture on top: fruit laboratory. Picture below: open field

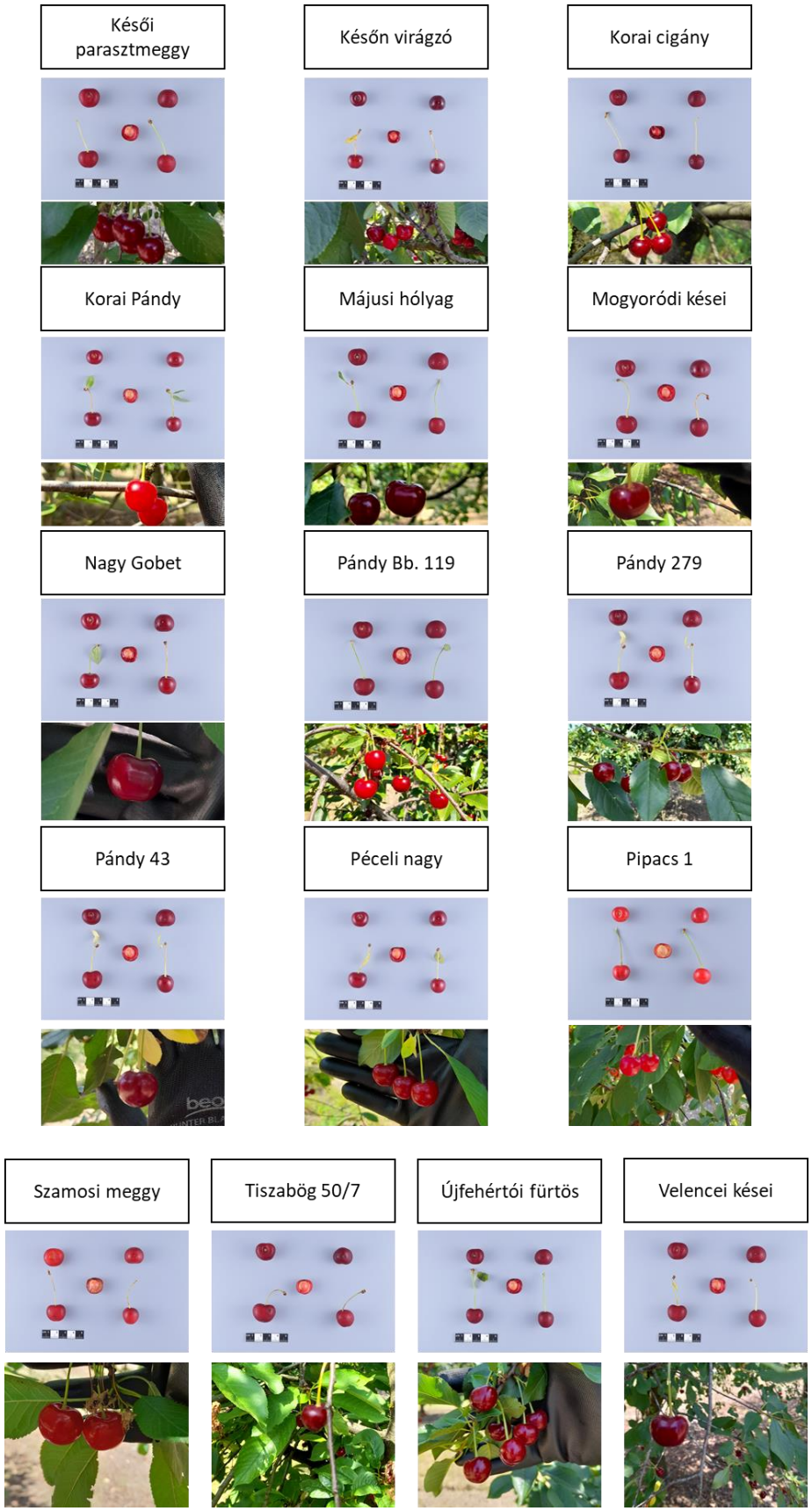


Figure 6 The 31 sour cherry selected accessions from the germplasm and reference cultivars analysed in this research. Picture on top: fruit laboratory. Picture below: open field

4.2 Fruit morphology and phenotyping

Several characteristics were measured for each stone fruit, in different years. For this reason, we indicate in detail each characteristic, to state clearly how the phenotypical analysis was conducted. Fruit characteristics were selected for sour cherry, where fruit size characteristics such as diameter, length, thickness, and weight were measured as well as the surface area of the fruit. The fruit shape of sour cherry was also analysed, skin color, flesh color, juice color, fruit firmness, acidity, sweetness, juiciness, and stone shape.

4.2.1 Measured characteristics of sour cherry

On average, appr. 500 g of fresh fruits were collected from 2 to 4 clone trees in the germplasm collection, depending on the availability and yearly production of each accession between June and July, period of maturation of sour cherry fruits. Then, 20 healthy fruits were selected and analysed for phenotyping for each accession for three consecutive years in 2021, 2022 and 2023 (n = 60). Fruit size characteristics such as diameter (ø), length, thickness, (mm), and weight (g) were measured with a digital calliper (Digital ABS Calliper, Mitutoyo Inc., Kawasaki, Japan) (Figure 7) and a scientific scale (Kern & Sohn GmbH, Albstadt, Germany), where pulp weight was measured as the difference between fruit and stone weight (g). To measure the surface area of the fruit, the three dimensions of fruit size were used. Ellipsoid area, also known as surface area (SA) was calculated with the formula $SA = 4 * \pi(\frac{a^p * b^p + a^p * c^p + b^p * c^p}{3})^{1/p}$ where a, b and c represent respectively the diameter, length and thickness of the fruit, p ≈ 1.6075 represents the relative error in the approximation of the spheroid (Thomsen, 2004). Fruit firmness was measured with a durometer (Hardness tester flat tip, T.R. Turioni s.r.l., Forlí, Italy). Soluble solid content (SSC) was measured with a refractometer (HI-96801, Hannah Instrument Ltd., UK). Measured characteristics recorded for three consecutive years were repeated 20 times each year for each sour cherry individual accession. Measured characteristics such as fruit diameter, length, thickness (Figure 6) each were measured with a calliper (mm), while fruit weight, seed weight and pulp weight were measured with a scientific scale (<0.01g). For the sour cherry plant material, the morphological characteristics were measured with the standard Union for the Protection of New Varieties of Plants (UPOV) scaling system, guidelines for sour cherry (CPVO-TP/230/1). Fruit characteristics such as fruit shape were indicated as reniform (1), oblate (2), circular (3) and elliptic (4), pistil end as pointed (1), flat (2) or depressed (3), skin color was indicated from orange-red (1) to blackish (6), flesh color from yellowish (1) to dark red (6), juice color from colorless (1) to dark red (5), fruit firmness from soft (1) to firm (7), acidity from very low (1) to very high (9), acidity from very low (1) to very high (9) sweetness from low (3) to high (7), juiciness from weak (1) to strong (7), and stone shape from narrow elliptic (1) to circular (3) were measured (Table 2).

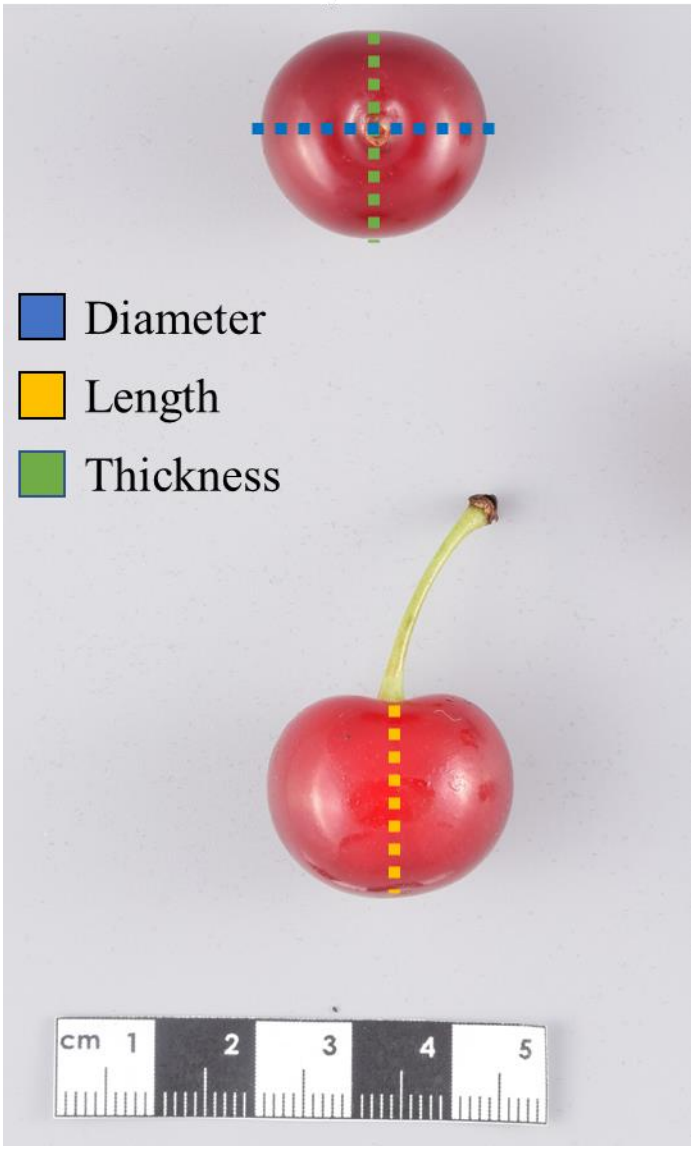


Figure 7 Sour cherry measurements for fruit size, in blue the diameter (mm), in green is indicated the thickness (mm), and in yellow the fruit length (mm). All measurements were performed with a standard calliper.

Table 2 Measured characteristics in sour cherry. Sources indicate the UPOV standard guideline used. Fruit characteristics are measured for fruit shape (1 to 4), pistil end (1 to 3), skin color (1 to 6), flesh color (1 to 4), juice color (1 to 5), firmness (1 to 7), acidity 1 to 9), sweetness (3 to 7), juiciness (1 to 7) and stone shape (1 to 3).

Specie	Source	Shape	Pistil end	Skin color	Flesh color	Juice color	Firmness	Acidity	Sweetness	Juiciness	Stone shape
Sour Cherry	UPOV CPVO-TP/230/1	reniform (1) oblate (2) circular (3) elliptic (4)	pointed (1) flat (2) depressed (3)	orange red (1) light red (2) medium red (3) dark red (4) brown red (5) blackish (6)	yellowish (1) pink (2) medium red (3) dark red (4)	colorless (1) light yellow (2) pink (3) medium red (4) dark red (5)	soft (1) medium (5) firm (7)	very low (1) low (3) medium (5) high (7) very high (9)	low (3) medium (5) high (7)	weak (1) medium (5) strong (7)	narrow elliptic (1) broad elliptic (2) circular (3)

4.2.2 Sour cherry colorimetric phenotyping

Fruit color was measured for three consecutive years from 2021 to 2023 with the Ctifl (Centre Technique Interprofessionnel des Légumes, Rungis, France) scaling system from light pink (1) to black (7), CIELab standard scaling system measured with a spectrophotometer CM-600d (Konica Minolta Inc., Osaka, Japan), using a D65 illuminant set and at 10° observer angle. L measures the lightness from 0 (black) to 100 (white), a measure the red (+a) to green (-a) spectrum and b measures the yellow (+b) to blue (-b). Chroma ($C = a^2 + b^2$) indicates the saturation or intensity of color (Pedisić et al., 2009) and a modified hue angle ($h^{\pm 33^\circ}$) indicates the amount of redness to yellowness [$H^\circ = \arctan(b/a)$], where 0° to 360° define colors from red to magenta, 90° to 180 °from yellow to green, and 180° to 270° defines blue. The modified hue angle was calculated with Color Conversion Centre 4.1 (http://ccc.orgfree.com/, accessed on 23rd May 2024). In total, ten fruits were measured for each accession, with 50 repeated measurements for each fruit, covering the surface in different measurement points for each individual cultivar (n = 500). Measured samples were then kept at -20 °C for further chemical analysis.

4.2.3 Chemical analysis of sour cherry

Sour cherry total polyphenolic content (TPC) was examined with the Folin-Ciocalteu colorimetric method according to Singleton and Rossi (1965) with some modifications. The samples (5 g) were mixed with 96% acidulate ethanol solution (30 mL) in a test tube. For the phenolic extraction, an ultrasonic water bath (EVO Sonic Ultrasonic Bath-POKA) was used for 30 min. After centrifuging the extracts for 20 min, liquid samples were passed through a 0.45uL filter. The extracts (1 mL), Folin-Ciocalteu reagent (5 mL), and distilled water (0.7 mL) were well combined in a test tube. After a 5-minute incubation at 25 °C, 5 mL of sodium carbonate solution (7.5%) was added. Mixtures were incubated in a dark room for 2 h at 25 °C. The absorbances were measured at 750 nm by a UV-VIS spectrophotometer (PerkinElmer Lambda 850+, Milan, Italy). The tests were performed in triplicates, where the output was given as the average value of these measurements. Gallic acid (GA) was used to draw the calibration curve and results were expressed for 1g of dried sample as the mg GA equivalent (GAE).

4.3 DNA extraction and SSR markers selection.

Selected germplasm material DNA was extracted from freshly collected young leaves. Around 100 mg of fresh plant tissue was used as starting material to extract total genomic DNA using the Plant Genomic DNA extraction miniprep system (Viogene, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out using the DreamTaq DNA polymerase (Thermo Scientific, USA) in 1x reaction buffer, optimizing the reaction as follows: 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.25 µM of each primer and 1ng of genomic DNA in a total volume of 25 µL. PCR conditions were optimized according to the manufacturer's protocol as follows: denaturation at 95 °C for 3 min followed by 35 cycles of 95 °C 30s, annealing temperature according to optimal primers temperature (Table 3) for 30s, 72 °C 15s; and a final extension at 72 °C for 3 min. To determine the exact size of the SSR fragment, forward primers were labelled with 5' 6-FAM fluorescent dye to detect the amplified fragment by capillary electrophoresis. The fluorescently labelled PCR products were analysed with an automated sequencer ABI prism 3100 Genetic Analyzer (Applied Biosystem, Budapest, Hungary). Allele size and evaluation were performed using the Thermo Fisher App, Peak Scanner (Thermo Fisher Scientific, USA) subsequently the haplotypes and genotypes of the accessions were determined.

Table 3 List of markers used in this study. Agronomical trait was indicated from previous literature as well as linkage group relationships. Forward markers were added to a probe/fluorescent dye for fragment (5' 6-FAM)

Primer name	Sequence (5'-3')	Trait	Group	Ta° C	product size (bp)	Reference
CPSCT038_F	CAGGAACCCTATTCCCACAA	Fruit size/weight (cherry)	LG2	54	200	(Mnejja et al., 2004)
CPSCT038_R	TCAATGGCACCCATTTTACA					
Ma039a-F	AGAAAGGCACTTTATCTAGG	Skin color (cherry)	LG3	50	188	(Sooriyapathirana et al., 2010)
Ma039a-R	TTTGTTTTGGGGATGGTAGT					
Pav-Rf-SSR_F	ATGCTGCATTGTGAAAGTGG	Skin color (cherry)	LG3	55	350	(Cmejla et al., 2021)
Pav-Rf-SSR_R	GGTGTCTACCCCAGTTAAAAACG					
LG3_13.146_F	ATGTGGCCAAAGGTCAGC	Flesh color (cherry)	LG3	55	218-220	(Stegmeir et al., 2015)
LG3_13.146_R	TGATCCCAATCACGTTTTCC					
BPPCT034_F	CTACCTGAAATAAGCAGAGCCAT	Fruit size/weight (cherry)	LG2	56	228	(Dirlewanger et al., 2002)
BPPCT034_R	CAATGGAGAATGGGGTGC					

4.4 Statistical analysis

Fruit characteristic data of 3 consecutive years were analysed between 2021, 2022 and 2023 for sour cherry. Landraces were classified according to their determined genotypes and checked whether the genotype differences resulted in fruit weight or color variation by analysis of variance (ANOVA) according to each stone fruit analysed and their phenotype to be divided in groups.

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

Physical characteristics for fruit weight, firmness, secondary characteristics (acidity, sweetness, juiciness), UPOV breeding scale values (skin color, flesh color and juice color), chemical components (soluble solid content, total polyphenolic content), CIELab values were correlated with a bivariate correlation matrix where Spearman"s rho model was used and significance level was set at a threshold of $p < 0.05$. Furthermore, to understand the underlying variation between single characteristics, a principal component analysis (PCA) was performed.

4.4.2 Phenotype and genotype analysis

Statistical analysis was conducted with SPSS, (IBM®, USA) where ANOVA was performed with Tukey's b test using a threshold of significance set at $p < 0.05$. The SSR reproducible fragments were classified as present (1) or absent (0) and typed as binary matrix one for each molecular marker and single alleles. The yearly phenotype data were inserted individually for as a unique value. The matrices were analysed on SPSS, where linear regression was performed to analyse the influence of single alleles with $p < 0.05$. Furthermore, to understand the correlation between phenotype and genotype of each single allele Pearson's correlation was performed with a threshold of significance set at $p < 0.05$. Linear regression was carried out between phenotype and single alleles, to identify the influence of each single allele, where the threshold of significance was set at $p < 0.05$. Boxplot data visualization for individual markers was performed in R studio with a script written for this analysis (Appendix A4).

4.4.3 SSR markers frequency analysis

Frequency analysis was performed in R studio with a script written for this analysis (Appendix A4). In the script, data were transformed into vectors for alleles analysis. After transformation, allele frequencies, expected heterozygosis (H_E), polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI) heterozygosity for polymorphic markers (H_o), resolving power (RP) and discriminating power (DP) (McDonald, 2008). Afterwards, a table was produced where the single SSR markers frequency was analysed for each stone fruit present in this study.

4.5 Sour cherry virus analysis

4.5.1 Plant material for viral analysis

Hungarian sour cherry (*Prunus cerasus* L.) varieties were investigated for the presence of possible harmful pathogens. Leaf samples of different Hungarian cultivars of sour cherry were collected in 2021 and 2023. In 2021, a total of 31 individual varieties were collected in Hungary, at the Fruit Growing Research stations of Érd, Elvira major. From the newly bred and standard variety cultivars (tested as a member of the pool 1_PC_E1, 2_PC_E2 and 3_PC_E3) only one representant of the existing 2 or 4 trees were sampled in 2021 and were investigated by HTS (Table 4). The test was repeated as an RT-PCR based survey in 2023, sampling all the trees alive at the time from this population and 58 sour cherry samples were thus collected in Érd and screened for viral presence and distribution in the open field.

Table 4 Testing of the 2021 sour cherry open field. Individual trees were tested with RT-PCR and subsequently in 2023 all the tree that were found alive.

Species	Place and year of sampling	Position of the tree in the open field		Variety	sRNA HTS library
		row	tree		
P. cerasus	Érd, open field, 2021	1	9	Késői parasztmeggy	1_PC_E1
		2	23	Helyi sötét	
		2	37	Késői Cigány	
		2	59	Édes pipacs	
		3	5	Fehérvári	
		3	29	Korai Cigány	
		3	49	Fűzlevelű kisszemű	
		3	51	Velencei kései	
		3	57	Mogyoródi kései	
		4	1	Májusi hólyag	
		4	25	Dunabogdányi	
		4	31	Későn virágzó	2_PC_E2
		5	1	Cigány késői	
		5	31	Korai Pándy	
		5	33	Bagi meggy	
		5	41	Péceli nagy	
		5	51	Tiszabög 50/7	
		6	17	Szamosi meggy	
		6	34	Nagy Gobet	
		6	49	Bosnyák	
		7	3	Hortenzia királynője	
		7	7	Pándy 43	
		19	13	Favorit	3_PC_E3
		19	18	Érdi Jubileum	
		20	9	Újfehértói fürtös	
		20	21	Cigánymeggy 7	
		21	6	Kantorjanosi 3	
		21	13	Pándy 279	
		21	17	Pándy Bb. 119	
		22	1	Pipacs1	
		13	9	Érdi bőtermő	

4.5.2 Samples preparation for HTS and RT-PCR

The samples for HTS analysis were divided into three groups according to the sampling (Table 4). The three groups contained samples of sour cherries: 22 cultivars of new breeds and 9 Hungarian standard cultivars, collected for the first time in 2021. The same samples were then collected again in Érd 2023 and were directly tested for RT-PCR. Four leaves were collected twice from each tree, to ensure the detection of all, possibly unevenly distributed viruses. After sample collection, leaves were stored in a -80°C freezer to avoid RNA degradation.

4.5.3 RNA extraction

RNA was extracted by combining materials of four leaves originating from one tree using the modified CTAB method (Gambino et al., 2008), where samples collected were weighted (150-200 mg each), submerged in liquid nitrogen and homogenized with mortar and pestle. Plant material was added to the 900 µL extraction buffer (EB) (4M guanidine isothiocyanate, 0.2 M sodium acetate pH 5.2, 25 mM EDTA, 1 M potassium acetate, 2,5 % PVP-40, 2% sarkosyl and) heated at 65°C and 17 µL of β-mercaptoethanol (1%) was previously added to it. Samples were incubated for 10 min at 65°C in a water bath and vortexed 3-5 times. Chloroform: isoamyl alcohol (24:1 v/v) was added to the samples and tubes were inverted a few times. Samples were then centrifuged at 10.000 rpm for 15 min at 4°C. The supernatant was transferred to new tubes and samples were centrifuged at 10.000 rpm for 10 minutes at 4°C. The upper phase was transferred to new tubes and 250 µL 9M LiCl (3M final concentration) was added. Tubes were inverted a few times and incubated on ice for 30 min. Samples were centrifuged at 13.000 rpm for 25 min at 4°C and the supernatant was removed. The pellet was then resuspended in 450 µL of SSTE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 1% SDS, 1 M NaCl) preheated at 65°C and the chloroform: isoamyl alcohol was added in the same volume. Samples were centrifuged at 10.000 rpm for 10 min at 4°C and afterwards, 30 µL of 4M NaAc and 280 µL of isopropanol were added to the mix and incubated for 5-10 min at room temperature. Samples were centrifuged at 13.000 rpm for 20 min at 4°C and afterwards, supernatant was removed. The pellet was washed with 1 mL 70% cold ethanol and samples were centrifuged at 14.500 rpm for 5 min at 4°C. The supernatant was removed and samples were dried for 10 min. The pellet was resuspended in 25 µL of sterile water and vortexed gently. Subsequently, each sample was quantified using Nanodrop and gel electrophoresis.

4.5.4 Small RNA library preparation and sequencing

Total RNA extracted previously was used for the small RNA library preparation. Samples were divided into three different libraries. RNA pools for small RNA library preparation were prepared by mixing equal amounts of RNA originating from different individuals from different cultivars. These pools were used for sRNA library preparation using the TruSeq Small RNA Library Preparation Kit (Illumina, USA) and our in-house protocol. The pooled RNA was purified to small RNA (sRNA) fraction from the total RNA following protocol from Jaksa-Czotter (2024). Between 10-30 µg of extracted total RNA was denatured at 65°C for 20 min and subsequently transferred on ice and spun down briefly. Samples were then loaded on an 8% TBE denaturing polyacrylamide gel containing urea, run at 100 V for 1-1.15 h. Subsequently, the gel was stained in 60 mL of 1xTBE containing 3 µl of ethidium bromide (EthBr) and visualized on a UV transilluminator. The small RNA was then excised under UV light with a sterile blade and carefully inserted in a punctured 0.5 mL microcentrifuge tube and then into a 2 mL microcentrifuge tube. The tube was centrifuged at room temperature for 2 min. Subsequently, the 0.5mL tube was removed and 350 µL of sterile 0.3 M NaCl was added to the gel debris. The tube was shaken overnight at 4°C to elute RNAs. The next day the eluate and

gel debris were transferred to a Spin X cellulose acetate filter tube and centrifuged at 12.000 revolutions per minute (RPM) for 2 min twice to ensure filtration. Afterwards, the Spin X is discarded and an equal volume of 100% isopropanol and 1 µL of GlycoBlue is added to the eluate. Samples were incubated at -70°C for at least 2-2.5h. The precipitated RNA was then centrifuged at full speed at 4°C for 20 min and the supernatant was carefully discarded. The pellet was subsequently washed with 1 mL of 70% cold ethanol twice. The pellet was then dried for 3-5 min at room temperature and resuspended in 12 µL MilliQ pure water. Here, the adapter ligation step followed as indicated. A total of 2.5 µL of small RNA was pipetted into a sterile PCR tube on ice and 0.5 µL RNA 3' adapter was added. Samples were denatured at 70°C for 2 min and immediately transferred into ice. 1 µL of ligation buffer (HML), 0.5 µL of RNase inhibitor and 0.5 µL T4 RNA ligase 2 (truncated) were added to a sterile PCR tube on ice and centrifuged briefly. A total of 2 µL of reaction were added and incubated at 28°C for 1 h. A 0.5 µL of ice-cold stop solution (STP) was added and incubation continued at 28°C for an additional 15 min where afterwards, tubes were placed on ice. A 0.5 µL of RNA 5' adapter (RA5) was pipetted into a sterile PCR tube on ice and subsequently incubated at 70°C for 2 min and transferred on ice. 0.5 µL 10 mM ATP and 0.5 µL T4 RNA ligase were added to the tube. The mix was added to the 3' adapter reaction and the total volume was incubated at 28°C for 1 h and then placed on ice. Here, reverse transcription is described. 1 µL of RT Primer (RTP) was added to the 3'-5' adapter-ligated reaction and placed at 70°C for 2 min and subsequently on ice. PCR reaction mixture was prepared with 1 µL ultrapure water, 2 µL 5x reaction buffer, 0.5 µL 12.5 mM dNTPs mixed with 1 µL of RNase inhibitor and 1 µL Revert Aid H-reverse transcriptase into a sterile PCR tube on ice. 5.5 µL of the RT-PCR reaction mixture was added to the previously prepared 3'-5' adapter-ligated mix and the RT-PCR reaction was incubated at 50°C for 1 h and the cDNA was placed on ice. The prepared libraries were purified from polyacrilamid gel and were sequenced on a HiScan2000 by UD GenoMed (Debrecen, Hungary) 50 bp long and, single-end reading. Fastq files of the sequenced libraries were deposited to the NCBI GEO database and can be accessed through the series accession number (GSE233558).

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

For the bioinformatic analysis, CLC Genomic Workbench (QUIAGEN®, NL) was used. The sequenced reads were trimmed and their quality was checked. From the good quality reads longer contigs were built *de novo*, using a CLC assembler (*de novo* assembly) with default options: word size 20, bubble size 50, and simple contig sequences with a minimum of 35nt length. To determine the presence of known viruses, we used two strategies as described previously (Barath et al., 2022), where longer contigs were built from non-redundant reads and the resulting contigs were annotated using the reference genomes of the plant-hosted viruses (downloaded from NCBI GenBank 16.03.2023) and to currently identified *Prunus* infecting viruses having no reference genomes (Hou et al., 2020). The contig annotation was performed with the BLASTN algorithm, with default options (thread 1, word size 11, match 2 mismatch 3, gap cost existence 5, and extension 2). In case of the presence of at least one virus-specific contig, the trimmed reads were directly mapped to the viral reference genomes and counted with and without redundancy (using the map to the reference command and enabling one mismatch). The number of normalized reads (read/1 million reads: RPM) was subsequently calculated from the mapped redundant reads and the number of total sequenced reads. Coverage (%) of the viral genome was calculated on the consensus sequence generated from this mapping. The viral presence was confirmed if at least two parameters of the three investigated ones were

present: (1) the presence of any viral-specific contigs, (2) the number of normalized redundant viral reads was >200, or (3) the viral genome coverage was >60%.

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

To validate the results of the bioinformatic analysis, RT-PCR was carried out with virus-specific primers. cDNA was synthesized from RNAs representing each library and each individual tree using random primers and RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) and Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA), following manufacturer's instructions. For the surveys, we used the cDNA obtained using the Revertaid cDNA kit (Thermo Fisher Scientific, USA). To amplify and Sanger sequence longer part of viral genome, cDNA obtained using Maxima First Strand cDNA Synthesis Kit was used and amplified using Q5 Polymerase (New England Biolabs) due to its proofreading superior quality. For the amplification we used previously published primers or primers, which were designed based on the sequenced sRNA reads (Table 5, Appendix A2). In case of PrVF, PNRSV and CVA the amplified products were purified from gel using NucleoSpin Gel and PCR Clean-up kit (Marcherey Nagel, DE). The purified product was cloned into a pJET vector System (Thermo Fisher Scientific, USA) and sent for Sanger sequencing. Sequences were deposited into GenBank (GenBank accession numbers: OR596712-OR596738). After identification through HTS, the validation process continues with primer testing. Primers previously designed for PrVF RNA1 called PrVF_CAF/CAR (James et al., 2018) and PrVF RNA2 Fabr2_1808_F/_2546_R (Villamor et al., 2017) were tested, amplifying a fragment of 462 and 738 bp respectively. Samples which were amplifying PrVF RNA1 and RNA2 were then sent for Sanger sequencing. BLASTn analysis confirmed that samples were infected with PrVF RNA1 and PrVF RNA2. New primers were designed according to the consensus sequences of the single libraries. Consensus sequences were based on PrVF RNA1 (NC_039077.1) and PrVF RNA2 (NC_039078.1) reference genomes, where each library was mapped to the reference genome and consensus was extracted. From those consensus-based files, we aligned with multiple alignments (Geneious® version 2023.0.1, NZ) and designed new primers accordingly.

4.5.7 Phylogenetic analysis

To better understand the relationship and variance of PNRSV RNA3, CVA and PrVF RNA1 and RNA2 a phylogenetic tree analysis was performed using Geneious. Alignment of the sequences was done using Geneious built-in function Geneious Alignment; alignment type global alignment with free end gaps, cost matrix 65% similarity, gap open penalty 12, gap extension penalty 3, refinement iteration 2. The reference sequences were used for PNRSV RNA3 (NC004364), CVA (NC_003689) PrVF RNA1 (NC_039077.1, MK834285, KX216775, and KX216779) and RNA2 (NC_039078.1, MK834286, KX269869, KX216779, MH998215). Obtained sequences from Sanger analysis were used for each virus analysed, and different phylogenetic trees were prepared, for each virus separately.

5 RESULTS AND DISCUSSION

5.1.1 Sour cherry size

For three consecutive years (2020, 2021, 2022) sour cherry trees were analysed. The smallest diameter between sour cherry fruits was recorded for 'Helyi sötét' (14.4 mm), while the biggest was 'Mogyoródi kései' (22.4 mm). The smallest length was of 'Helyi sötét' (12.4 mm), the biggest was Tiszabög 50/7 (). The least thick was 'Helyi sötét' (12 mm) while the thickest was 'Mogyoródi kései' (18.6 mm). The overall ellipsoid area/surface area was the smallest for 'Helyi sötét' (10.5 cm^3) and the largest fruit was of 'Mogyoródi kései' (25.15 cm^3). Fruit weight varied from the lightest, recorded for 'Helyi sötét' (2.31 g) to the heaviest 'Mogyoródi kései' (6.93 g). Seed weight was lightest in 'Helyi sötét' (0.2 g) to the heaviest in Pipacs 1 (0.5 g) and pulp weight varied from the lightest in 'Helyi sötét' (1 g) to the heaviest in 'Mogyoródi kései' (3.2 g) (Table 6, Appendix A2).

5.1.2 Firmness in sour cherry

Fruit firmness was measured for three consecutive years according to the UPOV scaling system and it varied from very soft accession 'Bosnyák' (3) to very hard accession 'Pipacs 1' (7). Furthermore, firmness was measured in parallel with a with a durometer to measure possible differences between the breeding standard scale system and the durometer data, and the firmness value was observed to be the lowest in 'Favorit' (19.8) while the firmest accession was determined to be 'Pipacs 1' (62.8).

5.1.3 Soluble solid content in sour cherry

Soluble solid content (SSC) was the lowest in 'Favorit' (16.3) and the highest score in 'Bagi meggy' (24.9). Fruit acidity varied from high acidity in 'Pipacs 1' (8) to low acidity in 'Favorit' (3). Sweetness was recorded from very sweet 'Érdi Jubileum' (7) to non-sweet 'Pipacs 1'. Juiciness was recorded as very juicy 'Szamosi meggy' (7) to non-juicy 'Bagi meggy' (3). Stone shape varied between 'Bosnyák' (1) and 'Cigány késői' (3). Fruit shape in ventral view varied from 3.5 in 'Bosnyák' to 1 in several accessions. Fruit pistil end varied from 1 in 'Érdi Jubileum' to 3 in 'Bagi meggy'.

5.1.4 Fruit color in sour cherry

Fruit color was also measured for each accession, where skin color (UPOV scale) scored lightest for 'Pipacs 1' (1) to darkest 'Érdi Jubileum' (6). CIELab scale system was also scored, where L score was the highest recorded in 'Hortenyia királynője' (33.4) and the darkest to 'Bosnyák' (24.7), a was highest in 'Hortenzia királynője' (31.9) and lowest in 'Bosnyák' (6.6), b was lowest in 'Bosnyák' (1.7) and highest in 'Hortenzia királynője' (15.8), Chroma was lowest in 'Bosnyák' (6.9) and highest in 'Hortenzia királynője' (35.7), hue was lowest in 'Bosnyák' (2.2) and highest in 'Hortenzia királynője' (19.3). Ctifl scale varied from very dark Bosnyák (6.5) to very pale 'Hortenzia királynője' (2.5). Flesh color was recorded as very dark for 'Bosnyák' (4) and very pale for 'Hortenzia királynője' (1). Juice color scored from lightest from 'Hortenzia királynője' (1) to very dark from 'Bosnyák' (5).

5.1.5 TPC content in sour cherry

Total polyphenolic content (TPC) was compared with CIELab values for each sour cherry accession in 2022. Our results indicated that accessions with the lowest TPC content was found in 'Kantorjanosi 3' (122.76 mgGAE/100g fresh cherries) while the cultivar 'Pipacs 1' had instead the highest TPC value (650.57 mgGAE/100g fresh cherries). Moreover, 'Pipacs 1' was

the accession with the highest a value (26.45) and Chroma (28.18) in 2022. ‘Hortenzia Királynője’ had the highest values for L, b and hue. Finally, ‘Bosnyák’ was the accession with the lowest L, a, b, Chroma, and hue of all the sour cherry analysed (Table 7, Appendix A2).

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

To better understand the relationship between individual characteristics analysed, a bivariate analysis was performed between each component described above. It is possible to observe that colorimetric data such as L, a, b, Chroma and hue correlate positively with one another, as expected (Table 8). A negative correlation is observed between CIELab and Ctifl, due to the fact that the scale is inverted. TPC positively correlate with SSC, firmness, and acidity. On the other hand, TPC negatively correlates with sweetness and weight-related characteristics, particularly with fruit weight. Bivariate analysis correlated positively TPC with L (0.281, $p < 0.01$), a (0.296, $p < 0.01$), b (0.266, $p < 0.05$), and C (0.291, $p < 0.01$) while the hue value shows not significant correlation (0.196). SSC has a negative correlation with acidity (-0.121, $p < 0.01$), juiciness (-0.189, $p < 0.01$), and weight (-0.473, $p < 0.01$), while is positively correlated with colorimetric values, such as L (0.236, $p < 0.01$), a (0.125, $p < 0.01$), b (0.120, $p < 0.01$), Chroma (0.124, $p < 0.01$), hue (0.108, $p < 0.01$) and TPC (0.294, $p < 0.01$).

Table 8 Bivariate correlation with Spearman's rho. Bold values indicate significant correlation. P value is considered significant at <0.05. Asterisk (*) indicate a significance level <0.05. Double asterisk (**) indicates significance level at <0.01.

Bivariate Correlations																			
		L	a	b	C	$h^{\pm 33°}$	TPC	UPOV Skin color	Ctifl scale	UPOV Flesh color	UPOV Juice color	Soluble solid content (SSC)	Firmness (d)	Acidity	Sweetness	Juiciness	Weight (g)	Seed weight (g)	Fruit pulp (g)
Spearman's rho	L	1.000																	
	a	**.694****	1.000																
	b	**.709****	**.992****	1.000															
	C	**.696****	**1.000****	**.993****	1.000														
	$h^{\pm 33°}$	**.718****	**.922****	**.960****	**.926****	1.000													
	TPC	**.281****	**.296****	**.266***	**.291****	0.196	1.000												
	UPOV Skin color	**-.322****	**-.493****	**-.502****	**-.494****	**-.495****	0.191	1.000											
	Ctifl scale	**-.314****	**-.518****	**-.524****	**-.519****	**-.507****	0.117	**.677****	1.000										
	UPOV Flesh color	**-.325****	**-.533****	**-.547****	**-.535****	**-.553****	0.143	**.802****	**.700****	1.000									
	UPOV Juice color	**-.322****	**-.542****	**-.552****	**-.544****	**-.546****	0.139	**.753****	**.666****	**.885****	1.000								
	Soluble solid content (SSC)	**.236****	**.125****	**.120****	**.124****	**.108****	**.294****	**.116****	**.213****	**.098***	0.033	1.000							
	Firmness (d)	0.054	**.244****	**.232****	**.243****	**.196****	**.444****	**-.255****	**-.275****	**-.312****	**-.242****	-0.023	1.000						
	Acidity	**.161****	**.208****	**.205****	.207**	**.195****	**.333****	**-.219****	**-.298****	**-.154****	**-.170****	**-.121****	**.347****	1.000					
	Sweetness	-0.012	0.000	0.026	0.004	0.066	**-.403****	**-.115****	**-.098***	0.005	0.028	0.062	**-.155****	-0.032	1.000				
	Juiciness	**.309****	**.397****	**.405****	**.398****	**.407****	-0.107	**-.605****	**-.559****	**-.625****	**-.445****	**-.189****	**.217****	**.280****	**.196****	1.000			
	Weight (g)	**-.219****	-0.068	-0.050	-0.066	-0.016	**-.409****	**-.404****	**-.277****	**-.312****	**-.281****	**-.473****	**.199****	**.297****	**.186****	**.267****	1.000		
	Seed weight (g)	-0.050	0.018	0.023	0.019	0.040	**-.232***	**-.343****	**-.277****	**-.250****	**-.220****	**-.270****	**.219****	**.396****	-0.032	**.227****	**.523****	1.000	
	Fruit pulp (g)	**-.229****	-0.078	-0.059	-0.076	-0.025	**-.409****	**-.386****	**-.262****	**-.300****	**-.268****	**-.473****	**.188****	**.280****	**.199****	**.261****	**.998****	**.467****	1.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Principal component analysis (PCA) was performed to better understand the underlying variation between the different characteristics analysed. The performed PCA indicated a positive correlation between L, a, b, Chroma, hue, and juiciness in the first component. The second component correlated TPC with L, a, b, Chroma and SSC positively (Table 9). In the Figure 8, it is possible to observe the different components clustering together, such as color physical parameters (Ctifl, UPOV flesh, skin and juice color), spectrophotometer measurements (L, a, b, Chroma, hue)

Table 9 Principal component analysis of color, physical characteristics flavour profile and fruit weight.

Principal Component Matrix		
Components	**PC1**	**PC2**
a	0.922	0.298
C	0.922	0.305
$h^{\pm 33°}$	0.914	0.092
b	0.905	0.333
UPOV Flesh color	-0.854	0.283
UPOV Skin color	-0.853	0.305
UPOV Juice color	-0.85	0.225
Ctifl scale	-0.808	0.277
L	0.791	0.432
Juiciness	0.644	-0.347
Weight (g)	0.1	-0.911
Fruit pulp (g)	0.095	-0.898
seed weight (g)	0.111	-0.545
Firmness (d)	0.256	-0.042
TPC	0.284	0.439
Acidity	0.292	-0.292
Sweetness	-0.21	-0.316
Soluble solid content (SSC)	-0.001	0.598

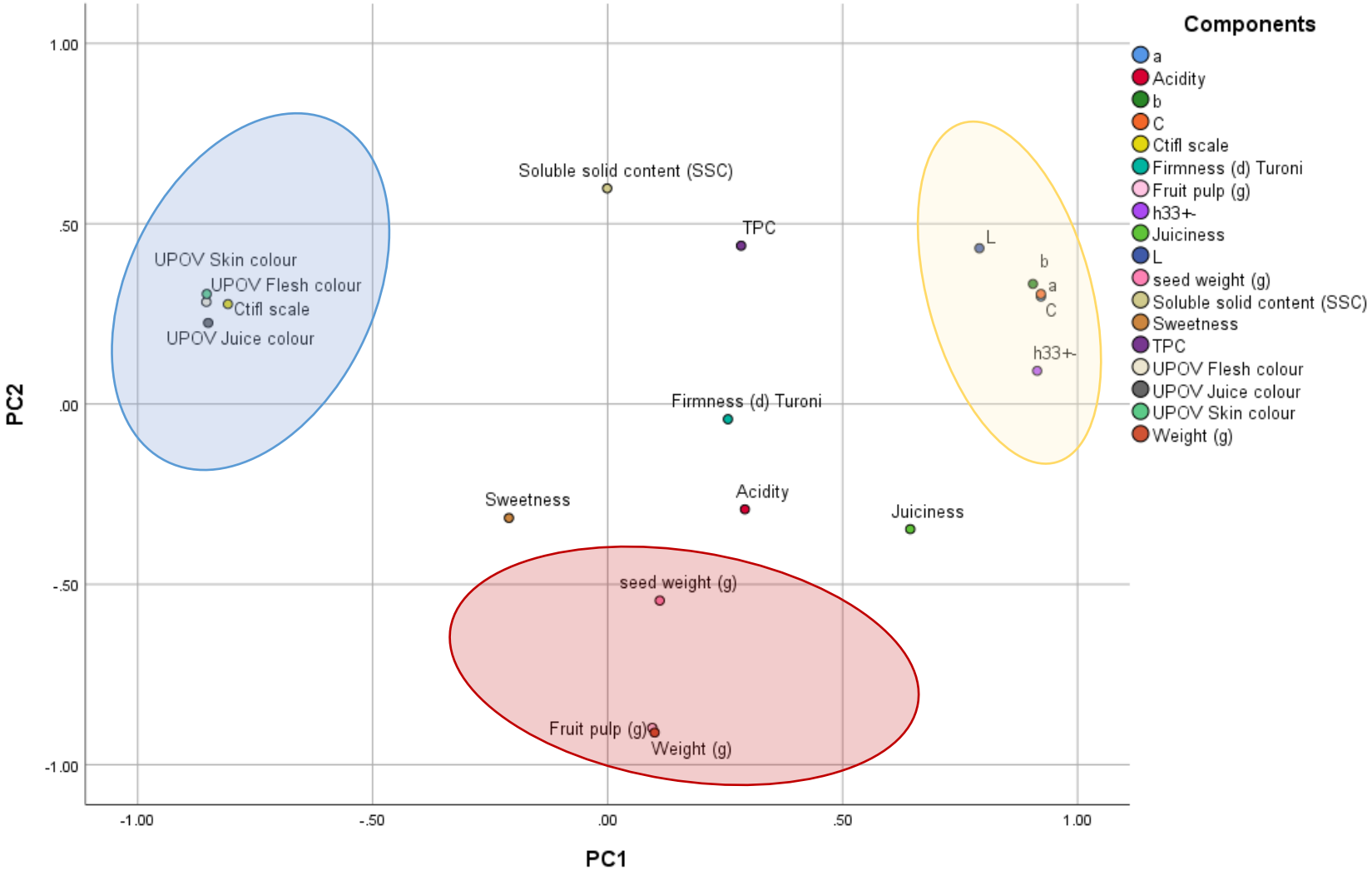


Figure 8 Principal component analysis performed on individual components measured in sour cherry. In blue is indicated the group of physical characteristics (Ctif and UPOV values), in red is indicated the group of weight characteristics (weight, pulp, seed weight) and in yellow is indicated the group containing CIELab values (L, a, b, Chroma, hue).

5.3 Results of genotyping data

5.3.1 Fruit size analysis

A total of 31 accessions of sour cherry were analysed with two SSR markers for fruit size, BPPCT034 and CPSCT038. The SSR markers size distribution varied between the two primers used. BPPCT034 size was observed between 204 and 251 bp. CPSCT038 was instead observed in allelic variations from 185 bp to 204 bp. Samples were analysed together and a total of 21 allelic combinations were observed. Of all the allelic combinations, 5 were shared by different accessions, determining five unique groups. 'Bagi meggy', 'Korai Cigány', 'Cigány kesői' were grouped together (208-208-226-230-190-190-190-190), 'Dunabogdányi', 'Májusi hólyag', 'Édes pipacs' had the same size (204-204-226-237-185-185-204-204), 'Késői parasztmeggy' and 'Velencei kései' (226-226-237-237-185-185-204-204), 'Szamosi meggy' and 'Tiszabög 50/7' (204-204-226-237-185-185-185-185) (Figure 9, Table 10 and Table 11, Appendix A2).

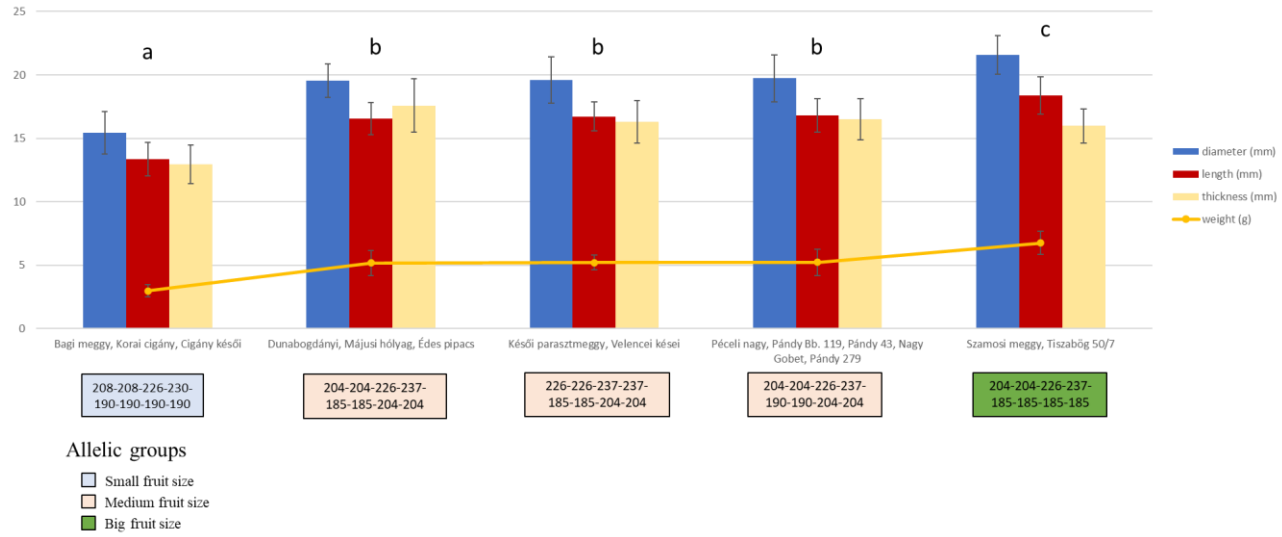


Figure 9 Sour cherry allelic groups based on BPPCT034 and CPSCT038 alleles. 5 groups were observed in total. Diameter is indicated in blue, length is indicated in red, thickness is indicated in light yellow. Weight is indicated in orange. Allelic group associated with small fruit size are indicated in light blue, medium fruit size association indicated in light orange while big fruit association is indicated in green.

5.3.1.1 Color analysis

SSR markers for fruit color were analysed as well. Ma039a was observed in a range of 157 bp to 205 bp, Pav-Rf-SSR was observed in range of 343 bp to 353 bp. Finally, LG3_13.146 was observed only at 218 bp. Each marker was analysed separately, and the allelic combination were observed. Pav-Rf-SSR showed 10 allelic combinations, where 5 were groups were observed, 'Bagi meggy', 'Cigánymeggy 7' and 'Késői Cigány' (343-343-351-353), 'Cigány késői', 'Dunabogdány', 'Édes pipacs', 'Favorit', 'Fehérvári', 'Fűzlevelű kisszemű', 'Helyi sötét', 'Mogyoródi kései' and 'Velencei kései' (351-351-353-353), 'Érdi bőtermő', 'Érdi Jubileum', 'Kantorjánosi 3', 'Késői paraszmeggy', 'Májusi hólyag', 'Pipacs 1' and 'Szamosi meggy' (349-349-351-353), 'Bosnyák', 'Hortenzia királynője', 'Nagy Gobet', 'Pándy Bb. 119' and 'Pándy 279' (347-349-351-353), 'Pándy 43' and 'Péceli nagy' (347-347-351-353) (Figure 10, Table 12 and Table 13 Appendix A2). SSR marker Ma039a exhibited 6 allelic combinations, where only four of those were shared, for 'Bagi meggy', 'Cigány késői', 'Cigánymeggy 7', 'Késői Cigány', 'Korai Cigány' and 'Pándy Bb. 119' (157-157-205-205), 'Érdi Jubileum', 'Fűzlevelű kosszemű', 'Helyi sötét' and 'Májusi hólyag' (157-157-175-175), 'Bosnyák', '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' and 'Velencei kései' (157-157-175-205) and 'Favorit' and 'Szamosi meggy' (175-175-205-205) (Figure 11, Table 14 and Table 15 Appendix A2). Finally, LG3_13.146 marker was observed in 'Édes pipacs', 'Érdi bőtermő', 'Érdi Jubileum', 'Késői parasztmeggy', 'Pándy Bb. 119', 'Pándy 279', 'Péceli nagy' and 'Újfehértói fürtös' at 218 bp (Table 16, Appendix A2).

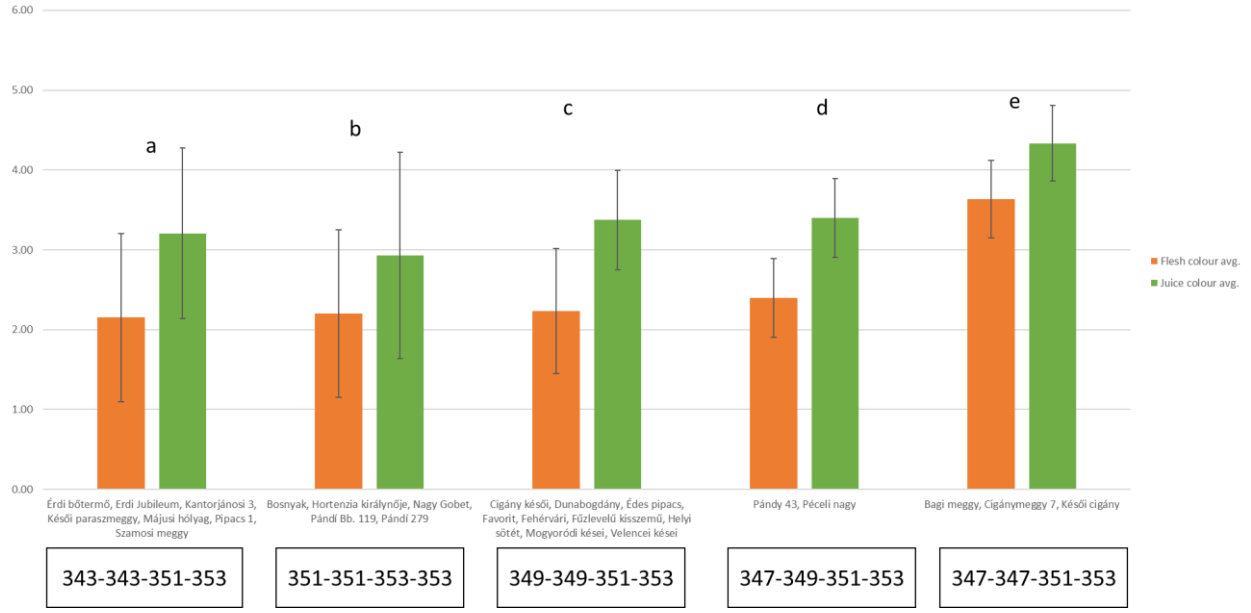


Figure 10 Sour cherry allelic groups based on Pav-Rf-SSR alleles. 5 groups were observed in total. Flesh color is indicated in orange while juice color is indicated in green

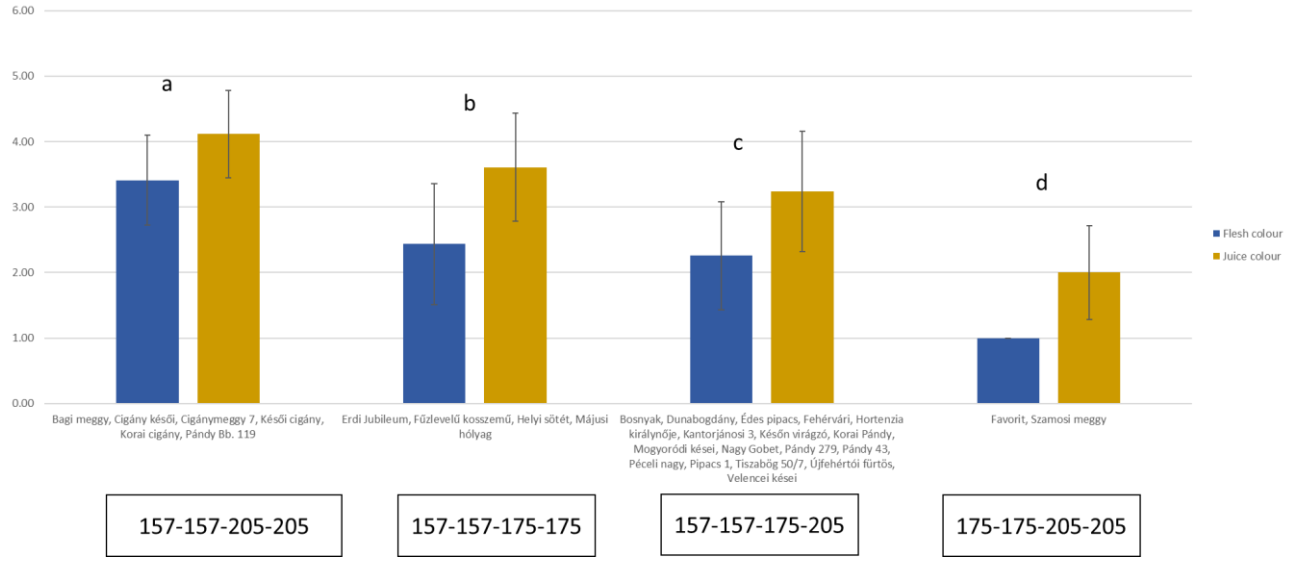
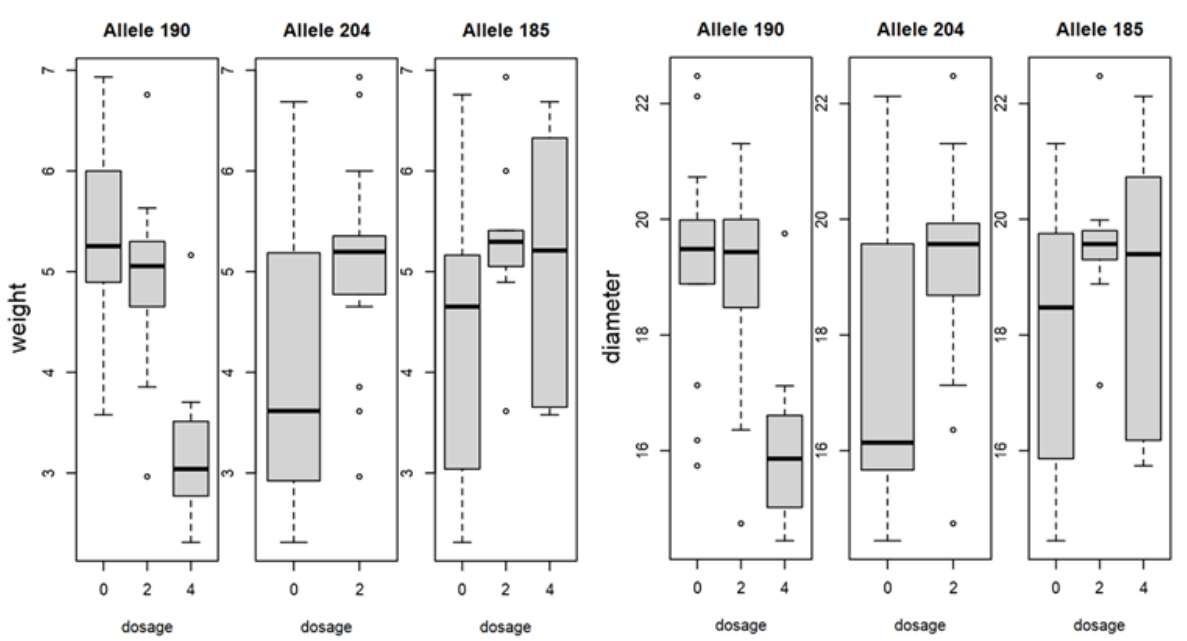


Figure 11 Sour cherry allelic groups based on Ma039a alleles. 4 groups were observed in total. Flesh color is indicated in blue while juice color is indicated in yellow.

5.4 Correlation between phenotype and genotype

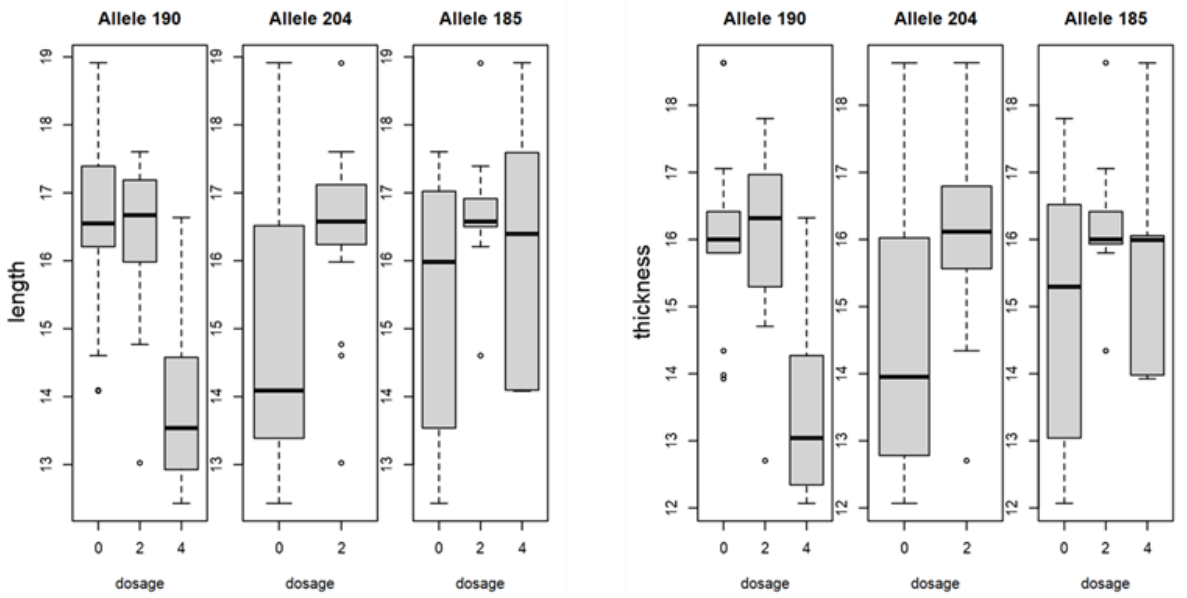
5.4.1 Sour cherry Pearson correlation for single alleles

Pearson correlation between markers and phenotype was performed for single alleles observed. Sour cherry fruit size (diameter, length, thickness, SA) correlated with BPPCT034$_{204}$ positively, while negatively with BPPCT034$_{208}$. BPPCT034$_{222}$ had a negative correlation with diameter, while BPPCT034$_{230}$ showed a negative correlation with diameter length and SA. BPPCT034$_{237}$ had a negative correlation with diameter, length, and SA. CPSCT038$_{185}$ positively correlated with diameter, length thickness and SA. CPSCT038$_{190}$ correlated negatively with diameter, length, thickness, and SA. CPSCT038$_{204}$ correlated positively with diameter, length, thickness, and SA. Fruit weight, seed weight and pulp weight were correlating positively with BPPCT034$_{204}$ while negatively with BPPCT034$_{208}$. Fruit weight had a positive correlation also with BPPCT034$_{237}$. CPSCT038$_{185}$ positively correlated with fruit and pulp weight. CPSCT038$_{190}$ showed a negative correlation with fruit and pulp weight, while finally, CPSCT038$_{204}$ showed a positive correlation with fruit, seed, and pulp weight (Figure 12, Figure 13, Figure 14, Figure 15 and Figure 16, Table 17 and Table 18). Linear regression was performed as well, indicating that single alleles were significantly influencing the diameter, length, thickness, SA, fruit weight, seed weight and pulp weight (Table 19, Appendix A2). Sour cherry color was analysed as well for single alleles. Pearson correlation indicated that Ma039a$_{157}$ correlated negatively with L, Ma039a$_{175}$ correlated positively with L, while negatively with Ctifl, flesh color and juice color. Ma039a$_{205}$ correlated negatively with Ctifl and flesh color. Pav-Rf-SSR$_{343}$ positively correlated with juice color (Table 20 and Table 21). Linear regression was performed for single alleles, showing significant influence on L, a, b, Chroma, hue, Ctifl, flesh color, juice color and SSC (Table 22, Table 23 and Table 24 Appendix A2).



Pearson Correlation	weight	Sign.	
CPSCT038 190	-0.393	0.014	*
CPSCT038 204	0.414	0.010	*
CPSCT038 185	0.393	0.014	*

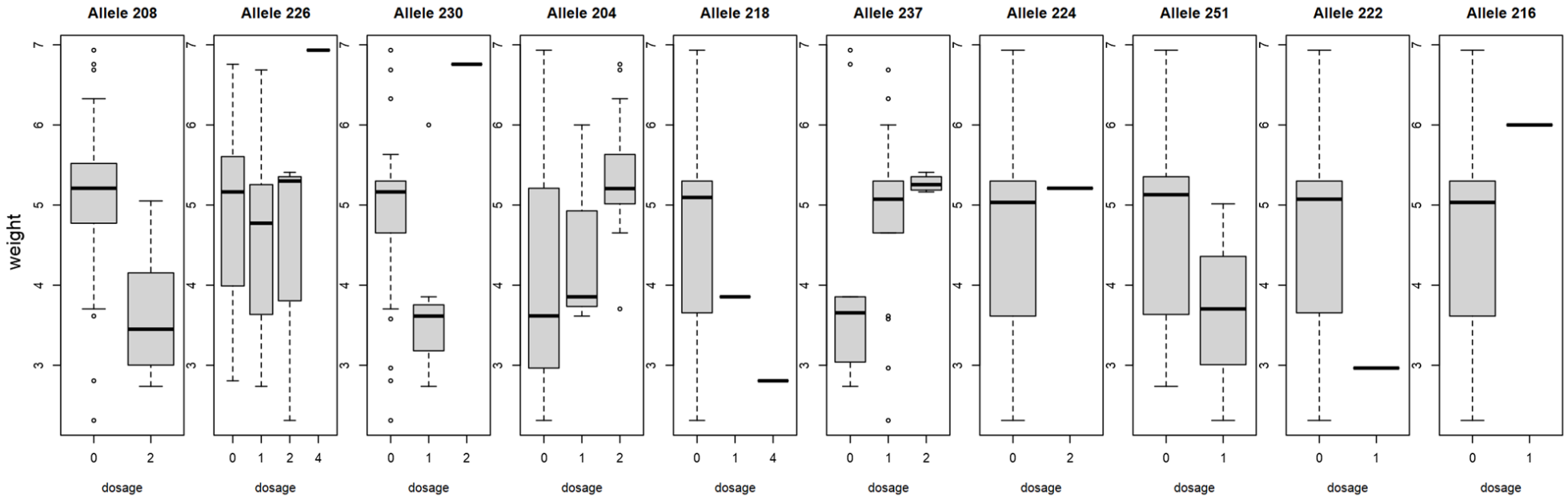
Pearson Correlation	diameter	Sign.	
CPSCT038 190	-0.351	0.026	*
CPSCT038 204	0.418	0.010	*
CPSCT038 185	0.351	0.026	*



Pearson Correlation	length	Sign.	
CPSCT038 190	-0.350	0.027	*
CPSCT038 204	0.440	0.007	*
CPSCT038 185	0.350	0.027	*

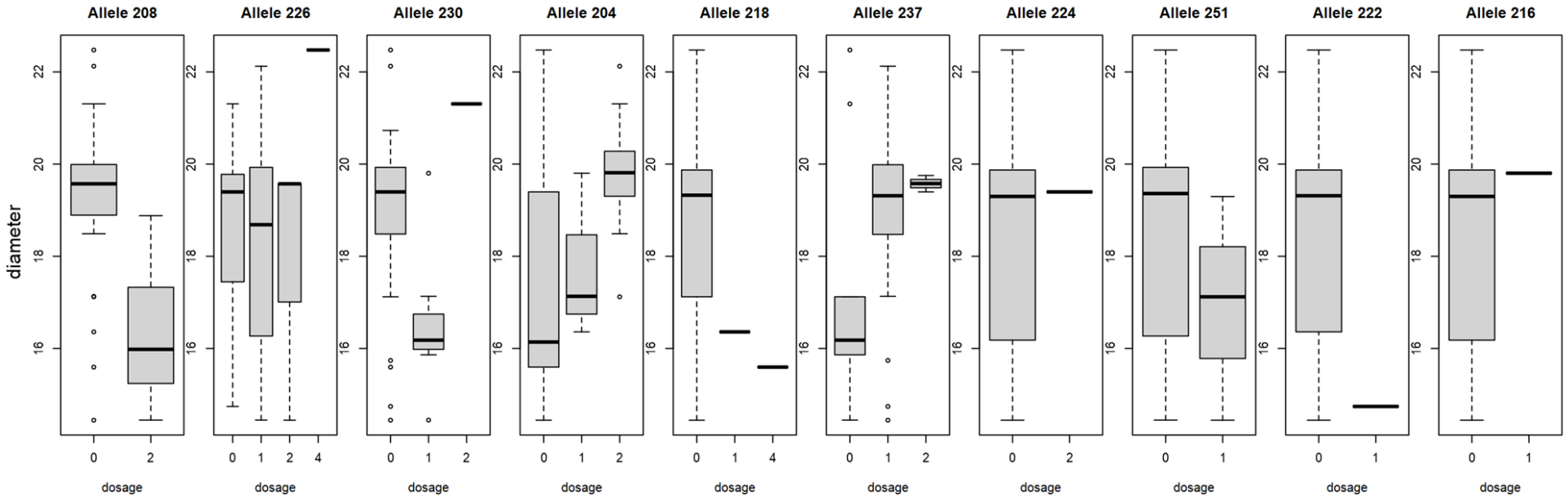
Pearson Correlation	thickness	Sign.	
CPSCT038 190	-0.325	0.037	*
CPSCT038 204	0.460	0.005	**
CPSCT038 185	0.325	0.037	*

Figure 12 Pearson correlation analysis for fruit size characteristics for CPSCT038. Boxplot indicate the single alleles influence for the individual characteristics, such as weight, diameter, length and thickness. Dosage indicates the number of allele copies observed. Significance level is indicated as a single asterisk (*) when $p < 0.05$ and double (**) when $p < 0.01$



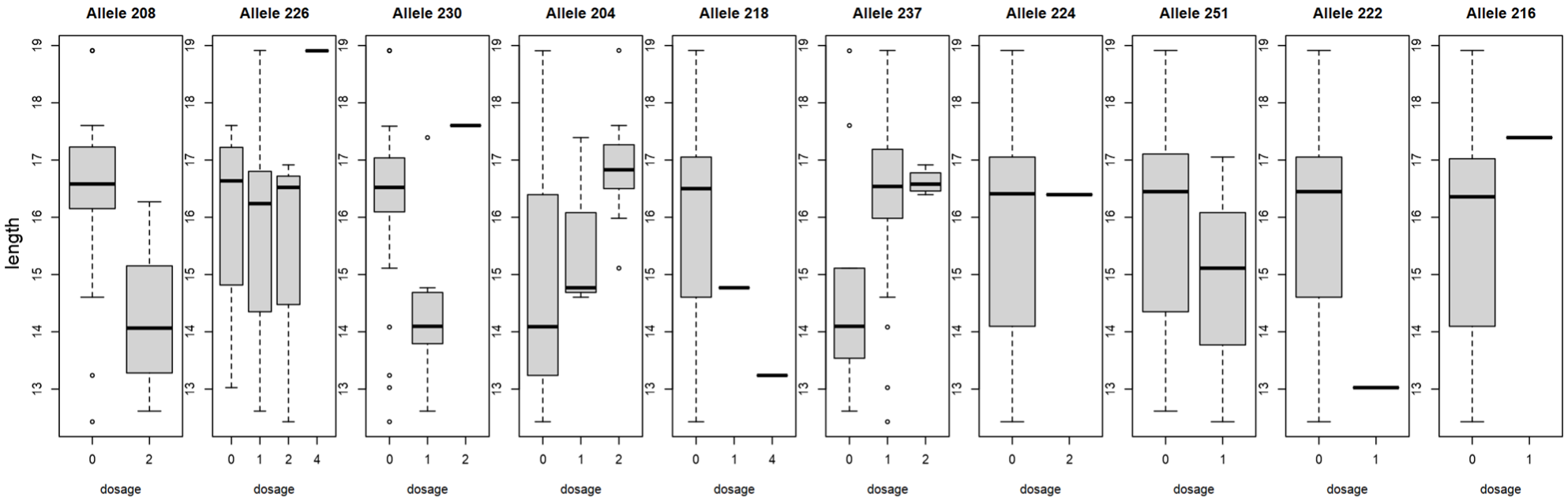
Pearson Correlation	weight	Sign.	
BPPCT034 208	-0.508	0.002	**
BPPCT034 226	-0.068	0.358	
BPPCT034 230	-0.270	0.071	
BPPCT034 204	0.455	0.005	**
BPPCT034 218	-0.289	0.058	
BPPCT034 237	0.310	0.045	*
BPPCT034 224	0.077	0.341	
BPPCT034 251	-0.268	0.072	
BPPCT034 222	-0.255	0.083	
BPPCT034 216	0.193	0.149	

Figure 13 Pearson correlation analysis for fruit weight characteristic for BPPCT034. Observed alleles are analysed separately. Boxplot indicate the single alleles influence for the individual characteristic. Dosage indicates the number of allele copies observed. Significance level is indicated as a single asterisk (*) when $p < 0.05$ and double (**) when $p < 0.01$



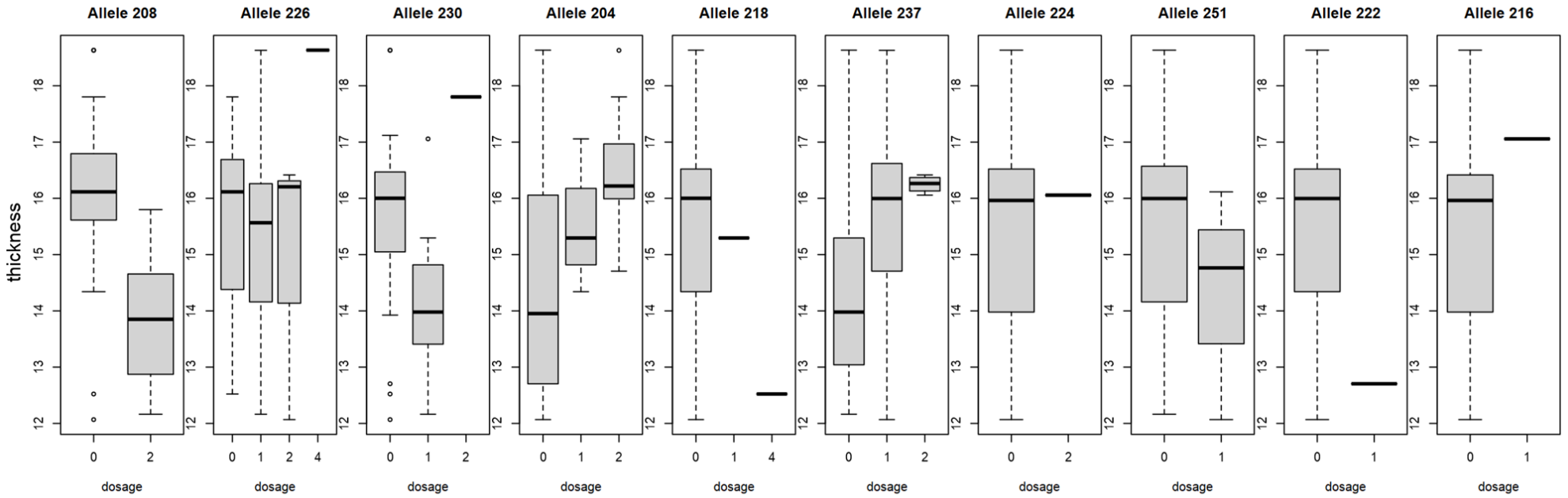
Pearson Correlation	diameter	Sign.	
BPPCT034 208	-0.575	0.000	**
BPPCT034 226	-0.026	0.445	
BPPCT034 230	-0.348	0.027	*
BPPCT034 204	0.494	0.002	**
BPPCT034 218	-0.295	0.054	
BPPCT034 237	0.342	0.030	*
BPPCT034 224	0.079	0.337	
BPPCT034 222	-0.307	0.046	*
BPPCT034 216	0.112	0.274	
BPPCT034 251	-0.222	0.115	

Figure 14 Pearson correlation analysis for fruit diameter characteristic for BPPCT034. Observed alleles are analysed separately. Boxplot indicate the single alleles influence for the individual characteristic. Dosage indicates the number of allele copies observed. Significance level is indicated as a single asterisk (*) when $p < 0.05$ and double (**) when $p < 0.01$



Pearson Correlation	length	Sign.	
BPPCT034 208	-0.549	0.001	**
BPPCT034 226	-0.017	0.464	
BPPCT034 230	-0.346	0.028	*
BPPCT034 204	0.509	0.002	**
BPPCT034 218	-0.280	0.064	
BPPCT034 237	0.358	0.024	*
BPPCT034 224	0.057	0.380	
BPPCT034 251	-0.186	0.158	
BPPCT034 222	-0.297	0.052	
BPPCT034 216	0.162	0.192	

Figure 15 Pearson correlation analysis for fruit length characteristic for BPPCT034. Observed alleles are analysed separately. Boxplot indicate the single alleles influence for the individual characteristic. Dosage indicates the number of allele copies observed. Significance level is indicated as a single asterisk (*) when $p < 0.05$ and double (**) when $p < 0.01$.



Pearson Correlation	thickness	Sign.	
BPPCT034 208	-0.544	0.001	**
BPPCT034 226	-0.020	0.456	
BPPCT034 230	-0.259	0.080	
BPPCT034 204	0.507	0.002	**
BPPCT034 218	-0.232	0.105	
BPPCT034 237	0.287	0.059	
BPPCT034 224	0.064	0.366	
BPPCT034 251	-0.212	0.126	
BPPCT034 222	-0.287	0.059	
BPPCT034 216	0.169	0.182	

Figure 16 Pearson correlation analysis for fruit thickness characteristic for BPPCT034. Observed alleles are analysed separately. Boxplot indicate the single alleles influence for the individual characteristic. Dosage indicates the number of allele copies observed. Significance level is indicated as a single asterisk (*) when $p < 0.05$ and double (**) when $p < 0.01$.

Table 17 Pearson correlation fruit size. Asterisk indicate significant correlation for p<0.05 (*) and p<0.01 (**). Green color indicates positive correlation, red color indicates negative correlation.

Pearson Correlation	**Diameter**	**Sign.**		**Length**	**Sign.**		**Thickness**	**Sign.**		**SA (cm^2)**	**Sign.**	
$BPPCT034_{204}$	0.494	0.002	**	0.509	0.002	**	0.507	0.002	**	0.347	0.028	*
$BPPCT034_{208}$	-0.575	0.000	**	-0.549	0.001	**	-0.544	0.001	**	-0.513	0.002	**
$BPPCT034_{216}$	0.112	0.274		0.162	0.192		0.169	0.182		0.183	0.162	
$BPPCT034_{218}$	-0.295	0.054		-0.280	0.064		-0.232	0.105		-0.186	0.158	
$BPPCT034_{222}$	-0.307	0.046	*	-0.297	0.052		-0.287	0.059		-0.222	0.115	
$BPPCT034_{224}$	0.079	0.337		0.057	0.380		0.064	0.366		0.104	0.289	
$BPPCT034_{226}$	-0.026	0.445		-0.017	0.464		-0.020	0.456		0.034	0.427	
$BPPCT034_{230}$	-0.348	0.027	*	-0.346	0.028	*	-0.259	0.080		-0.317	0.041	*
$BPPCT034_{237}$	0.342	0.030	*	0.358	0.024	*	0.287	0.059		0.339	0.031	*
$BPPCT034_{251}$	-0.222	0.115		-0.186	0.158		-0.212	0.126		-0.174	0.175	
$CPSCT038_{185}$	0.351	0.026	*	0.350	0.027	*	0.325	0.037	*	0.452	0.005	**
$CPSCT038_{190}$	-0.351	0.026	*	-0.350	0.027	*	-0.325	0.037	*	-0.452	0.005	**
$CPSCT038_{204}$	0.418	0.010	*	0.440	0.007	*	0.460	0.005	**	0.429	0.008	**

Table 18 Pearson correlation fruit weight. Asterisk indicate significant correlation for p<0.05 (*) and p<0.01 (**). Green color indicates positive correlation, red color indicates negative correlation.

Pearson Correlation	Weight	Sign.		Seed weight (g)	Sign.		Pulp weight (g)	Sign.	
BPPCT034$_{204}$	0.455	0.005	**	0.269	0.072		0.349	0.027	*
BPPCT034$_{208}$	-0.508	0.002	**	-0.093	0.310		-0.507	0.002	**
BPPCT034$_{216}$	0.193	0.149		0.007	0.486		0.248	0.089	
BPPCT034$_{218}$	-0.289	0.058		-0.149	0.212		-0.232	0.104	
BPPCT034$_{222}$	-0.255	0.083		-0.128	0.246		-0.217	0.121	
BPPCT034$_{224}$	0.077	0.341		0.080	0.334		0.120	0.260	
BPPCT034$_{226}$	-0.068	0.358		0.106	0.284		-0.014	0.470	
BPPCT034$_{230}$	-0.270	0.071		-0.225	0.112		-0.295	0.054	
BPPCT034$_{237}$	0.310	0.045	*	0.283	0.061		0.349	0.027	
BPPCT034$_{251}$	-0.268	0.072		-0.036	0.424		-0.254	0.084	
CPSCT038$_{185}$	0.393	0.014	*	0.301	0.050		0.507	0.002	**
CPSCT038$_{190}$	-0.393	0.014	*	-0.301	0.050		-0.507	0.002	**
CPSCT038$_{204}$	0.414	0.010	*	0.327	0.036	*	0.441	0.007	*

Table 20 Sour cherry SSR fruit color correlation with CIELab. Asterisk indicate significant correlation for p<0.05 (*) and p<0.01 (**). Green color indicates positive correlation, red color indicates negative correlation.

Correlations		L	Sign.		a	Sign.		b	Sign.		C	Sign.		$h^{\pm 33°}$	Sign.	
		1.000			1.000			1.000			1.000			1.000		
Pearson Correlation	$Pav\_Rf\_SSR_{343}$	-0.078	0.339		-0.210	0.129		-0.221	0.116		-0.215	0.122		-0.105	0.287	
	$Pav\_Rf\_SSR_{347}$	0.151	0.209		0.059	0.377		0.187	0.157		0.085	0.325		0.106	0.286	
	$Pav\_Rf\_SSR_{349}$	0.190	0.153		0.055	0.384		0.128	0.246		0.069	0.357		0.025	0.447	
	$Pav\_Rf\_SSR_{351}$	-0.100	0.297		-0.025	0.446		0.082	0.331		-0.006	0.487		0.202	0.137	
	$Pav\_Rf\_SSR_{353}$	0	0.000		0	0.000		0	0.000		0	0.000		0	0.000	
		1.000			1.000			1.000			1.000			1.000		
Pearson Correlation	$Ma039a_{157}$	-0.324	0.037	*	-0.181	0.164		-0.211	0.127		-0.186	0.158		0.085	0.325	
	$Ma039a_{175}$	0.314	0.043	*	0.252	0.085		0.236	0.100		0.252	0.086		0.016	0.465	
	$Ma039a_{205}$	0.172	0.178		0.112	0.273		0.099	0.299		0.112	0.275		-0.002	0.496	
		1.000			1.000			1.000			1.000			1.000		
Pearson Correlation	$LG3\_13.146_{218}$	-0.174	0.174		-0.249	0.089		-0.203	0.136		-0.240	0.096		-0.170	0.181	

Table 21 Sour cherry SSR markers correlation for fruit quality. Asterisk indicate significant correlation for p<0.05 (*) and p<0.01 (**). Green color indicates positive correlation, red color indicates negative correlation.

Correlations		Ctifl scale	Sign.		Flesh color	Sign.		Juice color	Sign.		Soluble content	Sign.	
		1.000			1.000			1.000			1.000		
Pearson Correlation	$Pav\_Rf\_SSR_{343}$	0.176	0.172		0.404	0.012		0.336	0.032	*	0.285	0.060	
	$Pav\_Rf\_SSR_{347}$	-0.159	0.197		-0.099	0.298		-0.191	0.152		-0.208	0.131	
	$Pav\_Rf\_SSR_{349}$	-0.122	0.257		-0.190	0.153		-0.244	0.093		0.043	0.410	
	$Pav\_Rf\_SSR_{351}$	-0.005	0.489		-0.145	0.219		-0.152	0.208		-0.205	0.134	
	$Pav\_Rf\_SSR_{353}$	0	0.000		0	0.000		0	0.000		0	0.000	
		1.000			1.000			1.000			1.000		
Pearson Correlation	$Ma039a_{157}$	0.181	0.165		0.285	0.060		0.273	0.069		-0.002	0.495	
	$Ma039a_{175}$	-0.372	0.020	*	-0.427	0.008	*	-0.342	0.030	*	-0.101	0.294	
	$Ma039a_{205}$	-0.302	0.049	*	-0.002	0.496	*	-0.106	0.285		-0.080	0.334	
		1.000			1.000			1.000			1.000		
Pearson Correlation	$LG3\_13.146_{218}$	0.261	0.078		0.122	0.256		0.164	0.189		-0.127	0.247	

5.5 Frequency analysis for the used markers

Frequency analysis was performed for the tested SSR markers. In sour cherry, the most polymorphic marker was BPPCT034 with 22 individual alleles observed, the same as CPSCT038. Ma039a and Pav_Rf_SSR markers had instead 3 and 5 unique alleles, respectively. Observed heterozygosity was high in sour cherry, with values from 0.94 to 1.00 (Table 25).

Table 25 Frequency analysis for the molecular markers used in this study. Number of alleles (AlleleN), expected (EH)and observed (OH) heterozygosity, polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI), discriminating (DP) and resolving power (RP) are indicated for each SSR marker analysed in this study.

Species	Marker	Allele N (AN)	ExpectedH (EH)	ObservedH (OH)	PIC	EMR	MI	Discriminating Power (DP)	Resolving Power (RP)	Frequency
Sour cherry	Ma039a	3	0.94	0.94	0.14	29	4.18	0.90	1.03	157:0.47; 175:0.27; 205:0.27
	Pav-Rf-SSR	5	0.98	1.00	0.09	31	2.66	0.96	2.13	343:0.09; 347:0.08; 349:0.19; 351:0.30; 353:0.35
	BPPCT034	22	0.99	0.94	0.03	29	0.98	0.99	4.39	204:0.25; 208:0.13; 216:0.01; 218:0.04; 222:0.01; 224:0.02; 226:0.24; 230:0.05; 237:0.11; 251:0.02
	CPSCT038	22	0.98	0.94	0.06	29	1.70	0.98	4.32	185:0.27; 190:0.34; 204:0.17

5.6 HTS base virological survey of sour cherry accessions

Three separate libraries were sequenced, respectively called 1_PC_E1, 2_PC_E2 and 3_PC_E3. The initial statistic showed an average of 18 million reads. Library 1_PC_E1 had more than 18 million reads, 2_PC_E2 had more than 20 million reads and 3_PC_E3 had 17 million reads, as indicated in Table 26. Trimmed reads varied from a maximum of 20 million (2_PC_E2) to a minimum of 16 million (3_PC_E3). Non-redundant reads ranged from a minimum of 1 million reads (2_PC_E2) to a maximum of 1.6 million reads (3_PC_E3). Number of contigs varied from a minimum of 1,597(2_PC_E2) to a maximum of 2,631 (3_PC_E3).

Table 26 Initial statistics for the 3 libraries analysed in this study. In bold are indicated the highest values recorded.

Library code	Sequenced reads	Trimmed reads all (containing redundant)	Non-redundant reads	Number of contigs
1_PC_E1	18,658,693	18,392,129	1,581,948	**2,631**
2_PC_E2	**20,657,588**	**20,288,965**	1,071,991	1,597
3_PC_E3	17,096,190	16,885,821	**1,676,263**	2,695

The small RNA HTS was compared to reference sequences of known viruses. As a result, three viruses were found as most probably present in our sample libraries; CVA, PNRSV and PrVF. In library 1_PC_E1, 2 contigs were found for CVA. For PNRSV 3 contigs for RNA1, 23 contigs for RNA2 and 1 single contig for RNA3 were found. RPM (reads per million) was above 400 for PNRSV RNA3 and the coverage of viral genome was above 60% for PNRSV RNA1 to RNA3. Coverage of PrVF RNA1 was also found above threshold limit of 60%. In library 2_PC_E2 one contig was found for CVA, while 1 contig for PNRSV RNA2 and 13 contigs for PNRSV RNA3. Viral genome coverage was above 60% for PNRSV RNA2 and PNRSV RNA3. For library 3_PC_E3 1 contig was found for CVA, 1 contig for PNRSV RNA2 and 2 contigs for PNRSV RNA3. Coverage of viral genome was above 60% for PNRSV RNA 3 (Table 27).

Table 27 Bioinformatic table for identified viruses. Cherry virus A, prunus necrotic ringspot virus and prunus virus F were found in several libraries. In grey, is indicated the threshold value above the pre-established limit.

Library	Viral family	Betaflexiviridae	Bromoviridae			Secoviridae	
	virus	Cherry virus A	Prunus necrotic ringspot virus RNA 1	Prunus necrotic ringspot virus RNA 2	Prunus necrotic ringspot virus RNA 3	Prunus virus F RNA1	Prunus virus F RNA2
	NCBI accession number	NC_003689	NC_004362	NC_004363	NC_004364	NC_039077.1	NC_039078.1
1_PC_E1	number of contigs	2	3	23	1	0	0
	number of non-redundant reads	635	626	775	2158	712	221
	number of redundant reads	2204	1895	2057	9219	1760	412
	RPM	118.1	101.6	110.2	494.1	94.3	22.1
	coverage of the viral genome (%)	45%	71%	93%	93%	72%	56%
2_PC_E2	number of contigs	1	0	1	13	0	0
	number of non-redundant reads	418	376	425	1361	192	101
	number of redundant reads	3223	2215	2707	13318	1069	456
	RPM	156	107.2	131	644.7	51.7	22.1
	coverage of the viral genome (%)	36%	56%	81%	87%	37%	33%
3_PC_E3	number of contigs	1	0	1	2	0	0
	number of non-redundant reads	353	122	151	404	341	110
	number of redundant reads	908	211	318	1129	793	232
	RPM	53.1	12.3	18.6	66	46.4	13.6
	coverage of the viral genome (%)	38%	39%	56%	71%	51%	33%

5.7 RT-PCR validation of HTS analysis

5.7.1 RT-PCR for three libraries

Pools were tested with RT-PCR for each virus previously identified with HTS. Validation indicated at first that 3/3 libraries were infected with PNRSV (RNA3), 1/3 libraries were infected with CVA and 3/3 libraries were infected with PrVF (RNA1 and RNA2) (Figure 17).

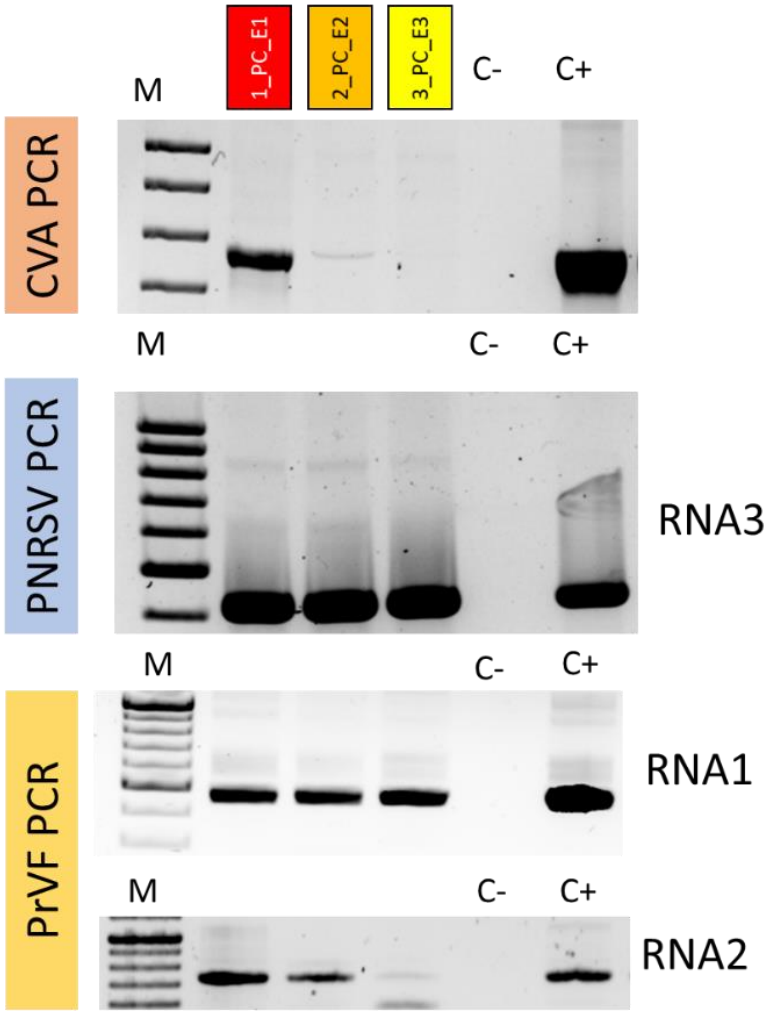


Figure 17 Preliminary PCR for each library. PrVF, CVA and PNRSV were analysed in each library to understand the presence of each virus in the libraries. C-: negative control. C+: positive control. M: ladder.

5.7.2 RT-PCR for individuals

After confirming the presence of PNRSV, CVA and PrVF in the 3 libraries, we started analysing the individuals contained in each library (Figure 18). In library 1_PC_E1, 6/11 samples were infected with CVA, while 7/11 were found positive for PRSV. 7/11 samples were positive for PrVF. Library 2_PC_E2 had 3/11 individuals infected with CVA, 7/11 individuals infected with PNRSV and 4/11 infected with PrVF. 3_PC_E3 library showed 1/9 samples infected with CVA, 1/9 samples infected with PNRSV and 5/9 samples infected with PrVF. Furthermore, we could observe that single infection was the most common (14 samples), while mixed infections were scarcer. CVA and PrVF infection was found in 2 samples, while PNRSV and PrVF in 4 samples and finally CVA and PNRSV in 2 samples. Triple infection was found in 4 cases, in one library (Table 28).

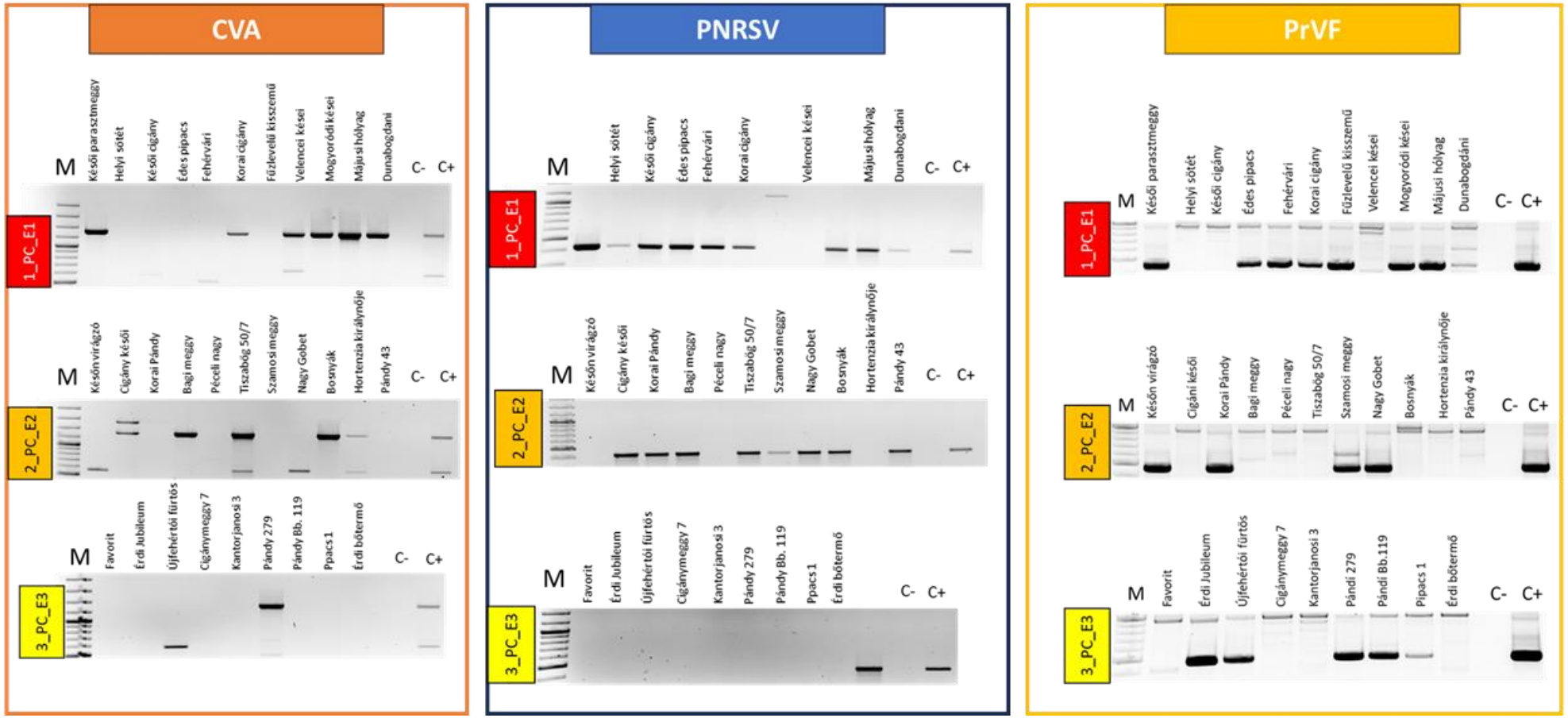


Figure 18 Individuals derived from the 3 libraries were tested for presence of PrVF, CVA and PNRSV. C-: negative control. C+: positive control. M: ladder.

Table 28 RT-PCR validation for individuals in each pool, where CVA, PNRSV and PrVF were analysed. In dark red are indicated pools that have only sweet cherry samples, while in light red the pools that contain only sour cherry samples.

Library	Diagnostic method	CVA	PNRSV			PrVF		virus infection					virus infected tree
			RNA 1	RNA 2	RNA 3	RNA1	RNA2	single infection	CVA + PNRSV	CVA + PrVF	PNRSV + PrVF	triple infection	
1_PC_E1	sRNA HTS	n	y	y	y	n	n	5	0	0	2	4	11
	RT-PCR	**6/11**	nd	nd	**7/11**	**7/11**	**5/11**						
2_PC_E2	sRNA HTS	n	n	y	y	n	n	4	2	1	2	0	9
	RT-PCR	**3/11**	nd	nd	**7/11**	**4/11**	**11/11**						
3_PC_E3	sRNA HTS	n	n	n	y	n	n	5	0	1	0	0	6
	RT-PCR	**1/9**	nd	nd	**1/9**	**5/9**	**9/9**						
Infection rate		32%	48%			52%	81%						

5.8 RT-PCR results for 2023 sour cherry collection

Individuals were collected again in 2023 and were analyzed for viral presence, and distribution. Each tree was tested for CVA, PNRSV and PrVF. Out of a total of 58 individual trees, 31% were positive for CVA, 45% were positive to PNRSV and 38% were positive to PrVF. Some of the trees previously collected and analyzed were found dead in 2023, thus they were not included in the analysis, as indicated in Table 29.

Table 29 RT-PCR analysis of samples collected in 2023 from sour cherry trees. Individual trees collected in 2021 are indicated in light blue. Trees that were dead in 2023 are indicated in grey. Positive samples were highlighted in orange.

small RNA library	cultivar	row	tree	CVA	PNRSV	PrVF
1_PC_E1_2023	Késői parasztmeggy	1	9	positive	positive	positive
	Késői parasztmeggy	1	10	negative	positive	positive
	Helyi sötét	2	23	not tested	not tested	not tested
	Helyi sötét	2	24	negative	negative	negative
	Késői Cigány	2	37	negative	negative	negative
	Késői Cigány	2	38	negative	negative	negative
	Édes pipacs	2	59	not tested	not tested	not tested
	Édes pipacs	2	60	positive	positive	positive
	Fehérvári	3	5	negative	positive	positive
	Fehérvári	3	6	negative	negative	negative
	Korai Cigány	3	29	positive	positive	positive
	Korai Cigány	3	30	DEAD		
	Fűzlevelű kisszemű	3	49	negative	negative	negative
	Fűzlevelű kisszemű	3	50	negative	negative	negative
	Velencei kései	3	51	negative	negative	negative
	Velencei kései	3	52	negative	negative	negative
	Mogyoródi kései	3	57	positive	positive	positive
	Mogyoródi kései	3	58	negative	positive	positive
	Májusi hólyag	4	1	DEAD		
	Májusi hólyag	4	2	positive	positive	positive
	Dunabogdányi	4	25	positive	positive	negative
	Dunabogdányi	4	26	negative	positive	negative
2_PC_E2_2023	Későn virágzó	4	31	negative	negative	positive
	Későn virágzó	4	32	DEAD		
	Cigány késői	5	1	positive	positive	negative
	Cigány késői	5	2	positive	positive	negative
	Korai Pándy	5	31	DEAD		
	Korai Pándy	5	32	DEAD		
	Bagi meggy	5	33	negative	positive	negative
	Bagi meggy	5	34	positive	positive	positive
	Péceli nagy	5	41	DEAD		
	Péceli nagy	5	42	DEAD		
	Tiszabög 50/7	5	51	positive	positive	negative
	Tiszabög 50/7	5	52	DEAD		
	Szamosi meggy	6	17	negative	negative	negative

	Szamosi meggy	6	18	negative	negative	negative
	Nagy Gobet	6	34	positive	positive	positive
	Nagy Gobet	6	35	positive	positive	positive
	Bosnyák	6	49	positive	positive	negative
	Bosnyák	6	50	positive	positive	negative
	Hortenzia királynője	7	3	negative	negative	negative
	Hortenzia királynője	7	4	positive	negative	negative
	Pándy 43	7	7	negative	positive	negative
	Pándy 43	7	8	DEAD		
3_PC_E3_2023	Favorit	19	13	negative	negative	negative
	Favorit	19	14	DEAD		
	Favorit	19	15	DEAD		
	Favorit	19	16	negative	negative	negative
	Érdi Jubileum	19	17	DEAD		
	Érdi Jubileum	19	18	negative	negative	positive
	Érdi Jubileum	19	19	negative	negative	positive
	Érdi Jubileum	19	20	positive	negative	positive
	Újfehértói fürtös	20	9	not tested	not tested	not tested
	Újfehértói fürtös	20	10	negative	positive	positive
	Újfehértói fürtös	20	11	negative	negative	positive
	Újfehértói fürtös	20	12	DEAD		
	Cigánymeggy 7	20	21	DEAD		
	Cigánymeggy 7	20	22	negative	positive	negative
	Cigánymeggy 7	20	23	negative	negative	negative
	Cigánymeggy 7	20	24	negative	negative	negative
	Kantorjanosi 3	21	5	DEAD		
	Kantorjanosi 3	21	6	negative	negative	negative
	Kantorjanosi 3	21	7	DEAD		
	Kantorjanosi 3	21	8	negative	negative	negative
	Pándy 279	21	13	negative	negative	negative
	Pándy 279	21	14	DEAD		
	Pándy 279	21	15	positive	negative	negative
	Pándy 279	21	16	negative	positive	negative
	Pándy Bb. 119	21	17	negative	negative	positive
	Pándy Bb. 119	21	18	negative	negative	positive
	Pándy Bb. 119	21	19	negative	positive	positive
	Pándy Bb. 119	21	20	negative	positive	positive
	Pipacs1	22	1	negative	negative	negative
	Pipacs1	22	2	negative	negative	negative
	Pipacs1	22	3	DEAD		
	Pipacs1	22	4	DEAD		
	Érdi bőtermő	13	9	positive	positive	positive

5.9 Phylogenetic analysis

To understand the variability and population size of CVA in our samples, a phylogenetic analysis was performed based on the movement protein gene. CVA movement protein was compared with reference sequences and sequences from other countries. CVA variants from Hungary were mainly in group I, where Hungarian, Indian, Czech, and Canadian variants of CVA grouped together. In Group III, two Hungarian variants from sweet cherry are grouped together with variants from China and Canada (Figure 19).

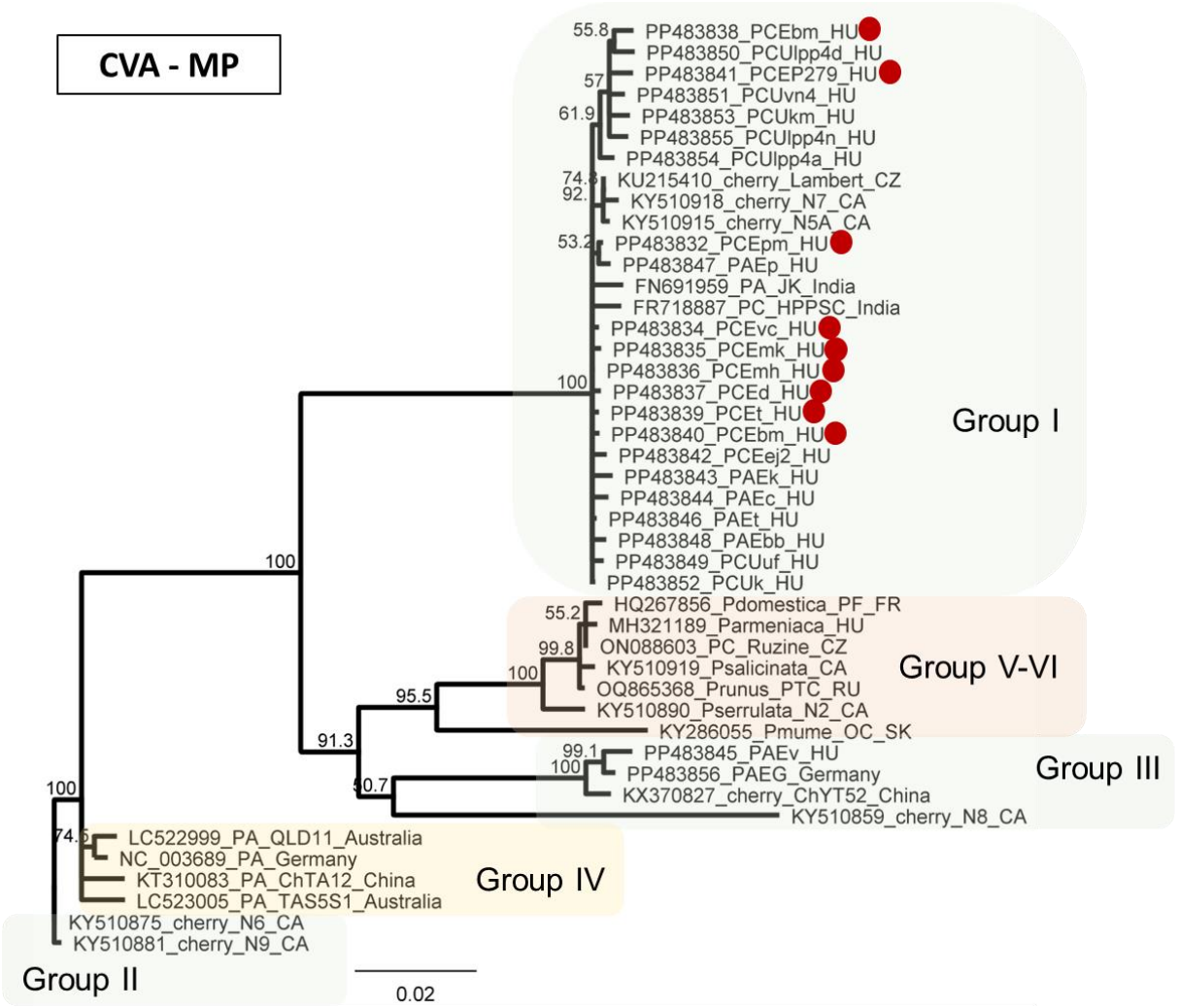


Figure 19 Phylogenetic analysis of CVA variants. In red: sour cherry infected with CVA in Hungary.

PNRSV variants were compared for RNA3 segment, where group PV96 represented all the Hungarian variants found in both sweet and sour cherry host trees, as shown in Figure 20.

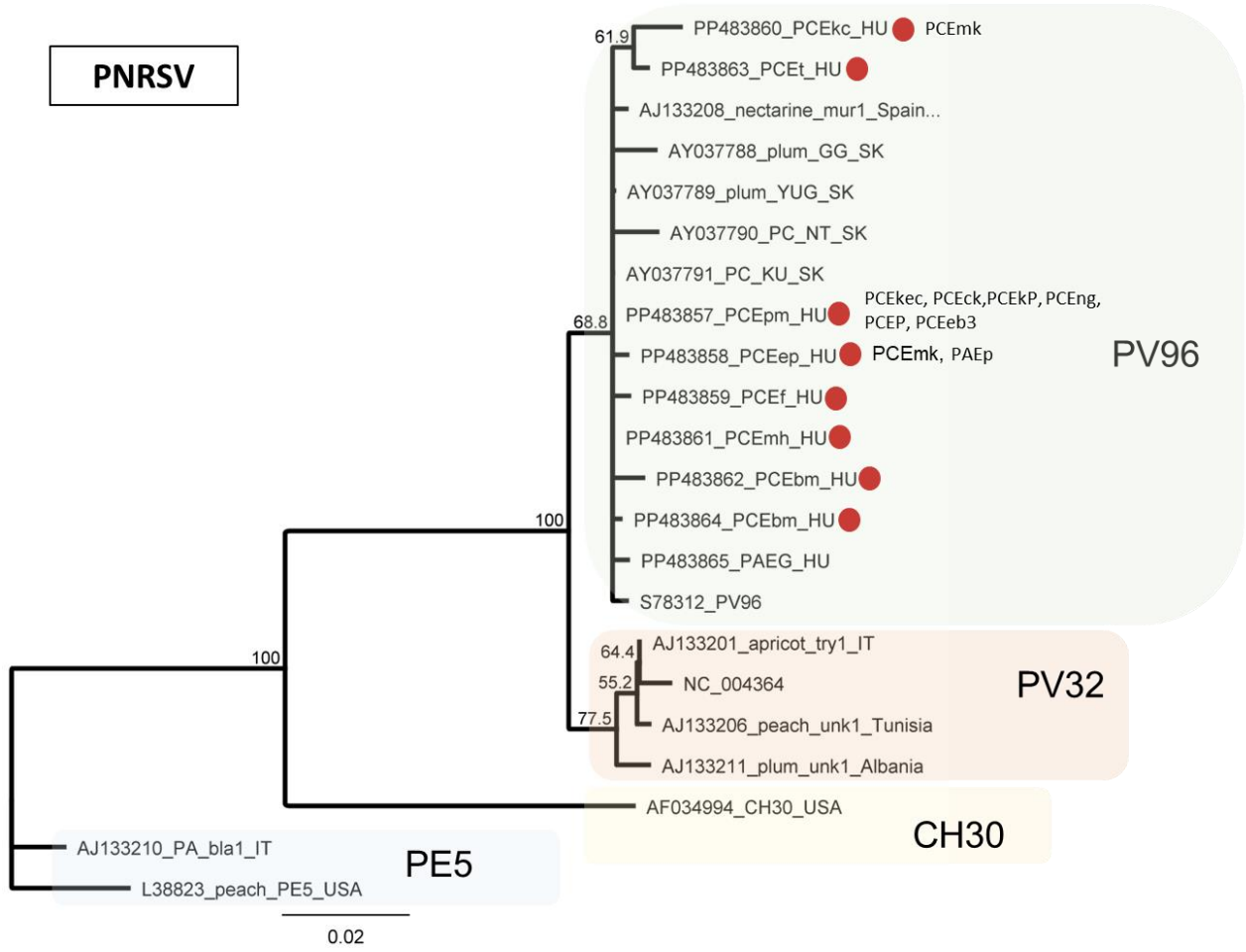


Figure 20 Phylogenetic tree of PNRSV. In red: sour cherry infected with PNRSV in Hungary

For PrVF we first compared the 5' UTR and 3' UTR. Consensus identity of 5'UTR suggested similarity for most of the Hungarian variants of PrVF, compared to higher diversification observed in 3'UTR (Figure 21, Appendix A2).

PrVF RNA1 phylogenetic analysis suggested that the Hungarian variants are quite close together, specifically, the PrVF variants found in sour cherry (Figure 22). PrVF RNA1 derived from sweet cherry, was instead closely related to PrVF infecting cultivar 'Staccato' found in Canada in previous research (James et al., 2018).

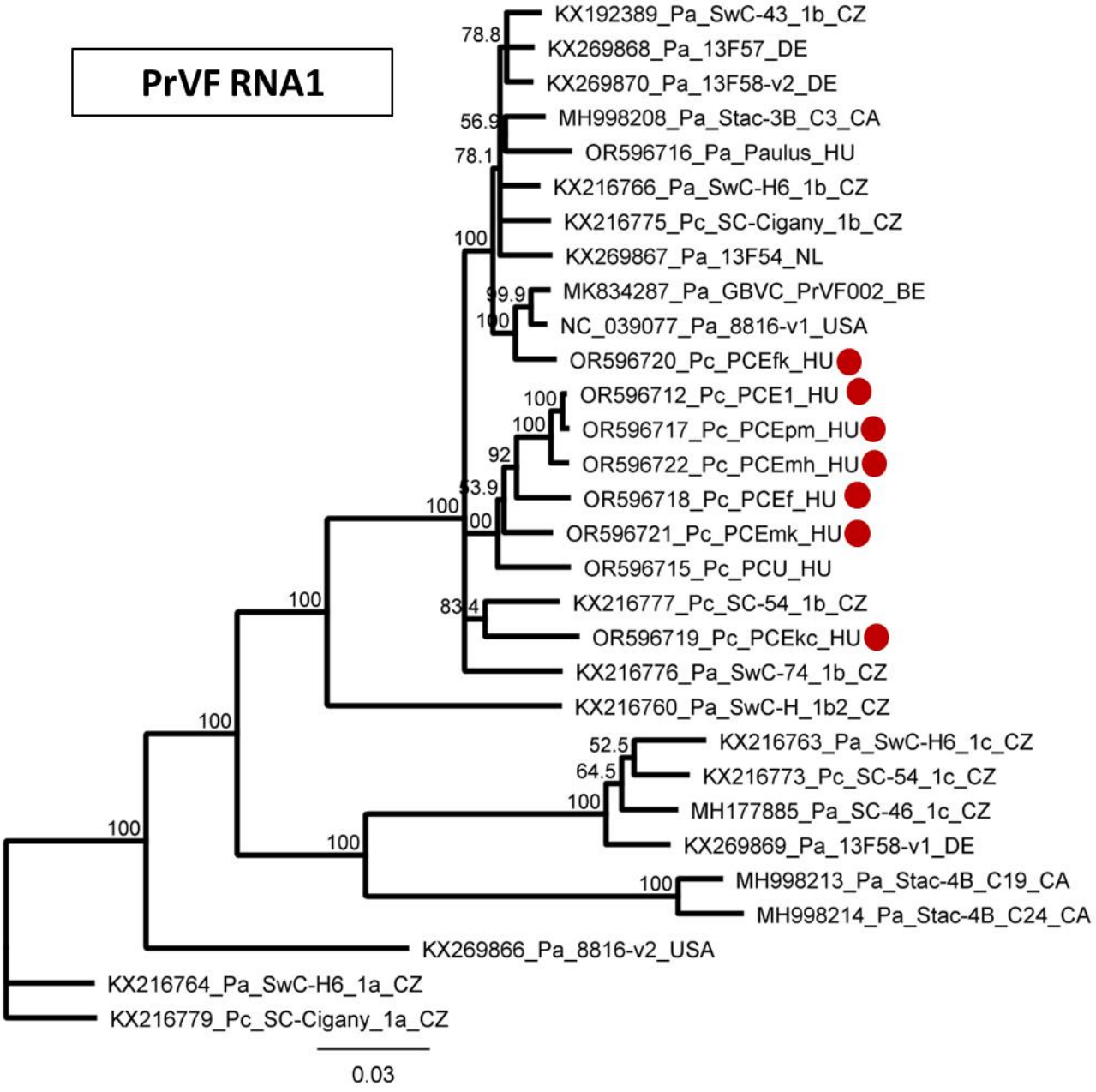


Figure 22 Phylogenetic analysis of PrVF RNA1. In red: sour cherry infected with PrVF in Hungary.

PrVF RNA2 phylogenetic tree for full sequence indicated a clustering of Hungarian variants and closely related to samples collected in Czech Republic, both from sweet and sour cherry as shown in Figure 23.

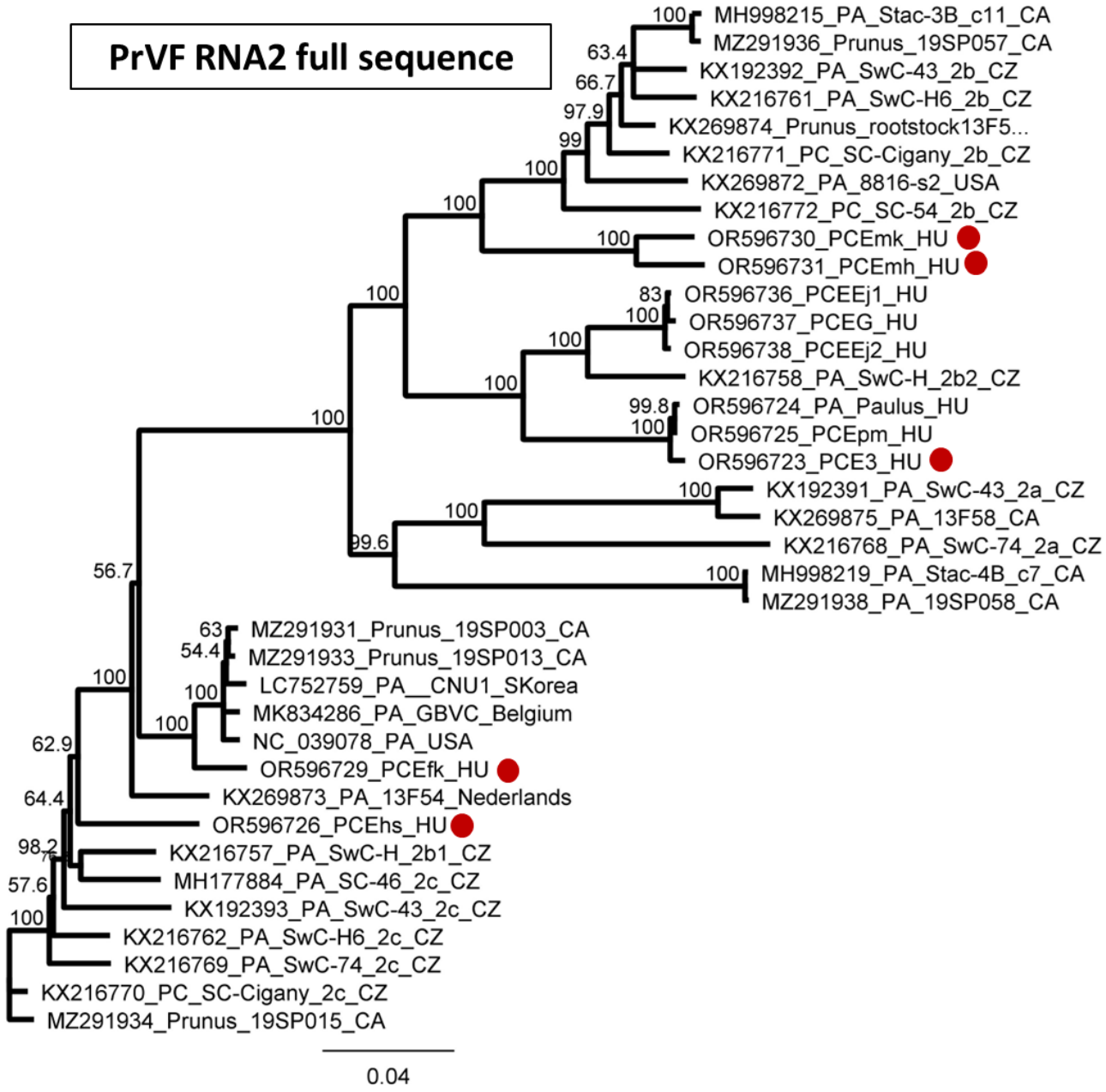


Figure 23 Phylogenetic tree for PrVF RNA2 full sequence. In red: sour cherry infected with PrVF in Hungary

PrVF RNA2 polyprotein was analysed with phylogenetic analysis, where again Hungarian variants were closely related to Czech variants found in previous research (Koloniuk et al., 2018; Šafářová et al., 2017) (Figure 24).

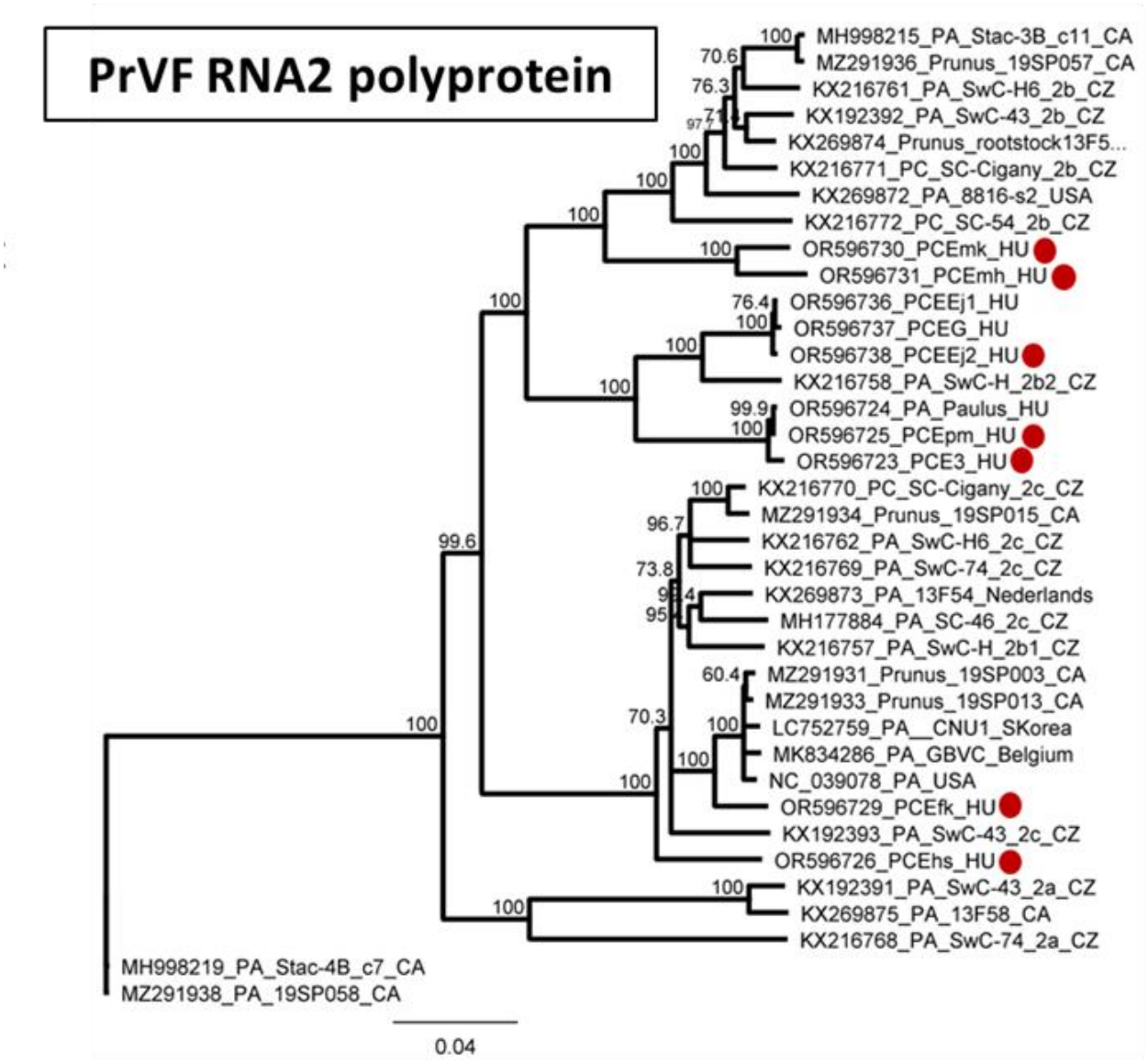


Figure 24 Phylogenetic analysis of PrVF RNA2 polyprotein. In red: sour cherry infected with PrVF in Hungary.

5.10 Discussion on sour cherry genotyping and phenotyping analysis

Total polyphenolic content (TPC) was analysed with CIELab to understand the relationship between the polyphenols and color of sour cherry. Following the results from previous literature, 'Érdi Bőtermő', 'Üjfehértói Furtös' and 'Pipacs1' had similar results regarding the TPC values suggested in this study (Papp et al., 2010). In our work, the highest TPC was found in 'Pipacs1' (650.5 mgGAE/100g fresh cherries) while the lowest was found in 'Kantorjanosi 3' (122.7 mgGAE/100g fresh cherries). 'Érdi Bőtermő' and 'Érdi Jubileum' also showed low TPC content (172.53 and 280.82 mgGAE/100g fresh cherries respectively), in line with previously published research (Khoo et al., 2011; Najafzadeh et al., 2014; Papp et al., 2010; Pissard et al., 2016; Viljevac et al., 2012). Fruit color measured with CIELab suggested that the darkest cultivar above all was 'Bosnyák', while the lightest accession between all was 'Hortenzia Királynője'. 'Bosnyák' was referred as a very dark fruit in previous research, when a population was compared for its antioxidant and anthocyanin content (Veres et al., 2006). In their study, Veres and colleagues understood that dark varieties such as 'Bosnyák' had high melatonin accumulation, making it a good candidate for further studies. Colorimetric data coincide with several previously published papers. Viljevac and colleagues (2012) in their research identified similar values for 'Cigány' cultivar, as in our research shown for 'Cigánymeggy 7', which is an accession derived from 'Cigány' population. 'Érdi Jubileum' was indicated as a very firm accession in a previous study (Najafzadeh et al., 2014). This characteristic is very important for the marketability of sour cherry in local and even more in international markets, making the fruit suitable for long-distance travel. 'Érdi Jubileum' and 'Érdi Bőtermő' were both tested in previous study, where weight and firmness of the fruit were comparable to results of our study. In our study, however, 'Pipacs1' was indicated as the firmest accession. The differences between accession firmness may be variable due to the collection and ripening time, which is critical for the identification of selectable cultivars. Principal component analysis (PCA) between categories indicated that there is a positive correlation between L, a, b, Chroma and hue, as expected. Negative correlation was observed with Ctifl, and UPOV as expected, since all the scaling systems have higher scores for darker colors, while CIELab is at the opposite end. In contrast with Viljevac (Viljevac et al., 2012), no negative correlation appeared when comparing TPC with L, a, b, Chroma and hue. Instead, as indicated by Najafzadeh and colleagues (2014), positive correlation is observed between TPC and fruit firmness. Probably this is due to the selection of the cultivars, accessions, and availability of color variants within the study. As in this study, in the same research was observed negative correlation between total polyphenolic content and total sugar, while in our study negative correlation between TPC and SSC appear similar. Negative correlation is observed as well between TPC and fruit weight. Fruit weight was highest recorded for 'Mogyoródi kései' (6.62 g). This variety could be investigated deeper for selection of fruit size in the future and integration in the breeding program. TPC content in fruits was comparatively similarly to other studies, as previously said, however fruit development may be influenced by other factors such as yearly waterfall, soil nutrients availability and temperature.

Genetic background information as well might influence fruit development, thus indicating that fruits from different accessions may differ from other grown in different countries (Magri et al., 2023; Wojdyło et al., 2014). Fruit size was analysed with two known SSR markers, CPSCT038 and BPPCT034. The first showed a range of alleles compared to previously published data. The biggest fruit observed were from 'Mogyoródi kései' (22.4 mm and 6.9 g), while the smallest fruits were bored by 'Helyi sötét' (14.4 mm and 2.3 g). The SSR marker CPSCT038 observed sizes were similar to the previously reported by our research in sweet cherry (Szilágyi et al., 2022), where in sour cherry haplotypes of 185, 190 and 204 bp were observed. Allelic combination of 185 and 204

bp seems to be correlated with bigger fruits, while 190 bp appears to be correlated with smaller fruits. Pearson correlation indicates that the CPSCT038$_{185}$ and CPSCT038$_{204}$ positively correlate with bigger fruits, while CPSCT038$_{190}$ correlate with smaller fruits. The second SSR marker, BPPCT034 showed a wider range of allelic variation, from 204 to 251 bp. Pearson correlation indicated a strong correlation between BPPCT034$_{204}$, BPPCT034$_{208}$, BPPCT034$_{230}$ alleles and smaller fruits. The BPPCT034$_{237}$ allele showed instead a positive correlation with fruit size and weight. These results are in line with our previous observation of sweet cherry (Szilágyi et al., 2022), as well as with Zhang and colleagues (2010), where it was suggested that the underlying mechanism relies on the functionality of fruit size, based on the cell number rather than size, since BPPCT034 and CPSCT038 flank the *PavCNR12* gene. Thus, it appears that BPPCT034 and CPSCT038 can be used in sour cherry fruit to characterize and select different fruit sizes. Linear regression of CPSCT038 and BPPCT034 indicate that both have a significant influence on fruit diameter (r^2 = 0.57), length (r^2 = 0.52), thickness (r^2 =0.45), ellipsoid area (r^2 =0.2), fruit weight (r^2 = 0.57), seed weight (r^2 =0.09), and pulp weight (r^2 =0.04). Standardized beta coefficient for allele BPPCT034$_{230}$ negatively correlated with all the mentioned characteristics, while BPPCT034$_{204}$ positively correlated with fruit size characteristics in sour cherry. CPSCT038$_{204}$ and CPSCT038$_{185}$ both have a positive correlation with fruit size and weight characteristics. Multiple regression analysis (MRA) was performed as in previous sweet cherry study, even though not as strong as previously indicated with different markers (Ganopoulos et al., 2011).

Fruit color in sour cherry when compared with Pav-Rf-SSR indicated a range between 347 to 353 bp. Only in MRA it was possible to observe that the beta coefficient was showing significant correlation with L (r^2 =0.01), a (r^2 =0.02), b (r^2 = 0.01), Chroma (r^2 = 0.02) and hue (r^2 = 0), however correlation was shown for Pav-Rf-SSR$_{343}$ which positively correlated with both flesh (r^2 = 0.18) and juice color (r^2 =0.15). As indicated in Čmejla and colleagues' work (2021), Pav-Rf-SSR$_{343}$ correlate with dark color fruits and can be used to predict the mahogany (red to dark-red) class of cultivars. The second fruit color marker tested, Ma039a showed an allelic range between 157 to 205 bp. A good correlation for L characteristic was also observed where Ma039a$_{157}$ negatively correlated with dark fruit color while Ma039a$_{175}$ positively correlated with lightness. Negative correlation could be observed also for Ma039a$_{175}$ for Ctifl scale flesh and juice color, while Ma039a$_{205}$ negatively correlates for Ctifl and flesh color only. MRA table indicated that Ma039a$_{175}$ has a positive correlation with L (r^2 =0.02), a (r^2 = 0.02), b (r^2 =0.12), and Chroma (r^2 =0.13), while it appears to be negatively correlating with flesh (r^2 = 0.16) and juice color (r^2 =0.10). This result is in accordance with the previous analysis of Ma039a in sweet cherry, where Ma039a was indicated as a possible candidate to identify lighter skin fruit in sweet cherry (Sooriyapathirana et al., 2010). The third and last tested SSR marker for fruit color was LG3_13.146, which exhibited only one allele a 218 bp. As mentioned in previous literature, 218 bp allele was defined as D1 haplotype and present in sour cherries with darker fruit color (Stegmeir et al., 2015). In our analysis, L, a, b and Chroma were negatively correlating with LG3_13.146$_{218}$ and juice color was instead positively correlated as well as Ctifl, suggesting indeed that LG3_13.146$_{218}$ can be regarded as a possible indicator of darker fruits in sour cherry.

5.11 Discussion on sour cherry virus analysis

5.11.1 High throughput sequencing and bioinformatic analysis

Three libraries were analysed in this study, where all samples were from sour cherry. Sequenced reads were in range of 18 million, with a trimmed read count within 18 million. Non-redundant reads ranged between 1 to 1.6 million reads, with single contigs ranging between 1.5 to 2.6 thousand. This indicated a high number of sequences, commonly found in stone fruits. Filtering out the redundant reads, it was possible to maintain a good number in the different libraries, with quite many contigs. Size distribution of the sRNA was of 21, 22 and 24 nt long, as indicated in previous research (Pooggin 2018). The size distribution is a result of DICER enzyme activity in the host, where the enzyme can slice the substrate dsRNA into specific sized products. Slight differences in the size distribution can arise from the different activity of DCL1, DCL2, DCL3 and DCL4. In case of the three sour cherry library, size distribution peaks were at 22 nt, due to the fact that probably environmental factors such as temperature at the moment of sample collection were the same. When compared to viral genome, many contigs were observed in several libraries matching cherry virus A, prunus necrotic ringspot virus and prunus virus F. These, and the values above threshold – more than 200 RPM and coverage of the viral genome above 60% - gave us some preliminary results regarding the viruses we could find in each library. These preliminary data were then followed by validation through RT-PCR, to confirm the presence of each virus in each sample.

5.11.2 RT-PCR validation strategy

To validate the presence of those viruses PNRSV, CVA and PrVF, several primers were designed. Primers design strategy was to design primer from the reference genome of CVA (NC_003689) PNRSV RNA3 (NC_004364) PrVF RNA1 (NC_039077) and PrVF RNA2 (NC_039078). Diagnostic primers used for CVA were from previously published CVamp-Fm/Rm (Baráth et al., 2018). PNRSV diagnostic primers called PNcip F/R were developed by Jarosova and Kundu (2010). Two diagnostic primers were instead selected for PrVF since it has high variability within isolates; PVF1-CAF/CAR developed by James and colleagues (2018), and Fab-R2_1808_F/_2546_R developed by Villamor (2017). Furthermore, primer design strategy integrated consensus sequences as references to better understand different variants. This work was conducted particularly on PrVF since the variance of the virus was quite high. Fifteen different primers were used for PrVF, five of which were used for sequencing (primers table). A previously tested primer pair was also inserted in this study since it had an almost fully amplified product, called PrVF2_5_F/_R (Koloniuk et al., 2018). With this strategy established, it was possible to analyse the samples, starting from the pools and then with the single individuals. The sRNA HTS could detect the infection with PNRSV, however detection of CVA and PrVF only RT-PCR validation confirmed the spread of those viruses. The cause could be in the latency of those viruses, as identified before (Jaksai-Czotter et al., 2018, Demian et al., 2020).

5.11.3 RT-PCR validation of three libraries and individuals

Individual libraries were tested for the presence of CVA, PNRSV and PrVF. PNRSV was found in all three libraries. This does not surprise us since the three libraries are located in the same open field and PNRSV has been proven to be pollen-transmissible (EFSA, 2014). CVA was found in all libraries except for 3_PC_E3. It is still not completely clear which vector might be involved in the spread of CVA, however grafting from infected propagation material has been indicated as one of the reasons for the disease spread (Baráth et al., 2018; Jelkmann, 1995). To our surprise, PrVF was also widespread. To our knowledge, this was the first time that PrVF has been found in Hungary. After identifying each of the three viruses in the libraries, we observed the distribution in each sample. CVA and PNRSV were found in more than 30% and 40% of samples analysed

respectively, indicating a possible problem for propagation material. Meanwhile, PrVF was found at a staggering rate of 81% (PrVF RNA2) in each individual library, suggesting that it is widespread in sour cherry. Mixed infection of whole three viruses was observed in 4 cases, as well as CVA and PrVF. Single infection was the most common. This could suggest that even if less frequent, in case of mixed infection the three viruses can coexist on the host, as well as infect separately. The latter seems to be more often represented according to our study.

5.11.4 Comparison between 2021 and 2023 sweet and sour cherry collection

In 2023 cherry samples were recollected and analysed for libraries 1_PC_E1, 2_PC_E2 and 3_PC_E3, this time including all the plants of the same cultivar available. In 2023 fifty-eight samples collected showed 31% CVA infection, 45% PNRSV infection and 38% PrVF. This indicated that 1 out of three plants were infected with CVA in a comparable size both in 2021 and 2023, almost half of the plants were infected with PNRSV and PrVF instead seems to be present in fewer samples in 2023. This last information could be misinterpreted since the high variance of PrVF has been a challenge to identify PrVF in the first place. We expect to find more PrVF-positive samples in the future since it could be more widespread than we think, as suggested in previous research (Koloniuk et al., 2018). Furthermore, when collecting samples in 2023, 19 trees were found dead. It is not clear if the presence and distribution of the mentioned viruses might have had an impact on the decline and death of the trees. Trees are planted in open fields without irrigation and thus are quite prone to biotic and abiotic stress whole year round, hence we cannot exclude external causes as main or at least contributing factors to the trees' death.

5.11.5 Phylogenetic analysis of CVA, PNRSV and PrVF

Phylogenetic analysis indicated that CVA had a high identity percentage for the MP coding region, where all the sour cherries were represented in Group I. PNRSV RNA3 analysis suggested that all the variants were present in group PV96, closely related to Slovakian and Spanish variants, indicating a common origin. Infected trees were kept at the same field plot, suggesting that the source of origin of infection is indeed local, and the spreading could have come from infected trees in the same area. PNRSV is a pollen-transmitted virus, and it has been detected in open fields in cultivar candidate testing orchards where trees were allowed to bloom. The infection could have easily spread by pollen of a single PNRSV-infecting tree. In the case of PrVF, analysing the 5' and 3' UTR coding regions it was possible to observe that besides the high divergency in the protein-coding region, the UTR coding regions of 5' and 3' are quite different, due to the diverse sets of INDEL (insertion-deletion) regions. The clustering of PrVF RNA1 region show the close relationship between Hungarian variants, and with Czech variants. With more variants probably the diversity would have increased inside the group. When analysing the PrVF RNA2, the divergency was much higher, both in the case of the full sequencing of PrVF RNA2 and the PrVF RNA2 polyprotein coding region. Since PrVF shows a high divergency and variance, a single set of primers could have not been used in this case, making more difficult the identification of variances. In the future, by using more sets of primers it would be possible to amplify more variants, but no more information would be added except the length of the new variants. Since PrVF was found in different mixed infections, no information could be retrieved about the effect or symptomatology related to the presence of PrVF. The high variability of PrVF suggests that its latent infection does not trigger a fast and severe host response now, but it will leave possibilities for frequent mutation which could accumulate and lead to a very diverse genome composition. PrVF poses a risk since it is not yet understood completely, and more strict containment and thorough test should be carried on in isolators and screen houses.

6 CONCLUSIONS AND RECOMMENDATIONS

Sour cherry is a valuable horticultural crop, holding a significant market margin in Hungary. For this reason, the analysis focused on the main two characteristics of fruit size and color, two factors that are crucial for breeders, farmers, and consumers' choices. Overall, the analysis showed lower correlation and significance level when compared with previous studies (Ganopoulos et al., 2011; Szilágyi et al., 2022). This could be explained due to the difference between diploid and tetraploid samples we faced. Due to the qualitative traits, the sour cherry polygenicity could be expressed differently when compared with diploid cherry. As shown in sour cherry analysis for fruit size characteristics, sour cherry results suggest the presence of more intense background noises and the possibility of having more QTLs to be included in the color analysis.

MRA analysis for fruit color showed generally a lower standardised beta coefficient, when compared with fruit size, probably because the colorimetric analysis did not show linkage between the physical characteristics and the markers tested. TPC play an important role in fruit development, and in the future, it would be important to evaluate other components such as antioxidant activity and volatile compounds.

Colorimetric and TPC analysis suggested possible future candidates for the close future breeding programme. Data collected indicated not only that the TPC and color positively correlated, but as well TPC positively correlate with acidity and firmness. Further study will be conducted to evaluate the correlation between soil, climatic factors, genetic profile over the chemical composition of Hungarian sour cherry.

We believe that this study helped us to characterize candidates in the germplasm material for future breeding selection. Since breeding is a vital part of the development for new and better varieties, we would emphasize that this work is useful for Hungarian breeders in particular, giving them a tool to use and shortening the time of selection with early screenings. The development of new varieties with desirable characteristics, such as fruit color, size, and flavour profile, have been a strong focus in Hungarian breeding, as well as resistance to biotic and abiotic stress factor (Apostol, 2014; Kappel et al., 2012; Schuster et al., 2017).

So far, no breeding program in cherry focuses on the development of virus-resistant varieties, nor it has been identified as a source of resistance to stone fruit viruses naturally occurring (Kappel et al., 2012). Viruses are being continuously discovered at a high rate since the development and further improvement of advanced diagnostic tools such as high throughput sequencing (Hou et al., 2020). For the first time, PrVF was detected in Hungary. Since its first description, it has been suggested that multiple strains may be found on the same tree (Villamor et al., 2017). The presence and distribution of several strains in the same host might influence the development of symptoms, having in some cases a positive or negative effect on the health condition of the host, with fluctuation of symptoms intensity, complexity and heterogeneity of viral population throughout the year (Maliogka et al., 2018). PrVF high number of variants is something peculiar, as observed in a recent article by Koloniuk and colleagues. In the same study, a high incidence of PrVF was observed as well, exclusively in cherry plants (Koloniuk et al., 2018).

In our case, we focused on sour cherry plantations, where the incidence of PrVF in the open field was generally high where several strains were found. Sour cherry plants exhibited high levels of infection, and to our surprise mother plants that were propagated and tested negative, were found positive in isolator screen houses. To ensure that screen houses are a hundred percent virus-tested, routine screening should be adopted. Furthermore, as suggested by several authors, meristematic propagation in combination with thermotherapy and chemotherapy offers a valid alternative to

propagate virus-tested plant material, however, this method is still challenging in cherry (Ahmad et al., 2020; Preece, 2003; Szabó et al., 2024a). Regulation of plant viruses should also come in aid, since the effect and presence of PrVF in infected plants is not completely understood. Aphids are known vectors of *Fabaviruses* and may be harbouring the PrVF virus, thus being a vector; we should also point out that it is well known that PrVF can be transmitted by infected grafting material (Koloniuk et al., 2018; Villamor et al., 2017). Implementation of insect-proof nets and the use of sterilized tools seem also necessary to maintain virus-tested areas. Regarding CVA and PNRSV, only the latter has been associated with symptoms, however, the varieties of symptoms shown by the plants can be only used as a preliminary indicator of the presence of the virus (Kamenova & Borisova, 2021). PNRSV can be found quite commonly since it is transmitted by infected pollen (Milusheva & Borisova, 2005). According to EFSA, the voluntary plant certification schemes significantly reduce the risk of infection by PNRSV, thus it is indicated as a limited-impact virus (EFSA, 2014). Routine screening may be an easy solution and early detection might help mitigate the presence of PNRSV in open fields. CVA does not show any symptoms and is generally considered latent (Noorani et al., 2022). Symptoms could be related to the presence of other viruses, which could enhance or mitigate the symptomatology (Komorowska et al., 2020). It seems difficult thus to eliminate a latent virus, however, the use of early screening could help us to reduce the spread of this virus. CVA has been found to spread through infected graft material only, thus the chances of control are quite high if the virus is identified in time. Elimination of infected material and use of thermo and chemotherapy to ensure virus-tested plant material seems to be a possibility in the future (Szabó et al., 2024a, 2024b). This however cannot be said for open fields, where the viral population reservoir seems to be alive and well. CVA infection was considerably high in open fields while PNRSV seemed to be contained, suggesting pollen transmission as the main cause. Monitoring and screening populations when transferring them into screen houses seem the most probable solution for the future. High throughput sequencing may be a useful tool in the future when costs will decrease and massive screening will be easier to conduct. However, due to the sRNA limited information, only through RT-PCR validation it had been possible to identify the distribution of the viruses analysed in this work. Possibly the use of non-coding RNA or total RNA might have a better function for RNA viruses escaping the RNAi machinery of the host, as in the case of CVA and PrVF.

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 color 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 SUMMARY

In this research, we investigated the role of a gene bank collection for genetic and virus screening purposes. Germplasm plant collection has a lot of potential for future research and studies. Molecular and phenotypical analysis identified possible candidates for breeding new Hungarian sour cherry varieties, with bigger fruits and desirable color. Germplasm material from the sour cherry screening highlighted the high values of cultivars such as ‘Mogyoródi kései’, ‘Bosnyák’ and ‘Pipacs 1’ for their size, color, and secondary metabolites. SSR markers CPSCT038 and BPPCT034 appear to be useful for molecular screening of sour cherry fruit size. Furthermore, Ma039a, Pav-Rf-SSR and LG_13.146 can be used for fruit color screening and selection. This could significantly shorten the selection period and identify rapidly new varieties to be introduced in the market. Virological screening of sour cherry indicated that the presence of PNRSV and CVA should be further monitored since the level of infection was high in an open field, and thus infected trees removed. PNRSV is known to be transmitted by pollen thus increasing the challenges of a complete removal in open fields. The analysis performed with high throughput sequencing and subsequent RT-PCR validation, where PrVF was found for the first time in Hungary, is raising concerns about the health status of plant material and the possibility of infection both nationally and internationally through the exportation of infected plant material. Early diagnostic of plant material could positively reduce the proliferation of infected material, maintaining the plants’ health and the propagation of virus-tested plant material. Monitoring of plant material can positively impact the plant propagation process, for this, it is recommended to be implemented in the future.

ÖSSZEFOGLALÁS

Ebben a kutatásban egy génbanki gyűjtemény szerepét vizsgáltuk genetikai és vírusszűrési célokra. A csíraplazma növénygyűjtemény sok lehetőséget rejt magában a jövőbeli kutatások és vizsgálatok számára. A molekuláris és fenotípusos elemzéssel lehetséges jelölteket azonosítottunk új magyar meggyfajták nemesítésére, amelyek nagyobb gyümölcsökkel és kívánatos színnel rendelkeznek. A meggy szűrésből származó csíraplazma kiemelte többek közt a 'Mogyoródi kései', 'Bosnyák' és 'Pipacs 1' fajták magas értékét a méret, a szín és a másodlagos anyagcseretermékek tekintetében. A CPSCT038 és BPPCT034 SSR markerek használhatónak tűnnek a meggy gyümölcsméretének molekuláris szűrésére. Továbbá a Ma039a, a Pav-Rf-SSR és az LG_13.146 használható a gyümölcs színének szűrésére és szelekciójára. Ez jelentősen lerövidítheti a szelekciós időszakot és gyorsan azonosíthat új fajokat, melyek bevezethetők a piacra. A meggy virológiai szűrése alapján a PNRSV és a CVA jelenlétét tovább kell figyelni, mivel a fertőzés mértéke magas volt a nyílt területen, ezért a fertőzött fákat el kellett távolítani. A PNRSV ismereteink szerint pollen útján terjed, ami növeli a nyílt termesztő területeken történő teljes eltávolítás kihívásait. A nagy áteresztőképességű szekvenálással végzett elemzés és az azt követő RT-PCR validálás, ahol a PrVF-et először került azonosításra Magyarországon, aggodalomra ad okot a növényi anyag egészségügyi állapota és a fertőzött növényi anyag exportja révén történő fertőzés lehetősége miatt mind nemzeti, mind nemzetközi szinten. A növényi anyag korai diagnosztikája pozitívan csökkenthetné a fertőzött anyag terjedését, megőrizve a növények egészségét és a vírus vizsgálattal rendelkező növényi anyag szaporítását. A növényanyag monitorozása pozitívan befolyásolhatja a szaporítási folyamatot, ezért a jövőben javasolt ennek megvalósítása.

SOMMARIO

In questo studio abbiamo analizzato il ruolo di una collezione di banca genica per lo screening genetico e virale. La collezione del germoplasma vegetale ha un grande potenziale per ricerche e studi futuri. L'analisi molecolare e fenotipica ha identificato possibili candidati per la selezione di nuove varietà di amarene ungheresi, con frutti più grandi e di colore desiderabile. Il materiale di germoplasma proveniente dallo screening delle amarene ha evidenziato cultivar come 'Mogyoródi kései', 'Bosnyák' e 'Pipacs 1' per quanto riguarda dimensioni, colore e metaboliti secondari. I marcatori moleculari SSR CPSCT038 e BPPCT034 appaiono utili per lo screening molecolare delle dimensioni dei frutti di amarena. Inoltre, Ma039a, Pav-Rf-SSR e LG_13.146 possono essere utilizzati per lo screening e la selezione del colore dei frutti. Ciò potrebbe abbreviare significativamente il periodo di selezione e identificare rapidamente nuove varietà da introdurre sul mercato. Lo screening virologico delle amarene ha indicato che la presenza di PNRSV e CVA i quali dovrebbero essere ulteriormente monitorati, poiché il livello di infezione é risultato elevato in campo aperto. Le popolazioni di PNRSV e CVA dovrebbero essere ulteriormente monitorate e gli alberi infetti rimossi. Il PNRSV è noto per essere trasmesso dal polline, il che aumenta le difficoltà di una rimozione completa in campo aperto. L'analisi effettuata con il sequenziamento high throughput e la successiva validazione RT-PCR, dove PrVF è stato trovato per la prima volta in Ungheria, solleva preoccupazioni sullo stato di salute del materiale vegetale e sulla possibilità di infezione sia a livello nazionale che internazionale attraverso l'esportazione di materiale vegetale infetto e non monitorato. La diagnosi precoce del materiale vegetale potrebbe ridurre positivamente la proliferazione di materiale infetto, mantenendo la salute delle piante e la propagazione di materiale vegetale esente da virus. Il monitoraggio annuale del materiale vegetale può avere un impatto positivo sul processo di propagazione delle piante, per questo se ne raccomanda l'implementazione in futuro.

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9 APPENDICES

A1 Appendix: Bibliography

Acero, N., Gradillas, A., Beltran, M., García, A., & Muñoz Mingarro, D. (2019). Comparison of phenolic compounds profile and antioxidant properties of different sweet cherry (Prunus avium L.) varieties. *Food Chemistry*, *279*(December 2018), 260–271. https://doi.org/10.1016/j.foodchem.2018.12.008

Ahmad, R., Anjum, M. A., Naz, S., & Balal, R. M. (2020). Applications of molecular markers in fruit crops for breeding programs—a review. *Phyton*, *90*(1), 17–34. https://doi.org/10.32604/phyton.2020.011680

Apostol, J. (2014). New varieties and selections of the Hungarian cherry breeding program. *Acta Horticulturae*, *1020*, 83–88. https://doi.org/10.17660/actahortic.2014.1020.10

Aranzana, M. J., Decroocq, V., Dirlewanger, E., Eduardo, I., Gao, Z. S., Gasic, K., Iezzoni, A., Jung, S., Peace, C., Prieto, H., Tao, R., Verde, I., Abbott, A. G., & Arús, P. (2019). Prunus genetics and applications after de novo genome sequencing: achievements and prospects. *Horticulture Research*, *6*(1). https://doi.org/10.1038/s41438-019-0140-8

Avgelis, A., & Barba, M. (2011). CHAPTER 30: Epirus cherry virus. In Virus and Virus-Like Diseases of Pome and Stone Fruits (pp. 151-152). The American Phytopathological Society.

Balcan, D., & Erzan, A. (2004). Random model for RNA interference yields scale free network. *European Physical Journal B*, *38*(2), 253–260. https://doi.org/10.1140/epjb/e2004-00055-7

Barath, D., Czako, D., Zsovakne-hangyal, R., Varga, T., Furton, A., Omran, M., Nyerges, K., & Varallyay, E. (2018). *Comparison of biotest and smallRNA NGS as a virus diagnostics method on fruit trees*. *November*, 1–2.

Baráth, D., Jaksa-Czotter, N., Molnár, J., Varga, T., Balássy, J., Szabó, L. K., Kirilla, Z., Tusnády, G. E., Preininger, É., & Várallyay, É. (2018). Small RNA NGS revealed the presence of cherry virus A and little cherry virus 1 on apricots in hungary. *Viruses*, *10*(6), 1–12. https://doi.org/10.3390/v10060318

Barath, D., Jaksa-Czotter, N., Varga, T., & Varallyay, E. (2022). Viromes of Hungarian Peach Trees Identified by High-Throughput Sequencing of Small RNAs. *Plants*, *11*(12). https://doi.org/10.3390/plants11121591

Barba, M., Ilardi, V., & Pasquini, G. (2015). Control of pome and stone fruit virus diseases. In *Advances in Virus Research* (1st ed., Vol. 91, Issue 1). Elsevier Inc. https://doi.org/10.1016/bs.aivir.2014.11.001

Baris, E., Bedő, J., Edosa, O. S., Kiss, E., & Veres, A. (2017). Preliminary results of SSR based characterization of sour (Prunus cerasus L.) and sweet cherry (Prunus avium L.) genotypes cultivated in Hungary. *Columella : Journal of Agricultural and Environmental Sciences*, *4*(1), 31–45. https://doi.org/10.18380/SZIE.COLUM.2017.4.1.31

Baulcombe, D. (2004). RNA silencing in plants. *Nature*, *431*(7006), 356–363. https://doi.org/10.1038/nature02874

Bedő, J., Tóth-Lencsés, A. K., Kovács, Z., Pápai, B., Szőke, A., Kiss, E., & Veres, A. (2023). Microsatellite-Based Molecular Diversity in Sour Cherry Genotypes (Prunus cerasus L.) Cultivated in Hungary. *Horticulturae*, *9*(8), 892. https://doi.org/10.3390/horticulturae9080892

Bi, Y., Tugume, A. K., & Valkonen, J. P. T. (2012). Small-RNA deep sequencing reveals Arctium tomentosum as a natural host of Alstroemeria virus X and a new putative emaravirus. *PLoS ONE*, *7*(8). https://doi.org/10.1371/journal.pone.0042758

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., & Mumford, R. (2014). Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research*, *186*, 20–31. https://doi.org/10.1016/j.virusres.2013.12.007

Borowiec, K., Stachniuk, A., Szwajgier, D., & Trzpil, A. (2022). Polyphenols composition and the biological effects of six selected small dark fruits. *Food Chemistry*, *391*(February), 133281. https://doi.org/10.1016/j.foodchem.2022.133281

Bragard, C., Dehnen-Schmutz, K., Gonthier, P., Jacques, M., Jaques Miret, J. A., Justesen, A. F., MacLeod, A., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Reignault, P. L., Thulke, H., Van der Werf, W., Vicent Civera, A., Yuen, J., Zappalà, L., Candresse, T., … Rubino, L. (2019). Pest categorisation of non-EU viruses and viroids of Prunus L. *EFSA Journal*, *17*(9). https://doi.org/10.2903/j.efsa.2019.5735

Bretting, P. K., & Widrlechner, M. P. (1995). Genetic markers and horticultural germplasm management. *HortScience*, *30*(7), 1349–1356. https://doi.org/10.21273/hortsci.30.7.1349

Brewer, E., Cao, M., Gutierrez, B., Bateman, M., & Li, R. (2020). Discovery and molecular characterization of a novel trichovirus infecting sweet cherry. Virus genes, 56(3), 380-385.

Bujdosó, G., & Hrotkó, K. (2017). Cherry production. In Cherries: botany, production and uses (pp. 1-13). Wallingford UK: Cabi.

Bujdosó, G., Hrotkó, K., Feldmane, D., Giovannini, D., Demirsoy, H., Tao, R., Ercisli, S., Ertek, N., & Malchev, S. (2020). What kind of sweet cherries do the final consumers prefer? *South-Western Journal of Horticulture, Biology and Environment*, *11*(1), 37–48.

Burgyán, J., & Havelda, Z. (2011). Viral suppressors of RNA silencing. *Trends in Plant Science*, *16*(5), 265–272. https://doi.org/10.1016/j.tplants.2011.02.010

Bodin, M., Glasa, M., Verger, D., Costes, E., & Dosba, F. (2003). Distribution of the sour cherry isolate of Plum pox virus in infected Prunus rootstocks. Journal of Phytopathology, 151(11-12), 625-630.

Çağlayan, K., Roumi, V., Gazel, M., Elçi, E., Acioğlu, M., Mavric Plesko, I., ... & Massart, S. (2019). Identification and characterization of a novel Robigovirus species from sweet cherry in turkey. Pathogens, 8(2), 57.

Cai, L., Quero-García, J., Barreneche, T., Dirlewanger, E., Saski, C., & Iezzoni, A. (2019). A fruit firmness QTL identified on linkage group 4 in sweet cherry (Prunus avium L.) is associated with domesticated and bred germplasm. *Scientific Reports*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-41484-8

Cao, X., Yu, K., Liu, S., Geng, G., Li, X., & Yuan, X. (2017). First report of Cucumber mosaic virus infecting cherry (Prunus avium) in Shandong province of China. Plant Disease, 101(10), 1828-1828.

Calle, A., Serradilla, M. J., & Wünsch, A. (2021). QTL mapping of phenolic compounds and fruit color in sweet cherry using a 6+9K SNP array genetic map. *Scientia Horticulturae*, *280*(January), 109900. https://doi.org/10.1016/j.scienta.2021.109900

CDFA. (2020). *California Pest Rating Proposal for Cherry virus A*.

Çevik, B., Yardimci, N., & Çulal-Klllç, H. (2011). Detection of viruses infecting stone fruits in western Mediterranean region of Turkey. *Plant Pathology Journal*, *27*(1), 44–52. https://doi.org/10.5423/PPJ.2011.27.1.044

Cmejla, R., Zdarska, I., Suran, P., Paprstein, F., & Cmejlova, J. (2021). A new allelic discrimination real-time PCR assay for PavMYB10.1 genotyping to predict sweet cherry fruit color: a comparison with the Pav-Rf-SSR assay. *Journal of Horticultural Science and Biotechnology*, *96*(2), 260–269. https://doi.org/10.1080/14620316.2020.1837021

Cui, H., & Wang, A. (2017). An efficient viral vector for functional genomic studies of Prunus fruit trees and its induced resistance to Plum pox virus via silencing of a host factor gene. *Plant Biotechnology Journal*, *15*(3), 344–356. https://doi.org/10.1111/pbi.12629

Demian, E., Jaksa-Czotter, N., Molnar, J., Tusnady, G. E., Kocsis, L., and Varallyay, (2020) Grapevine rootstocks can be a source of infection with non-regulated viruses. European Journal of Plant Pathology 156:897-912

Deng, Z., Ma, L., Zhang, P., & Zhu, H. (2022). Small RNAs Participate in Plant–Virus Interaction and Their Application in Plant Viral Defense. *International Journal of Molecular Sciences*, *23*(2). https://doi.org/10.3390/ijms23020696

Desiderio, F., Szilagyi, S., Békefi, Z., Boronkay, G., Usenik, V., Milić, B., Mihali, C., & Giurgiulescu, L. (2023). Polyphenolic and Fruit Colorimetric Analysis of Hungarian Sour Cherry Genebank Accessions. *Agriculture*, *13*(7), 1287. https://doi.org/10.3390/agriculture13071287

Di Serio F, Ambrós S, Sano T, Flores R, Navarro B. (2018) Viroid Diseases in Pome and Stone Fruit Trees and Koch's Postulates: A Critical Assessment. Viruses.; 10(11):612. https://doi.org/10.3390/v10110612

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M. J., Poizat, C., Zanetto, A., Arús, P., & Laigret, F. (2002). Development of microsatellite markers in peach [Prunus persica (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (Prunus avium L.). *Theoretical and Applied Genetics*, *105*(1), 127–138. https://doi.org/10.1007/s00122-002-0867-7

EFSA. (2014). Scientific Opinion on the pest categorisation of Prunus necrotic ringspot virus. *EFSA Journal*, *12*(10), 3849. https://doi.org/10.2903/j.efsa.2014.3849

EPPO. (2023a). *Cherry virus A (CVA000)*. https://gd.eppo.int/taxon/CVA000

EPPO. (2023b). *Prunus necrotic ringspot virus (PNRSV0)*. https://gd.eppo.int/taxon/PNRSV0

EPPO. (2024a). *Cherry virus A*.

EPPO. (2024b). *Regulated non-quarantine pest Project*. https://rnqp.eppo.int/

Fang, X., & Qi, Y. (2015). Rnai in plants: An argonaute-centered view. In *Plant Cell* (Vol. 28, Issue 2, pp. 272–285). American Society of Plant Biologists. https://doi.org/10.1105/tpc.15.00920

FAOSTAT. (2024). *Hungarian sour cherry, peach and almond production from 2012 to 2022*. https://www.fao.org/faostat/en/#data/QCL

Fanigliulo, A., Comes, S., Maiss, E., Piazzolla, P., & Crescenzi, A. (2003). The complete nucleotide sequence of Plum pox virus isolates from sweet (PPV-SwC) and sour (PPV-SoC) cherry and their taxonomic relationships within the species. Archives of virology, 148, 2137-2153.

Faust, M., & Surány, D. (1997). Origin and dissemination of cherry. In *Horticultural Reviews* (pp. 263–270).

Ferretti, G., Bacchetti, T., Belleggia, A., & Neri, D. (2010). Cherry antioxidants: From farm to table. *Molecules*, *15*(10), 6993–7005. https://doi.org/10.3390/molecules15106993

Gambino, G., Perrone, I., & Gribaudo, I. (2008). A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. *Phytochemical Analysis*, *19*(6), 520–525. https://doi.org/10.1002/pca.1078

Ganopoulos, I. V., Kazantzis, K., Chatzicharisis, I., Karayiannis, I., & Tsaftaris, A. S. (2011). Genetic diversity, structure and fruit trait associations in Greek sweet cherry cultivars using microsatellite based (SSR/ISSR) and morpho-physiological markers. *Euphytica*, *181*(2), 237–251. https://doi.org/10.1007/s10681-011-0416-z

Głowacka, A., Rozpara, E., & Hallmann, E. (2020). The dynamic of polyphenols concentrations in organic and conventional sour cherry fruits: Results of a 4-year field study. Molecules, 25(16), 3729.

Hadersdorfer, J., Neumüller, M., Treutter, D., & Fischer, T. C. (2011). Fast and reliable detection of Plum pox virus in woody host plants using the Blue LAMP protocol. *Annals of Applied Biology*, *159*(3), 456–466. https://doi.org/10.1111/j.1744-7348.2011.00510.x

Halász, J., Balogh, E., Makovics-Zsohár, N., & Hegedűs, A. (2019). S -genotyping of Hungarian sour cherry cultivars. *Acta Horticulturae*, *1231*(1231), 161–166. https://doi.org/10.17660/ActaHortic.2019.1231.27

Hamilton, A. J., & Baulcombe, D. C. (1975). A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants. In *Proc. Natl. Acad. Sci. U.S.A* (Vol. 124). Univ. of California Press. www.sciencemag.org/feature/data/l042575.shl

Hily, J. M., Scorza, R., Webb, K., & Ravelonandro, M. (2005). Accumulation of the long class of siRNA is associated with resistance to Plum pox virus in a transgenic woody perennial plum tree. *Molecular Plant-Microbe Interactions*, *18*(8), 794–799. https://doi.org/10.1094/MPMI-18-0794

Hou, W., Li, S., & Massart, S. (2020). Is There a "Biological Desert" With the Discovery of New Plant Viruses? A Retrospective Analysis for New Fruit Tree Viruses. *Frontiers in Microbiology*, *11*(November), 1–15. https://doi.org/10.3389/fmicb.2020.592816

Isogai, M., Aoyagi, J., Nakagawa, M., Kubodera, Y., Satoh, K., Katoh, T., ... & Yoshikawa, N. (2004). Molecular detection of five cherry viruses from sweet cherry trees in Japan. Journal of General Plant Pathology, 70, 288-291.

Jaksa-Czotter, N., Molnar, J. Szabó, E. Demina, E., Kontra, L., Baksa, I., Szittya, G., Kocsis, L., Deak, T., Bisztray, G., Tusnady, G. E., Burgyan, J., and Varallyay, E., (2018). "NGS of Virus Derived Small RNAs as a Diagnostic Method Used to Determine Viromes of Hungarian Vineyards" Frontiers in Microbiology 9

Jaksa-Czotter, N., Nagyné Galbács, Z., Jahan, A., Demián, E., & Várallyay, É. (2024). Viromes of Plants Determined by High-Throughput Sequencing of Virus-Derived siRNAs. In *Viral Metagenomics: Methods in Molecular Biology* (pp. 179–198). Humana. https://doi.org/10.1007/978-1-0716-3515-5_13

James, D., & Mukerji, S. (1996). Comparison of ELISA and immunoblotting techniques for the detection of cherry mottle leaf virus. Annals of applied biology, 129(1), 13-23.

James, D. (2003). Nucleotide sequence analysis and detection of Cherry rasp leaf virus. In XIX International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops-Fruit Tree Diseases 657 (pp. 99-101).

James, D., Varga, A., & Lye, D. (2014). Analysis of the complete genome of a virus associated with twisted leaf disease of cherry reveals evidence of a close relationship to unassigned viruses in the family Betaflexiviridae. Archives of virology, 159, 2463-2468.

James, D., Phelan, J., & Jesperson, G. (2018). First Report of Prunus virus F Infecting Sweet Cherry (Prunus avium cv. Staccato) in Canada. *Plant Disease*, *102*(7), 1468. https://doi.org/10.1094/PDIS-12-17-1883-PDN

James, D., Phelan, J., & Sanderson, D. (2019). Detection by high-throughput sequencing and molecular characterization of complexes of fabaviruses infecting 'Staccato (R) ' sweet cherry (Prunus avium) in Canada. *Canadian Journal of Plant Pathology*, *41*(4), 519–534. https://doi.org/10.1080/07060661.2019.1566179

Jarošová, J., & Kundu, J. K. (2010). Simultaneous detection of stone fruit tree viruses by one-step multiplex RT-PCR. *Scientia Horticulturae*, *125*(1), 68–72. https://doi.org/10.1016/j.scienta.2010.02.011

Jelkmann, W. (1995). Cherry virus A: cDNA cloning of dsRNA, nucleotide sequence analysis and serology reveal a new plant capillovirus in sweet cherry. *Journal of General Virology*, *76*(8), 2015–2024. https://doi.org/10.1099/0022-1317-76-8-2015

Jelkmann, W., Sanderson, D., Berwarth, C., & James, D. (2018). First detection and complete genome characterization of a Cherry (C) strain isolate of plum pox virus from sour cherry (Prunus cerasus) in Germany. *Journal of Plant Diseases and Protection*, *125*(3), 267–272. https://doi.org/10.1007/s41348-018-0155-7

Jiao, J., Kong, K., Han, J., Song, S., Bai, T., Song, C., Wang, M., Yan, Z., Zhang, H., Zhang, R., Feng, J., & Zheng, X. (2021). Field detection of multiple RNA viruses/viroids in apple using a CRISPR/Cas12a-based visual assay. *Plant Biotechnology Journal*, *19*(2), 394–405. https://doi.org/10.1111/pbi.13474

Johnson, A. M. A., Gopal, D. V. R. S., & Sudhakar, C. (2021). GM Crops for Plant Virus Resistance: A Review. In *Genetically Modified Crops*. https://doi.org/10.1007/978-981-15-5932-7_11

Kamenova, I., & Borisova, A. (2021). Molecular variability of the coat protein gene of prunus necrotic ringspot virus on sweet and sour cherry in Bulgaria. *Journal of Plant Pathology*, *103*(1), 97–104. https://doi.org/10.1007/s42161-020-00659-3

Kamenova, I., Borisova, A., & Popov, A. (2019). Incidence and genetic diversity of Prune dwarf virus in sweet and sour cherry in Bulgaria. *Biotechnology and Biotechnological Equipment*, *33*(1), 980–987. https://doi.org/10.1080/13102818.2019.1637278

Kaponi M, Kyriakopoulou PE, Hadidi A. (2024). Viroids of the Mediterranean Basin. Viruses; 16(4):612. https://doi.org/10.3390/v16040612

Kappel, F., Fisher-Fleming, B., & Hogue, E. (1996). Fruit characteristics and sensory attributes of an ideal Sweet Cherry. *HortScience*, *31*(3), 443–446. https://doi.org/10.21273/hortsci.31.3.443

Kappel, F., Granger, A., Hrotkó, K., & Schuster, M. (2012). Cherry. In *Fruit Breeding* (pp. 459–504). Springer US. https://doi.org/10.1007/978-1-4419-0763-9_13

Kashif, M., Pietilä, S., Artola, K., Jones, R. A. C., Tugume, A. K., Mäkinen, V., & Valkonen, J. P. T. (2012). Detection of viruses in sweetpotato from Honduras and Guatemala augmented by deep-sequencing of small-RNAs. *Plant Disease*, *96*(10), 1430–1437. https://doi.org/10.1094/PDIS-03-12-0268-RE

Katsiani, A., Maliogka, V. I., Katis, N., Svanella-Dumas, L., Olmos, A., Ruiz-García, A. B., ... & Candresse, T. (2018). High-throughput sequencing reveals further diversity of little cherry virus 1 with implications for diagnostics. Viruses, 10(7), 385.

Kelley, R. D. (1983). Location of prune dwarf and prunus necrotic ringspot viruses in sweet cherry pollen and fruit.

Kelley, D. S., Adkins, Y., & Laugero, K. D. (2018). A review of the health benefits of cherries. *Nutrients*, *10*(3), 1–22. https://doi.org/10.3390/nu10030368

Khadivi-Khub, A. (2015). Physiological and genetic factors influencing fruit cracking. *Acta Physiologiae Plantarum*, *37*(1). https://doi.org/10.1007/s11738-014-1718-2

Khoo, G. M., Clausen, M. R., Pedersen, B. H., & Larsen, E. (2011). Bioactivity and total phenolic content of 34 sour cherry cultivars. *Journal of Food Composition and Analysis*, *24*(6), 772–776. https://doi.org/10.1016/j.jfca.2011.03.004

Kołodziejczyk, K., Sójka, M., Abadias, M., Viñas, I., Guyot, S., & Baron, A. (2013). Polyphenol composition, antioxidant capacity, and antimicrobial activity of the extracts obtained from industrial sour cherry pomace. Industrial crops and products, 51, 279-288.

Koloniuk, I., Sarkisova, T., Petrzik, K., Lenz, O., Přibylová, J., Fránová, J., Špak, J., Lotos, L., Beta, C., Katsiani, A., Candresse, T., & Maliogka, V. I. (2018). Variability studies of two Prunus-infecting fabaviruses with the aid of high-throughput sequencing. *Viruses*, *10*(4), 1–13. https://doi.org/10.3390/v10040204

Komorowska, B., Hasiów-Jaroszewska, B., & Czajka, A. (2020). Occurrence and detection of little cherry virus 1, little cherry virus 2, cherry green ring mottle virus, cherry necrotic rusty mottle virus, and cherry virus A in stone fruit trees in Poland. *Acta Virologica*, *64*(01), 100–103. https://doi.org/10.4149/av_2020_112

Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., & Simon, R. (2009). Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. *Virology*, *388*(1), 1–7. https://doi.org/10.1016/j.virol.2009.03.024

Lenz, O., Přibylová, J., Fránová, J., Koloniuk, I., & Špak, J. (2017). Identification and characterization of a new member of the genus Luteovirus from cherry. Archives of Virology, 162(2), 587-590.

Liberti, D., Marais, A., Svanella-Dumas, L., Dulucq, M. J., Alioto, D., Ragozzino, A., ... & Candresse, T. (2005). Characterization of Apricot pseudo-chlorotic leaf spot virus, a novel Trichovirus isolated from stone fruit trees. Phytopathology, 95(4), 420-426.

Lin, L., Li, R., Bateman, M. et al. (2013). Development of a multiplex TaqMan real-time RT-PCR assay for simultaneous detection of Asian prunus viruses, plum bark necrosis stem pitting associated virus, and peach latent mosaic viroid. Eur J Plant Pathol 137, 797–804https://doi.org/10.1007/s10658-013-0289-1

Lindbo, J. A. (2012). A historical overview of RNAi in plants. In *Methods in Molecular Biology* (Vol. 894, pp. 1–16). Humana Press Inc. https://doi.org/10.1007/978-1-61779-882-5_1

Magri, A., Malorni, L., Cozzolino, R., Adiletta, G., Siano, F., Picariello, G., Cice, D., Capriolo, G., Nunziata, A., Di Matteo, M., & Petriccione, M. (2023). Agronomic, Physicochemical, Aromatic and Sensory Characterization of Four Sweet Cherry Accessions of the Campania Region. *Plants*, *12*(3), 610. https://doi.org/10.3390/plants12030610

Maliogka, V. I., Minafra, A., Saldarelli, P., Ruiz-García, A. B., Glasa, M., Katis, N., & Olmos, A. (2018). Recent advances on detection and characterization of fruit tree viruses using high-throughput sequencing technologies. *Viruses*, *10*(8), 1–23. https://doi.org/10.3390/v10080436

Mandic, B., Matić, S., Rwahnih, M. Al, Jelkmann, W., & Myrta, A. (2007). VIRUSES OF SWEET AND SOUR CHERRY IN SERBIA. *Journal of Plant Pathology*, *89*(1), 103–108.

Marais, A., Faure, C., Svanella-Dumas, L., & Candresse, T. (2008). First Report of Cherry virus A in Prunus mume in China. Plant disease, 92(11), 1589-1589.

Marais, A., Faure, C., Mustafayev, E., Barone, M., Alioto, D., & Candresse, T. (2015). Characterization by deep sequencing of Prunus virus T, a novel Tepovirus infecting Prunus species. Phytopathology, 105(1), 135-140.

Marais, A., Šafářová, D., Navrátil, M., Faure, C., Cornaggia, D., Brans, Y., Suchá, J., & Candresse, T. (2020). Complete genome sequence of cherry virus T, a novel cherry-infecting tepovirus. *Archives of Virology*, *165*(7), 1711–1714. https://doi.org/10.1007/s00705-020-04656-w

Massart, S., Candresse, T., Gil, J., Lacomme, C., Predajna, L., Ravnikar, M., Reynard, J. S., Rumbou, A., Saldarelli, P., Škoric, D., Vainio, E. J., Valkonen, J. P. T., Vanderschuren, H., Varveri, C., & Wetzel, T. (2017). A framework for the evaluation of biosecurity, commercial, regulatory, and scientific impacts of plant viruses and viroids identified by NGS technologies. *Frontiers in Microbiology*, *8*(JAN). https://doi.org/10.3389/fmicb.2017.00045

Massart, S., Olmos, A., Jijakli, H., & Candresse, T. (2014). Current impact and future directions of high throughput sequencing in plant virus diagnostics. *Virus Research*, *188*, 90–96. https://doi.org/10.1016/j.virusres.2014.03.029

McDonald, D. (2008). *Lecture 4. Population Genetics II*. Https://Www.Uwyo.Edu/Dbmcd/Molmark/Lect04/Lect4.Html. https://www.uwyo.edu/dbmcd/molmark/lect04/lect4.html

Mekuria, T. A., Zhang, S., & Eastwell, K. C. (2014). Rapid and sensitive detection of Little cherry virus 2 using isothermal reverse transcription-recombinase polymerase amplification. *Journal of Virological Methods*, *205*, 24–30. https://doi.org/10.1016/j.jviromet.2014.04.015

Messmer, A., Sanderson, D., Braun, G., Serra, P., Flores, R., & James, D. (2017). Molecular and phylogenetic identification of unique isolates of hammerhead viroid-like RNA from 'Pacific Gala' apple (Malus domestica) in Canada. Canadian Journal of Plant Pathology, 39(3), 342–353. https://doi.org/10.1080/07060661.2017.1354334

Milusheva, S. A., & Borisova, A. Z. (2005). The Incidence of Prunus Necrotic Ringspot and Prune Dwarf Viruses in Prunus Species in South Bulgaria. *Biotechnology & Biotechnological Equipment*, *19*(sup1), 42–45. https://doi.org/10.1080/13102818.2005.10817260

Mnejja, M., Garcia-Mas, J., Howad, W., Badenes, M. L., & Arús, P. (2004). Simple-sequence repeat (SSR) markers of Japanese plum (Prunus salicina Lindl.) are highly polymorphic and transferable to peach and almond. *Molecular Ecology Notes*, *4*(2), 163–166. https://doi.org/10.1111/j.1471-8286.2004.00603.x

Myrta, A., Di Terlizzi, B., Savino, V., & Martelli, G. P. (2003). Virus diseases affecting the Mediterranean stone fruit industry: a decade of surveys. Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region. Options Méditerranéennes: Série B. Etudes et Recherches, 45, 15-23.

Najafzadeh, R., Arzani, K., Bouzari, N., & Hashemi, J. (2014). Identification of new Iranian sour cherry genotypes with enhanced fruit quality parameters and high antioxidant properties. *New Zealand Journal of Crop and Horticultural Science*, *42*(4), 275–287. https://doi.org/10.1080/01140671.2014.918044

Navratil, M., Safarova, D., Karesova, R., & Petrzik, K. (2005). First incidence of Plum pox virus on apricot trees in China. Plant Disease, 89(3), 338-338.

Nawaz, H. H., Umer, M., Bano, S., Usmani, A., & Naseer, M. (2014). A research review on Tomato bushy stunt virus disease complex. J. Nat. Sci. Res, 4, 18-23.

Németh, M. (1986). *Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees*. Akadémiai Kiadó.

Noorani, M. S., Khan, J. A., & Khursheed, S. (2022). Molecular characterization of cherry virus a and prunus necrotic ringspot virus and their variability based on nucleotide polymorphism.

Archives of Phytopathology and Plant Protection, *55*(4), 387–404. https://doi.org/10.1080/03235408.2021.1914358

Olmos, A., Boonham, N., Candresse, T., Gentit, P., Giovani, B., Kutnjak, D., Liefting, L., Maree, H. J., Minafra, A., Moreira, A., Nakhla, M. K., Petter, F., Ravnikar, M., Rodoni, B., Roenhorst, J. W., Rott, M., Ruiz-García, A. B., Santala, J., Stancanelli, G., … Massart, S. (2018). High-throughput sequencing technologies for plant pest diagnosis: challenges and opportunities. *EPPO Bulletin*, *48*(2), 219–224. https://doi.org/10.1111/epp.12472

Pallas, V., Aparicio, F., Herranz, M. C., Amari, K., Sanchez-Pina, M. A., Myrta, A., & Sanchez-Navarro, J. A. (2012). Ilarviruses of Prunus spp.: A continued concern for fruit trees. *Phytopathology*, *102*(12), 1108–1120. https://doi.org/10.1094/PHYTO-02-12-0023-RVW

Papp, N., Szilvássy, B., Abrankó, L., Szabó, T., Pfeiffer, P., Szabó, Z., Nyéki, J., Ercisli, S., Stefanovits-Bányai, É., & Hegedüs, A. (2010). Main quality attributes and antioxidants in Hungarian sour cherries: Identification of genotypes with enhanced functional properties. *International Journal of Food Science and Technology*, *45*(2), 395–402. https://doi.org/10.1111/j.1365-2621.2009.02168.x

Pavliuk, L., Udovychenko, K., Riaba, I., & Bublyk, M. (2021). Detection of sour and sweet cherry viruses in Ukraine. *Agronomy Research*, *19*(X).

Peace, C., Bassil, N., Main, D., Ficklin, S., Rosyara, U. R., Stegmeir, T., Sebolt, A., Gilmore, B., Lawley, C., Mockler, T. C., Bryant, D. W., Wilhelm, L., & Iezzoni, A. (2012). Development and Evaluation of a Genome-Wide 6K SNP Array for Diploid Sweet Cherry and Tetraploid Sour Cherry. *PLoS ONE*, *7*(12). https://doi.org/10.1371/journal.pone.0048305

Pedisić, S., Levaj, B., Verica, D. U., Škevin, D., & Babojelić, M. S. (2009). Color parameters and total anthocyanins of sour cherries (Prunus Cerasus L.) during ripening. *Agriculturae Conspectus Scientificus*, *74*(3), 259–262.

Pérez-Sánchez, R., Morales-Corts, M. R., & Gómez-Sánchez, M. Á. (2017). Sour and duke cherry viruses in South-West Europe. *Phytopathologia Mediterranea*, *56*(1), 62–69. https://doi.org/10.14601/Phytopathol_Mediterr-15326

Pfeilstetter, E., Kunze, L., & Zinkernagel, V. (1996). Viral twig necrosis of sweet cherry. Modes of transmission and spread of petunia asteroid mosaic virus (PeAMV). Annals of applied biology, 128(2), 285-301.

Pissard, A., Lateur, M., Baeten, V., Magein, H., Dupont, P., Tabart, J., Pincemail, J., & Kevers, C. (2016). Determination of total phenolic compound content and antioxidant activity in cherry species and cultivars. *Journal of Berry Research*, *6*(1), 81–91. https://doi.org/10.3233/JBR-150109

Pooggin, M. M. (2018). Small RNA-omics for plant virus identification, virome reconstruction, and antiviral defense characterization. *Frontiers in Microbiology*, *9*(NOV), 1–20. https://doi.org/10.3389/fmicb.2018.02779

Potter, D. (2012). Basic Information on the Stone Fruit Crops. In C. Kole & A. G. Abbott (Eds.), *Genetics, Genomics and Breeding of Stone Fruits* (p. 418). Taylor & Francis.

Poudel, B., & Scott, S. W. (2017). A report of cherry rusty mottle-associated virus in South Carolina. Australasian Plant Disease Notes, 12, 1-4.

Preece, J. E. (2003). *A Century of Progress with Vegetative Plant Propagation*. *38*(5), 1015–1025.

Přibylová, J., Lenz, O., Fránová, J., Koloniuk, I., & Špak, J. (2020). Comparison of the incidence of viruses within germplasm, orchards and wild cherry trees in the Czech Republic. *Annals of Applied Biology*, *176*(2), 138–146. https://doi.org/10.1111/aab.12577

Quero-García, J., Lezzoni, A., Puławska, J., & Lang, G. (Eds.). (2017). Cherries: botany, production and uses. Cabi.

Rodrigues, L. C., Morales, M. R., Fernandes, A. J. B., & Ortiz, J. M. (2008). Morphological characterization of sweet and sour cherry cultivars in a germplasm bank at Portugal. *Genetic Resources and Crop Evolution*, *55*(4), 593–601. https://doi.org/10.1007/s10722-007-9263-0

Rozsnyay, Z., & Apostol, J. (2005). Breeding for sweet and sour cherry disease resistance in Hungary. *Acta Horticulturae*, *667*, 117–122. https://doi.org/10.17660/ActaHortic.2005.667.15

Rubio, M., Martínez-Gómez, P., Marais, A., Sánchez-Navarro, J. A., Pallás, V., & Candresse, T. (2017). Recent advances and prospects in Prunus virology. *Annals of Applied Biology*, *171*(2), 125–138. https://doi.org/10.1111/aab.12371

Sabanadzovic, S., Ghanem-Sabanadzovic, N. A., Rowhani, A., Grant, J. A., & Uyemoto, J. K. (2005). Detection of Cherry virus A, Cherry necrotic rusty mottle virus and Little cherry virus 1 in California orchards. Journal of Plant Pathology, 173-177.

Šafářová, D., Faure, C., Marais, A., Suchá, J., Paprštein, F., Navrátil, M., & Candresse, T. (2017). First Report of Prunus virus F Infecting Sour Cherry in the Czech Republic. *Plant Disease*, *101*(10), 1828. https://doi.org/10.1094/PDIS-04-17-0469-PDN

Santala, J., & Valkonen, J. P. T. (2018). Sensitivity of small RNA-based detection of plant viruses. *Frontiers in Microbiology*, *9*(MAY). https://doi.org/10.3389/fmicb.2018.00939

Šarić, A., Sobočanec, S., Balog, T., Kušić, B., Šverko, V., Dragović-Uzelac, V., ... & Marotti, T. (2009). Improved antioxidant and anti-inflammatory potential in mice consuming sour cherry juice (Prunus Cerasus cv. Maraska). Plant foods for human nutrition, 64, 231-237.

Schuster, M., Apostol, J., Iezzoni, A., Jensen, M., & Milatović, D. (2017). Sour cherry varieties and improvement. In *Cherries: botany, production and uses* (pp. 95–116). CABI. https://doi.org/10.1079/9781780648378.0095

Schröder, M., & Petruschke, M. (2010). Occurrence of Little cherry virus-1 on Prunus species in the State of Baden-Württemberg, Germany. Julius-Kühn-Archiv, (427), 268.

Shulaev, V., Korban, S. S., Sosinski, B., Abbott, A. G., Aldwinckle, H. S., Folta, K. M., Iezzoni, A., Main, D., Arús, P., Dandekar, A. M., Lewers, K., Brown, S. K., Davis, T. M., Gardiner, S. E., Potter, D., & Veilleux, R. E. (2008). Multiple models for Rosaceae genomics. *Plant Physiology*, *147*(3), 985–1003. https://doi.org/10.1104/pp.107.115618

Singh, K., Dardick, C., & Kumar Kundu, J. (2019). RNAi-Mediated Resistance Against Viruses in Perennial Fruit Plants. *Plants*, *8*(10), 359. https://doi.org/10.3390/plants8100359

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.

Soltani, N., Hayati, J., Babaei, G., & Ebrahim Qomi, M. (2013). Serological and molecular detection opfr une dwarf virus infecting stone fruits of Charmahal-va-Bakhtiari province, a central region of Iran. *International Journal of Plant Biology*, *4*(1), 14–17. https://doi.org/10.4081/pb.2013.e4

Soltész, M., Nyéki, J., Szabó, Z., & Papp, J. (2003). Hungaricum as a quality of fruits and fruit products. *International Journal of Horticultural Science*, *9*(2). https://doi.org/10.31421/ijhs/9/2/396

Sooriyapathirana, S. S., Khan, A., Sebolt, A. M., Wang, D., Bushakra, J. M., Lin-Wang, K., Allan, A. C., Gardiner, S. E., Chagné, D., & Iezzoni, A. F. (2010). QTL analysis and candidate gene

mapping for skin and flesh color in sweet cherry fruit (Prunus avium L.). *Tree Genetics and Genomes*, *6*(6), 821–832. https://doi.org/10.1007/s11295-010-0294-x

Sottile, F., Caltagirone, C., Giacalone, G., Peano, C., & Barone, E. (2022). Unlocking Plum Genetic Potential: Where Are We At? *Horticulturae*, *8*(2), 1–23. https://doi.org/10.3390/horticulturae8020128

Stegmeir, T., Cai, L., Basundari, F. R. A., Sebolt, A. M., & Iezzoni, A. F. (2015). A DNA test for fruit flesh color in tetraploid sour cherry (Prunus cerasus L.). *Molecular Breeding*, *35*(7), 1–10. https://doi.org/10.1007/s11032-015-0337-y

Szabó, L. K., Desiderio, F., Kirilla, Z., Hegedűs, A., Várallyay, É., & Preininger, É. (2024a). A mini-review on in vitro methods for virus elimination from Prunus sp. fruit trees. *Plant Cell, Tissue and Organ Culture*, *156*(2), 1–11. https://doi.org/10.1007/s11240-023-02670-9

Szabó, L. K., Desiderio, F., Kirilla, Z., Hegedűs, A., Várallyay, É., & Preininger, É. (2024b). Elimination of cherry virus A from Prunus domestica 'Besztercei Bt. 2' using in vitro techniques. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *157*(2), 45. https://doi.org/10.1007/s11240-024-02770-0

Szilágyi, S., Horváth-Kupi, T., Desiderio, F., & Bekefi, Z. (2022). Evaluation of sweet cherry (Prunus avium L.) cultivars for fruit size by FW_G2a QTL analysis and phenotypic characterization. *Scientia Horticulturae*, *292*(October 2021), 110656. https://doi.org/10.1016/j.scienta.2021.110656

Tahzima, R., Foucart, Y., Massart, S., & De Jonghe, K. (2019). First report of Prunus virus F infecting sweet cherry cultivars using high-throughput sequencing in Belgium. *New Disease Reports*, *40*(1), 7–7. https://doi.org/10.5197/j.2044-0588.2019.040.007

Tamisier, L., Haegeman, A., Foucart, Y., Fouillien, N., Al Rwahnih, M., Buzkan, N., Candresse, T., Chiumenti, M., De Jonghe, K., Lefebvre, M., Margaria, P., Reynard, J. S., Stevens, K., Kutnjak, D., & Massart, S. (2021). Semi-artificial datasets as a resource for validation of bioinformatics pipelines for plant virus detection. *Peer Community Journal*, *1*(December). https://doi.org/10.24072/pcjournal.62

Thomsen, K. (2004). *Surface Area of an Ellipsoid*. https://www.numericana.com/answer/ellipsoid.htm#thomsen

Ulubas, C., & Ertunc, F. (2004). RT-PCR Detection and Molecular Characterization of Prunus necrotic ringspot virus Isolates Occurring in Turkey. *Journal of Phytopathology*, *152*(8–9), 498–502. https://doi.org/10.1111/j.1439-0434.2004.00886.x

UPOV. (2006). *PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS Prunus cerasus L. and Prunus x gondouinii (Poit. & Turpin) Rehder SOUR CHERRY and DUKE CHERRY*. 1–29.

UPOV. (2024). *What is UPOV?* https://www.upov.int/overview/en/index.html

Vaucheret, H. (2006). Post-transcriptional small RNA pathways in plants: Mechanisms and regulations. In *Genes and Development* (Vol. 20, Issue 7, pp. 759–771). https://doi.org/10.1101/gad.1410506

Veres, Zs., Holb, I. J., Nyéki, I. J., Szabó, Z., Remenyik, J., & Fári, M. G. (2006). High antioxidant - and anthocyanin contents of sour cherry cultivars may benefit the human health: international and Hungarian achievements on phytochemicals. *International Journal of Horticultural Science*, *12*(3). https://doi.org/10.31421/IJHS/12/3/657

Viljevac, M., Dugalic, K., Jurkovic, V., Mihaljevic, I., Tomas, V., Puskar, B., Lepedus, H., Sudar, R., & Jurkovic, Z. (2012). Relation between polyphenols content and skin color in sour cherry

fruits. *Journal of Agricultural Sciences, Belgrade*, *57*(2), 57–67. https://doi.org/10.2298/jas1202057v

Villamor, D. E. V., Pillai, S. S., & Eastwell, K. C. (2017). High throughput sequencing reveals a novel fabavirus infecting sweet cherry. *Archives of Virology*, *162*(3), 811–816. https://doi.org/10.1007/s00705-016-3141-z

ViralZone. (2024). *Ilarvirus*. https://viralzone.expasy.org/136

Wani, L. A., Jawa, P., & Khan, J. A. (2023). Development of one step colorimetric RT-LAMP assays for rapid detection of Apple mosaic virus and Prunus necrotic ringspot virus. *Journal of Virological Methods*, *316*(September 2022), 114729. https://doi.org/10.1016/j.jviromet.2023.114729

Wöhner, T. W., Emeriewen, F. O., Wittenberg, A. H. J., Nijbroek, K., Wang, R. P., Blom, E.-J., Keilwagen, J., Berner, T., Hoff, K. J., Gabriel, L., Thiefeldt, H., Almolla, O., Barchi, L., Schuster, M., Lempe, J., Peil, A., & Flachowsky, H. (2023). *The structure of the tetraploid sour cherry 'Schattenmorelle' (Prunus cerasus L.) genome reveals insights into its segmental allopolyploid nature*. https://doi.org/10.1101/2023.03.28.534503

Wojdyło, A., Nowicka, P., Laskowski, P., & Oszmiański, J. (2014). Evaluation of Sour Cherry (Prunus cerasus L.) Fruits for Their Polyphenol Content, Antioxidant Properties, and Nutritional Components. *Journal of Agricultural and Food Chemistry*, *62*(51), 12332–12345. https://doi.org/10.1021/jf504023z

Woldring, H. (1997). On the origin of plums: a study of sloe, damson, cherry plum, domestic plums and their intermediate forms. *Palaeohistoria*, *39*(0), 535–562.

Yazbek, M. M., & Al-Zein, M. S. (2014). Wild almonds gone wild: revisiting Darwin's statement on the origin of peaches. *Genetic Resources and Crop Evolution*, *61*(7), 1319–1328. https://doi.org/10.1007/s10722-014-0113-6

Zhang, G., Sebolt, A. M., Sooriyapathirana, S. S., Wang, D., Bink, M. C., Olmstead, J. W., & Iezzoni, A. F. (2010). Fruit size QTL analysis of an F1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. *Tree Genetics and Genomes*, *6*(1), 25–36. https://doi.org/10.1007/s11295-009-0225-x

Zhao, D., & Song, G. (2014). Rootstock-to-scion transfer of transgene-derived small interfering <scp>RNA</scp> s and their effect on virus resistance in nontransgenic sweet cherry. *Plant Biotechnology Journal*, *12*(9), 1319–1328. https://doi.org/10.1111/pbi.12243

A2 Appendix: Additional figures and tables

Table 5 Primers used in our study. In grey are indicated the primers that were used for diagnostic purpose. Sequencing samples were amplified with the same primers and have different sizes.

Virus	Primer Name	Primer Sequence (5'-3')	Position on the reference genome	Reference genome	Annealing temperature using Q5 or Phire	Product size (bp)	Reference
CVA	CVAmp-Fm (5400)	ATGTCGATCATACCAGTYAAG	5400-5421	NC_003689	Q5 62 / Phire 57	1391	(Barath et al., 2018)
	CVAMP-Rm (6791)	TTACCTTCTGCACCAACYAC	6791-6772				
PNRSV	PNcpinF	GAGTATTGACTTCACGACCAC	1402-1422	NC_004364	Q5 56 / Phire 60	425	(Jarošová & Kundu, 2010)
	PNcpR	CTTTCCATTCGGAGAAATTCG	1827-1807				
PrVF RNA1	PVF1-CAF	GARAGTTTCCTGARTGGATGCG	2815-2831	MK834285	Q5 65 / Phire 61.9/	450	(James et al., 2018)
	PVF1-CAR	ACACTTGGGCAACATAACTGC	3265-3245				
	PrVF1_3448_F	GGTTAGACAGGTTCCACAGAGTG	3448-3470	NC_039077	Q5 68	1998	This work
	PrVF1_5446_R	CAAATCACAGACCAGGTTGTAGG	5446-5424				
	PrVF1_232_3272_F	ATTGGCTGTCTACCGATGTTCC	3272-3293	KX216779	Q5 62	2138	This work
	PrVF1_231_233_5410_R	TTGTAGGGTCTAGTCTCTCCTC	5410-5389	KX216775			This work
PrVF RNA2	Fab-R2 1808F	ATYTTTTGGAATCCAGCTTGTC	1833-1854	MK834286	Q5 62 / Phire 60.3	738	(Villamor et al., 2017)
	Fab-R2 2546R	ATTCAAGGTTTTCAACYCGGGA	2571-2550				
	PrVF2_5_F	TAAGAGATTAAACAACCGCTTTC	5-3622	NC_039078	Q5 60	3617	(Koloniuk et al., 2018)
	PrVF2_Rev	GCTTTCACCAATTCTCAACA					
	PrVF1_4293F	AGGGCAAGTCACGATATCTGGAG	4293-4315	NC_039078	Q5 60 / Phire 65	sequencing	This work
	PrVF2_655F	ACCCGGTTCATGCGTCTTGG	655-674			sequencing	This work
	PrVF2_3284R	CAAACAGAATAAGTGTCTGCC	3284-3264			sequencing	This work
	PrVF2_2534R	CCATCCACACTGGAAGTAACAC	2534-2515			sequencing	This work
	PrVF2_1159F	ATCACTCAGTTCACTCGTGGGTC	1159-1181			sequencing	This work
	PrVF2_1184_F	TCGAATCCACTCACACTGCC	1184-1203			2064	This work
	PrVF2_3248_R	CTCCAGTAGTTCCAATTATACG	3227-3248				
	PrVF1_113_3321_F	CTGGACAGATGCATTTTTGATG	3321-3342	KX269869	Q5 62	sequencing	This work
	PrVF1_113_5471_R	CTGGATCATTCAAGGGCCCAAG	5450-5471				This work
	PrVF1_232_5316_R	CAAGCAATGCTGGATTAAGCATC	5294-5316	KX216779			This work
	PrVF2_14_F	AAACAACCGCTTTCGTTACCAG	14-35	MH998215			This work

Table 6 Fruit size measurements between 2020 and 2023. In italics are indicated the smallest values, in bold are shown the biggest values.

Variety name	Diameter (mm)	Length (mm)	Thickness (mm)	Weight (g)	Seed weight (g)	Pulp weight (g)
Bagi meggy	14.45 ± 1.15	12.62 ± 0.82	12.16 ± 1.06	2.74 ± 0.57	0.29 ± 0.15	1.23 ± 1.28
Bosnyák	16.36 ± 1.78	14.77 ± 1.96	15.30 ± 1.27	3.86 ± 0.75	0.40 ± 0.21	1.73 ± 1.81
Cigány késői	16.10 ± 1.41	14.05 ± 1.33	13.78 ± 1.03	3.32 ± 0.51	0.40 ± 0.15	1.22 ± 1.49
Cigánymeggy 7	15.74 ± 1.35	14.08 ± 0.93	13.93 ± 1.25	3.58 ± 0.60	0.36 ± 0.10	1.61 ± 1.67
Dunabogdány	19.30 ± 1.11	16.50 ± 1.19	16.00 ± 1.08	4.90 ± 0.50	0.45 ± 0.15	2.22 ± 2.26
Édes pipacs	19.99 ± 1.21	16.58 ± 1.59	15.93 ± 1.14	5.30 ± 0.75	0.45 ± 0.12	2.42 ± 2.48
Érdi bőtermő	19.80 ± 1.35	17.39 ± 1.21	17.06 ± 1.16	6.00 ± 0.61	0.41± 0.12	2.79 ± 2.84
Érdi Jubileum	19.75 ± 2.04	16.64 ± 1.51	16.32 ± 1.70	5.16 ± 0.99	0.28 ± .08	2.04 ± 2.50
Favorit	21.31 ± 1.18	17.60 ± 1.42	17.80 ± 1.17	6.76 ± 0.89	0.36 ± 0.12	2.13 ± 3.07
Fehérvári	19.40 ± 1.25	16.40 ± 1.24	16.06 ± 1.16	5.21 ± 0.56	0.44 ± 0.19	2.38 ± 2.42
Fűzlevelű kisszemű	15.59 ± 1.05	13.24 ± 0.92	12.52 ± 1.39	2.81 ± 0.40	0.33 ± 0.15	1.24 ± 1.29
Helyi sötét	*14.44 ± 0.91*	*12.43 ± 1.05*	*12.07 ± 0.77*	*2.31 ± 0.24*	*0.24 ± 0.06*	*1.04 ± 1.05*
Hortenzia királynője	14.74 ± 1.45	13.03 ± 1.20	12.70 ± 1.27	2.97 ± 0.49	0.36 ± 0.36	1.30 ± 1.38
Kantorjánosi 3	18.88 ± 1.60	16.21 ± 0.96	15.80 ± 1.76	5.05 ± 0.87	0.47 ± 0.08	2.29 ± 2.38
Késői Cigány	16.18 ± 1.49	14.10 ± 1.16	13.98 ± 1.41	3.66 ± 0.54	0.45 ± 0.18	1.60 ± 1.65
Késői parasztmeggy	19.57 ± 1.85	16.52 ± 1.29	16.21 ± 1.72	5.30 ± 0.75	0.45 ± 0.11	2.42 ± 2.50
Későn virágzó	17.12 ± 1.39	15.11 ± 1.27	14.76 ± 1.27	3.70 ± 0.71	0.37 ± 0.20	1.39 ± 1.71
Korai Cigány	15.86 ± 1.81	13.54 ± 1.36	13.04 ± 1.86	3.04 ± 0.55	0.39 ± 0.10	1.33 ± 1.39
Korai Pándy	18.47 ± 1.62	16.27 ± 1.47	15.33 ± 0.83	4.65 ± 1.09	0.48 ± 0.21	1.39 ± 2.04
Májusi hólyag	19.33 ± 1.51	16.58 ± 0.95	16.00 ± 1.75	5.20 ± 0.65	0.46 ± 0.14	2.37 ± 2.42
Mogyoródi kései	**22.48 ± 1.48**	18.91 ± 0.96	**18.63 ± 1.36**	**6.93 ± 0.75**	0.40 ± 0.09	**3.27 ± 3.32**
Nagy Gobet	20.00 ± 2.97	17.02 ± 1.89	16.62 ± 2.43	5.10 ± 1.82	0.32 ± 0.14	2.39 ± 2.69
Pándí Bb. 119	19.57 ± 1.35	16.32 ± 1.19	16.52 ± 1.03	5.30 ± 0.59	0.47 ± 0.12	2.42 ± 2.46
Pándy 279	20.28 ± 1.15	17.27± 0.77	17.12 ± 0.95	5.63 ± 0.54	0.51 ± 0.14	2.56 ± 2.60
Pándy 43	19.87 ± 1.17	17.19 ± 1.00	16.97 ± 1.12	5.22 ± 0.72	0.39 ± 0.14	2.41 ± 2.47
Péceli nagy	18.49 ± 1.32	15.98 ± 1.08	14.71 ± 1.07	4.65 ± 0.74	0.45 ± 0.16	1.40 ± 2.02
Pipacs 1	19.30 ± 1.70	17.05 ± 1.51	16.12 ± 1.96	5.02 ± 0.98	**0.59 ± 0.14**	2.21 ± 2.32
Szamosi meggy	20.73 ± 1.38	17.59 ± 1.48	15.99 ± 1.27	6.33 ± 1.01	0.49 ± 0.18	1.95 ± 2.81
Tiszabög 50/7	22.12 ± 1.35	**18.91 ± 1.21**	18.63 ± 1.87	6.69 ± 0.82	0.51 ± 0.12	3.09 ± 3.15
Újfehértói fürtös	17.13 ± 1.53	14.60 ± 1.38	14.34 ± 1.37	3.61 ± 0.60	0.36 ± 0.08	1.63 ± 1.69
Velencei kései	19.58 ± 1.82	16.92 ± 0.98	16.42 ± 1.64	5.41 ± 0.76	0.37 ± 0.09	2.52 ± 2.59

Table 7. TPC compared with CIELab values in 2022. Letters of the same column indicate significantly different values at p<0.05. Bold and italic values represent the highest and lowest, respectively. Asterisk (*) indicate the commercial cultivars.

Accession	TPC	L	a	b	C	h$^{\pm 33°}$
Bosnyák	283.59 ± 0.74	*24.52 ± 0.78 a*	*2.75 ± 1.15 a*	*-0.05 ± 0.33 a*	*2.77 ± 1.15 a*	*-36.08 ± 6.97 a*
Késői parasztmeggy	220.11 ± 0.42	25.05 ± 1.20 a,b	17.29 ± 3.46 m	5.08 ± 1.51 k	18.03 ± 3.75 k	-16.87 ± 1.35 h,i
Érdi Bőtermő*	172.53 ± 0.69	25.36 ± 0.43 b,c	7.35 ± 1.83 b	1.50 ± 0.56 c	7.50 ± 1.90 b	-21.74 ± 1.66 c
Érdi Jubileum*	280.82 ± 0.77	25.66 ± 0.90 b,c,d	4.21 ± 1.97 a	0.68 ± 0.68 b	4.28 ± 2.06 a	-25.08 ± 4.36 b
Korai Pándy	289.19 ± 0.64	25.71 ± 0.87 c,d	14.30 ± 1.51 h,i,j,k	3.88 ± 0.60 f,g,h,i,j	14.82 ± 1.62 g,h,i	-17.90 ± 0.86 e,f,g,h
Nagy Gobet	329.83 ± 0.76	26.05 ± 0.64 d,e	12.85 ± 2.60 d,e,f,g,h,i	3.32 ± 0.99 d,e,f,g,h	13.27 ± 2.76 d,e,f,g,h	-18.80 ± 1.48 e,f,g
Mogyoródi kései	247.26 ± 0.95	26.24 ± 0.83 d,e,f	12.53 ± 2.66 d,e,f,g,h	3.23 ± 1.00 d,e,f,g,h	12.94 ± 2.82 d,e,f,g	-18.86 ± 1.74 e,f,g
Korai Cigány	400.43 ± 1.24	26.27 ± 0.56 d,e,f	9.19 ± 1.44 c	2.07 ± 0.51 c	9.43 ± 1.51 c	-20.43 ± 1.33 d
Pándy Bb. 119*	213.65 ± 0.98	26.40 ± 0.53 e,f,g	11.46 ± 1.50 d,e	2.93 ± 0.52 d,e	11.83 ± 1.58 d	-18.75 ± 0.99 e,f,g
Dunabogdányi	267.71 ± 0.29	26.40 ± 0.60 e,f,g	11.79 ± 2.17 d,e,f	2.97 ± 0.79 d,e	12.16 ± 2.29 d,e	-19.03 ± 1.55 e,f,g
Pándy 43*	195.16 ± 1.11	26.43 ± 0.61 e,f,g	13.56 ± 1.79 f,g,h,i,j,k	3.52 ± 0.66 d,e,f,g,h,i	14.01 ± 1.90 e,f,g,h,i	-18.57 ± 0.85 e,f,g
Pándy 279*	202.28 ± 0.94	26.43 ± 0.86 e,f,g	12.14 ± 1.85 d,e,f,g	3.14 ± 0.62 d,e,f,g	12.54 ± 1.94 d,e,f	-18.55 ± 1.07 e,f,g
Velencei kései	245.19 ± 1.01	26.50 ± 0.63 e,f,g	13.18 ± 1.80 e,f,g,h,i,j	3.48 ± 0.68 d,e,f,g,h,i	13.63 ± 1.91 d,e,f,g,h,i	-18.30 ± 1.04 e,f,g,h
Cigány Késői	270.39 ±2.87	26.51 ± 0.65 e,f,g	12.72 ± 2.62 d,e,f,g,h,i	3.34 ± 0.96 d,e,f,g,h,i	13.15 ± 2.78 d,e,f,g	-18.51 ± 1.16 e,f,g
Késői Cigány	241.71 ± 0.47	26.51 ± 0.85 e,f,g	12.38 ± 2.87 d,e,f,g	3.13 ± 1.15 d,e,f,g	12.78 ± 3.07 d,e,f	-19.15 ± 1.69 d,e,f
Cigánymeggy 7*	294.43 ± 0.49	26.52 ± 0.60 e,f,g	11.68 ± 2.11 d,e	2.99 ± 0.73 d,e,f	12.06 ± 2.22 d,e	-18.81 ± 1.16 e,f,g
Fehérvári	296.12 ± 0.70	26.55 ± 0.54 e,f,g	13.56 ± 1.94 f,g,h,i,j,k	3.61 ± 0.77 d,e,f,g,h,i	14.03 ± 2.07 e,f,g,h,i	-18.23 ± 1.09 e,f,g,h
Édes Pipacs	297.35 ± 0.73	26.69 ± 0.63 e,f,g	13.81 ± 1.80 g,h,i,j,k	3.74 ± 0.76 e,f,g,h,i	14.31 ± 1.93 f,g,h,i	-17.98 ± 1.09 e,f,g,h
Újfehértói fürtös*	466.19 ± 0.37	26.77 ± 0.61 e,f,g	12.74 ± 1.86 d,e,f,g,h,i	3.19 ± 0.67 d,e,f,g,h	13.13 ± 1.96 d,e,f,g	-19.08 ± 1.09 d,e,f
Májusi hólyag	256.16 ± 0.51	26.83 ± 0.72 f,g	14.68 ± 2.08 j,k	4.06 ± 0.87 h,i,j	15.23 ± 2.24 h,i	-17.70 ± 1.22 f,g,h
Későn virágzó	293.32 ± 0.74	26.92 ± 0.75 f,g	15.01 ± 2.49 k,l	4.22 ± 1.11 i,j	15.60 ± 2.70 i,j	-17.51 ± 1.39 g,h
Bagi meggy	313.28 ± 0.75	26.94 ± 0.54 f,g	11.30 ± 2.46 d	2.81 ± 0.94 d	11.65 ± 2.61 d	-19.43 ± 2.06 d,e
Péceli nagy	281.76 ± 0.71	27.04 ± 0.54 g	14.64 ± 1.53 j,k	3.94 ± 0.56 g,h,i,j	15.16 ± 1.62 h,i	-17.97 ± 0.69 e,f,g,h
Kántorjánosi 3*	*122.76 ± 0.97*	27.11 ± 0.99 g,h	14.38 ± 3.45 i,j,k	3.89 ± 1.40 f,g,h,i,j	14.90 ± 3.70 g,h,i	-18.20 ± 1.43 e,f,g,h
Helyi Sötét	315.29 ± 0.82	27.68 ± 1.37 h,i	16.39 ± 5.11 l,m	4.62 ± 2.24 j,k	17.05 ± 5.52 j,k	-18.03 ± 2.93 e,f,g,h
Tiszabög 50/7	436.87 ± 0.86	27.94 ± 0.96 i	18.91 ± 2.87 n	5.84 ± 1.33 l	19.80 ± 3.12 l	-16.04 ± 1.52 i
Fűzlevelű Kisszemű	398.72 ± 0.42	28.25 ± 0.68 i	19.89 ± 2.17 n	6.12 ± 0.97 l	20.81 ± 2.36 l	-16.00 ± 0.95 i
Szamosi meggy	192.59 ± 0.93	30.21 ± 2.07 j	21.91 ± 4.39 o	8.46 ± 2.45 m	23.50 ± 4.97 m	-12.24 ± 1.85 j
Pipacs1*	**650.57 ± 1.41**	30.72 ± 1.51 j	**26.45 ± 4.05 p**	9.69 ± 2.50 n	**28.18 ± 4.64 n**	-13.18 ± 2.15 j
Hortenzia Királynője	179.11 ± 0.93	**31.89 ± 3.02 k**	25.65 ± 4.11 p	**11.09 ± 3.46 o**	28.01 ± 5.04 n	**-10.17 ± 3.76 k**

Table 10. Molecular markers for fruit size tested in sour cherry accessions. Indicated is the accession name, the allelic combination of BPPCT0034 and CPSCT038 compared with fruit size characteristics.

BPPCT034+CPSCT038					
Accessions	**Allelic combination observed**	**Diameter (mm)**	**Length (mm)**	**Thickness (mm)**	**SA (cm^2)**
Helyi sötét	226-226-237-251-190-190-190-190	*14.44 ± 0.91 a*	*12.43 ± 1.05 a*	*12.07 ± 0.77 a*	*10.59 ± 10.75 a*
Hortenzia királynője	208-208-222-237-190-190-204-204	14.74 ± 1.45 a,b	13.03 ± 1.20 a,b	12.70 ± 1.27 a,b	11.47 ± 11.72 a, b
Bagi meggy, Korai Cigány, Cigány késői	208-208-226-230-190-190-190-190	15.43 ± 1.65 b,c	13.36 ± 1.32 b	12.95 ± 1.53 b	11.56 ± 12.62 a, b
Fűzlevelű kisszemű	218-218-218-218-190-190-190-190	15.59 ± 1.05 c,d	13.24 ± 0.92 b	12.52 ± 1.39 a,b	11.94 ± 12.12 a, b, c
Cigánymeggy 7	208-208-226-237-185-185-185-185	15.74 ± 1.35 c,d	14.08 ± 0.93 c	13.93 ± 1.25 c	13.39 ± 13.64 a, b, c, d
Késői Cigány	208-208-226-230-185-185-185-185	16.18 ± 1.49 c,d	14.10 ± 1.16 c	13.98 ± 1.41 c	13.70 ± 13.95 a, b, c, d
Bosnyák	204-218-226-230-190-190-204-204	16.36 ± 1.78 d,e	14.77 ± 1.96 c,d	15.30 ± 1.27 e,f	15.14 ± 15.64 a, b, c, d
Későn virágzó	204-204-226-251-190-190-190-190	17.12 ± 1.39 e	15.11 ± 1.27 d	14.76 ± 1.27 d,e	12.89 ± 15.53 a, b, c
Újfehértói fürtös	204-226-230-237-185-185-204-204	17.13 ± 1.53 e	14.60 ± 1.38 c,d	14.34 ± 1.37 c,d	14.90 ± 15.37 a, b, c, d
Korai Pándy	208-208-226-237-190-190-204-204	18.47 ± 1.62 f	16.27 ± 1.47 e	15.33 ± 0.83 e,f	11.70 ± 16.84 a, b
Kantorjanosi 3	208-208-226-237-185-185-204-204	18.88 ± 1.60 f,g	16.21 ± 0.96 e	15.80 ± 1.76 f,g	18.13 ± 18.55 b, c, d, e
Pipacs1	204-204-237-251-190-190-204-204	19.30 ± 1.70 g,h	17.05 ± 1.51 f,g,h	16.12 ± 1.96 f,g	19.34 ± 20.01 c, d, e

Fehérvári	224-224-237-237-185-185-185-185	19.40 ± 1.25 g,h	16.40 ± 1.24 e,f	16.06 ± 1.16 f,g	18.78 ± 19.07 b, c, d, e
Dunabogdányi, Májusi hólyag, Édes pipacs	204-204-226-237-185-185-204-204	19.54 ± 1.32 g,h	16.55 ± 1.27 e,f	17.57 ± 2.10 i	18.94 ± 19.20 b, c, d, e
Késői parasztmeggy, Velencei kései	226-226-237-237-185-185-204-204	19.58 ± 1.83 g,h	16.72 ± 1.16 e,f,g	16.31 ± 1.68 g,h	19.40 ± 19.88 c, d, e
Péceli nagy, Pándy Bb. 119, Pándy 43, Nagy Gobet, Pándy 279	204-204-226-237-190-190-204-204	19.72 ± 1.84 g,h	16.81 ± 1.34 e,f,g	16.51 ± 1.64 g,h	18.42 ± 20.17 b, c, d, e
Érdi Jubileum	204-204-237-237-190-190-190-190	19.75 ± 2.04 g,h	16.64 ± 1.51 e,f	16.32 ± 1.70 g,h	16.26 ± 19.79 a, b, c, d
Érdi bőtermő	204-216-230-237-185-185-204-204	19.80 ± 1.35 h	17.39 ± 1.21 g,h	17.06 ± 1.16 h,i	20.56 ± 20.82 d, e
Favorit	204-204-230-230-190-190-204-204	21.31 ± 1.18 i	17.60 ± 1.42 h	17.80 1.17 i	14.98 ± 21.44 a, b, c, d
Szamosi meggy, Tiszabög 50/7	204-204-226-237-185-185-185-185	21.57 ± 1.52 i	18.39 ± 1.47 i	15.98 ± 1.35 f,g	19.32 ± 23.34 c, d, e
Mogyoródi kései	226-226-226-226-185-185-204-204	**22.48 ± 1.48 j**	**18.91 ± 0.96 i**	**18.63 ± 1.36 j**	**25.15 ± 25.45 e**

Table 11 BPPCT034 and CPSCT038 correlation with weight. In italics are indicated the smallest values. In bold are indicated biggest values.

BPPCT034+CPSCT038				
Accessions	**Allelic combination observed**	**Weight (g)**	**Seed weight (g)**	**Pulp weight (g)**
Helyi sötét	226-226-237-251-190-190-190-190	*2.31 ± 0.24 a*	*0.24 ± 0.06 a*	*1.04 ± 1.05 a*
Hortenzia királynője	208-208-222-237-190-190-204-204	2.81 ± 0.40 b	0.36 ± 0.36 b, c, d	1.30 ± 1.38 a, b, c
Bagi meggy, Korai Cigány, Cigány késői	208-208-226-230-190-190-190-190	2.97 ± 0.49 b	0.35 ± 0.14 b, c, d	1.26 ± 1.39 a, b
Fűzlevelű kisszemű	218-218-218-218-190-190-190-190	3.02 ± 0.59 b	0.33 ± 0.15 b, c	1.24 ± 1.29 a, b
Cigánymeggy 7	208-208-226-237-185-185-185-185	3.58 ± 0.60 c	0.36 ± 0.10 b, c, d	1.61 ± 1.67 a, b, c, d, e
Késői Cigány	208-208-226-230-185-185-185-185	3.61 ± 0.60 c	0.45 ± 0.18 d, e, f, g	1.60 ± 1.65 a, b, c, d, e
Bosnyák	204-218-226-230-190-190-204-204	3.66 ± 0.54 c	0.40 ± 0.21 c, d, e, f	1.73 ± 1.81 a, b, c, d, e
Későn virágzó	204-204-226-251-190-190-190-190	3.70 ± 0.71 c	0.37 ± 0.20 c, d, e	1.39 ± 1.71 a, b, c, d
Újfehértói fürtös	204-226-230-237-185-185-204-204	3.86 ± 0.75 c	0.36 ± 0.08 b, c, d	1.63 ± 1.69 a, b, c, d, e
Korai Pándy	208-208-226-237-190-190-204-204	4.65 ± 1.09 d	0.48 ± 0.21 f, g	1.39 ± 2.04 a, b, c, d
Kantorjanosi 3	208-208-226-237-185-185-204-204	5.02 ± 0.98 d, e	0.47 ± 0.08 e, f, g	2.29 ± 2.38 d, e, f
Pipacs1	204-204-237-251-190-190-204-204	5.05 ± 0.87 d, e	0.59 ± 0.14 h	2.21 ± 2.32 c, d, e, f
Fehérvári	224-224-237-237-185-185-185-185	5.13 ± 0.66 e	0.44 ± 0.19 d, e, f, g	2.38 ± 2.42 e, f
Dunabogdányi, Májusi hólyag, Édes pipacs	204-204-226-237-185-185-204-204	5.16 ± 0.99 e	0.46 ± 0.13 e, f, g	2.34 ± 2.38 e, f
Késői parasztmeggy, Velencei kései	226-226-237-237-185-185-204-204	5.21 ± 0.56 e	0.41 ± 0.11 c, d, e, f, g	2.47 ± 2.54 e, f, g
Péceli nagy, Pándy Bb. 119, Pándy 43, Nagy Gobet, Pándy 279	204-204-226-237-190-190-204-204	5.22 ± 1.06 e	0.42 ± 0.15 d, e, f, g	2.24 ± 2.49 d, e, f
Érdi Jubileum	204-204-237-237-190-190-190-190	5.35 ± 0.75 e	0.28 ± 0.08 a, b	2.04 ± 2.50 b, c, d, e, f
Érdi bőtermő	204-216-230-237-185-185-204-204	6.00 ± 0.61 f	0.41 ± 0.12 c, d, e, f, g	2.79 ± 2.84 f, g
Favorit	204-204-230-230-190-190-204-204	6.54 ± 0.92 g	0.36 ± 0.12 b, c, d	2.13 ± 3.07 b, c, d, e, f
Szamosi meggy, Tiszabög 50/7	204-204-226-237-185-185-185-185	6.76 ± 0.89 g	0.50 ± 0.15 g	2.52 ± 3.04 e, f, g
Mogyoródi kései	226-226-226-226-185-185-204-204	**6.93 ± 0.75 g**	**0.40 ± 0.09 c, d, e, f**	**3.27 ± 3.32 g**

Table 12. Genotype Pav-Rf-SSR compared with CIELab. In italics are indicated the smallest values. In bold are indicated the biggest values.

Pav-Rf-SSR						
Accessions	**Allelic size range**	**L**	**a**	**b**	**C**	**h$^{\pm 33°}$**
Bagi meggy, Cigánymeggy 7, Késői Cigány	343-343-351-353	*25.87 ± 2.78 a*	*14.61 ± 6.29 a*	4.61 ± 3.45 a, b	*15.38 ± 7.04 a*	10.51 ± 8.99 a, b, c
Korai Cigány	343-343-353-353	25.93 ± 2.21 a	14.79 ± 7.63 a	*4.25 ± 3.05 a*	15.41 ± 8.18 a	*9.24 ± 10.52 a, b*
Újfehértói fürtös	343-349-351-353	26.39 ± 3.51 a, b	14.76 ± 2.71 a	4.38 ± 1.68 a	15.42 ± 3.04 a	10.52 ± 9.38 a, b, c
Cigány késői, Dunabogdány, Édes pipacs, Favorit, Fehérvári, Fűzlevelű kisszemű, Helyi sötét, Mogyoródi kései, Velencei kései	351-351-353-353	26.73 ± 4.26 a, b, c	18.89 ± 8.92 b, c	6.15 ± 4.52 b, c	19.94 ± 9.86 b	10.99 ± 10.78 b, c
Későn virágzó	349-349-353-353	27.27 ± 3.17 a, b, c	21.32 ± 9.46 c	5.96 ± 2.28 b, c	22.17 ± 9.66 b	10.42 ± 14.26 a, b, c
Érdi bőtermő, Érdi Jubileum, Kantorjánosi 3, Késői paraszmeggy, Májusi hólyag, Pipacs 1, Szamosi meggy	349-349-351-353	27.45 ± 4.62 b, c	18.94 ± 10.24 b, c	6.75 ± 5.38 c	20.18 ± 11.44 b	11.11 ± 10.79 b, c
Bosnyák, Hortenzia királynője, Nagy Gobet, Pándí Bb. 119, Pándí 279	347-349-351-353	27.68 ± 5.74 b, c	18.19 ± 11.65 b	7.36 ± 7.72 c	19.78 ± 13.75 b	11.51 ± 9.89 b, c
Pándy 43, Péceli nagy	347-347-351-353	27.95 ± 5.20 c	20.21 ± 9.67 b, c	7.20 ± 6.51 c	21.57 ± 11.43 b	10.30 ± 10.15 a, b, c
Tiszabög 50/7	343-347-351-353	28.21 ± 2.78 c	**21.20 ± 4.91 b, c**	**7.37 ± 2.46 c**	**22.47 ± 5.40 b**	**13.36 ± 10.75 c**
Korai Pándy	343-349-353-353	**31.13 ± 8.67 d**	21.19 ± 11.04 b, c	7.04 ± 5.07 c	22.36 ± 12.09 b	7.44 ± 12.23 a

Table 13. Genotype Pav-Rf-SSR for colour characteristics. In italics are indicated the smallest values. In bold are indicated the biggest values.

Pav-Rf-SSR					
Accessions	**Allelic size range**	**Ctifl**	**Flesh colour**	**Juice colour**	**SSC**
Bagi meggy, Cigánymeggy 7, Késői Cigány	343-343-351-353	5.02 ± 0.70 b, c	3.63 ± 0.48 d	4.33 ± 0.47 d	21.37 ± 4.27 c, d
Korai Cigány	343-343-353-353	**5.30 ± 0.59 c**	**3.67 ± 0.48 d**	**4.33 ± 0.48 d**	**22.51 ± 2.46 d**
Újfehértói fürtös	343-349-351-353	4.88 ± 0.32 b, c	3.00 ± 0.00 c	4.00 ± 0.00 d	20.03 ± 2.61 a, b, c
Cigány késői, Dunabogdány, Édes pipacs, Favorit, Fehérvári, Fűzlevelű kisszemű, Helyi sötét, Mogyoródi kései, Velencei kései	351-351-353-353	4.30 ± 1.91 b, c	2.24 ± 0.78 a, b	3.37 ± 0.63 c	19.28 ± 3.28 a, b
Későn virágzó	349-349-353-353	4.64 ± 0.53 b, c	3.00 ± 0.00 c	4.00 ± 0.00 d	20.69 ± 5.14 b, c
Érdi bőtermő, Érdi Jubileum, Kantorjánosi 3, Késői paraszmeggy, Májusi hólyag, Pipacs 1, Szamosi meggy	349-349-351-353	4.39 ± 4.09 b, c	2.15 ± 1.05 a, b	3.21 ± 1.07 a, b, c	20.42 ± 4.27 b, c
Bosnyák, Hortenzia királynője, Nagy Gobet, Pándí Bb. 119, Pándí 279	347-349-351-353	4.08 ± 1.66 a, b	2.20 ± 1.05 a, b	2.93 ± 1.29 a	19.09 ± 3.48 a, b
Pándy 43, Péceli nagy	347-347-351-353	4.24 ± 0.62 b, c	2.40 ± 0.49 b	3.40 ± 0.49 c	*18.73 ± 2.57 a*
Tiszabög 50/7	343-347-351-353	4.18 ± 0.68 b	2.33 ± 0.48 a, b	3.33 ± 0.48 b, c	20.54 ± 2.58 b, c
Korai Pándy	343-349-353-353	*3.18 ± 0.38 a*	*2.00 ± 0.00 a*	*3.00 ± 0.00 a, b*	20.02 ± 1.58 a, b, c

Table 14. Genotype Ma039a correlated to CIELab. In italics are indicated the smallest values. In bold are indicated the biggest values.

Ma039a						
Accessions	**Allelic size range**	**L**	**a**	**b**	**Chroma**	**$h^{\pm 33°}$**
Késői parasztmeggy	157-157-157-157	*25.69 ± 3.43 a*	19.21 ± 4.88 c	6.89 ± 4.12 c	20.50 ± 6.06 c	**12.89 ± 7.69 b**
Érdi bőtermő	175-175-175-175	25.96 ± 1.26 a, b	*11.57 ± 4.18 a*	*2.90 ± 1.42 a*	*11.94 ± 4.38 a*	*7.90 ± 13.22 a*
Bagi meggy, Cigány késői, Cigánymeggy 7, Késői Cigány, Korai Cigány, Pándy Bb. 119	157-157-205-205	26.04 ± 2.76 a, b	15.59 ± 7.96 b	4.90 ± 3.81 b	16.39 ± 8.72 b	10.44 ± 9.43 b
Érdi Jubileum, Fűzlevelű kosszemű, Helyi sötét, Májusi hólyag	157-157-175-175	26.90 ± 3.55 b, c	18.42 ± 9.12 c	6.40 ± 4.58 c	19.58 ± 10.07 c	11.14 ± 9.75 b
Bosnyák, 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	27.47 ± 4.91 c	19.11 ± 9.59 c	6.64 ± 5.62 c	20.33 ± 10.93 c	11.11 ± 10.98 b
Favorit, Szamosi meggy	175-175-205-205	**31.65 ± 8.82 d**	**26.80 ± 14.34 d**	**11.24 ± 8.11 d**	**29.12 ± 16.36 d**	11.21 ± 11.91 b

Table 15. Genotype Ma039a correlated to colour characteristics. In italics are indicated the smallest values. In bold are indicated the biggest values.

Ma039a					
Accessions	**Allelic size range**	**Ctifl**	**Flesh colour**	**Juice colour**	**SSC**
Késői parasztmeggy	157-157-157-157	**5.73 ± 9.16 c**	1.67 ± 0.48 b	3.00 ± 0.00 b	*17.89 ± 2.63 a*
Érdi bőtermő	175-175-175-175	4.95 ± 0.65 b	**3.00 ± 0.00 d**	4.00 ± 0.00 d	18.92 ± 4.05 a, b
Bagi meggy, Cigány késői, Cigánymeggy 7, Késői Cigány, Korai Cigány, Pándy Bb. 119	157-157-205-205	4.87 ± 0.78 b	3.41 ± 0.69 e	**4.11 ± 0.67 d**	20.71 ± 3.75 c, d
Érdi Jubileum, Fűzlevelű kosszemű, Helyi sötét, Májusi hólyag	157-157-175-175	4.65 ± 2.84 b	2.43 ± 0.93 c	3.61 ± 0.82 c	**21.22 ± 4.26 d**
Bosnyák, 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	4.12 ± 1.50 b	2.26 ± 0.83 c	3.24 ± 0.92 b	19.47 ± 3.31 b, c
Favorit, Szamosi meggy	175-175-205-205	*3.30 ± 0.83 a*	*1.00 ± 0.00 a*	*2.00 ± 0.71 a*	20.47 ± 4.93 c, d

Table 16. Genotype LG3_13.146 correlated to colour characteristics.

Accessions	LG3_13.146 218	Flesh colour		Juice colour		SSC	
Édes pipacs, Érdi bőtermő, Érdi Jubileum, Késői parasztmeggy, Pándy Bb. 119, Pándy 279, Péceli nagy, Újfehértói fürtös	**Present**	2.58 ± 0.80		3.62 ± 0.68		19.43 ± 3.37	
Bagi meggy, Bosnyák, Cigány késői, Cigánymeggy 7, Dunabogdány, Favorit, Fehérvári, Fűzlevelű kisszemű, Helyi sötét, Hortenzia királynője, Kantorjánosi 3, Késői Cigány, Későn virágzó, Korai Cigány, Korai Pándy, Májusi hólyag, Mogyoródi kései, Nagy Gobet, Pándy 43, Pipacs 1, Szamosi meggy, Tiszabög 50/7, Velencei kései	**Absent**	2.42 ± 1.01	$p < 0.05$	3.35 ± 1.01	$p < 0.05$	20.09 ± 3.81	$p < 0.05$

Table 19. Linear regression sour cherry size and weight with BPPCT034 and CPSCT038. Individual alleles are compared to single phenotype characteristics and alleles with $p < 0.05$ are shown

Phenotype	SSR markers (alleles)	r	R^2	R^2 change	F change	Standard error	Standardized beta coefficients	*t* value	*P* value
Diameter	$BPPCT034_{208}$	0.48	0.23	0.23	521.93	2.36	-0.21	-7.61	0
	+ $BPPCT034_{218}$	0.58	0.34	0.11	278.55	2.19	-0.39	-19.55	0
	+ $BPPCT034_{251}$	0.66	0.44	0.1	314.67	2.02	-0.3	-16.12	0
	+ $BPPCT034_{230}$	0.67	0.45	0.02	47.79	1.99	-0.41	-17.87	0
	+ $BPPCT034_{222}$	0.69	0.47	0.02	69.32	1.96	-0.27	-12.79	0
	+ $CPSCT038_{204}$	0.7	0.49	0.02	64.25	1.92	0.24	12.15	0
	+ $BPPCT034_{237}$	0.72	0.51	0.02	81.76	1.88	-0.36	-13.92	0
	+ $BPPCT034_{226}$	0.73	0.54	0.02	89.84	1.83	-0.22	-11.4	0
	+ $BPPCT034_{204}$	0.74	0.55	0.01	54.28	1.8	0.24	9.79	0
	+ $CPSCT038_{185}$	0.75	0.57	0.02	62.66	1.77	0.16	7.92	0
Length	$BPPCT034_{208}$	0.45	0.21	0.21	454.28	1.92	-0.11	-3.83	0.0001
	+ $BPPCT034_{218}$	0.55	0.3	0.09	230.33	1.81	-0.33	-15.57	0
	+ $BPPCT034_{251}$	0.61	0.38	0.08	217.39	1.71	-0.24	-12.07	0
	+ $CPSCT038_{204}$	0.63	0.39	0.02	51.22	1.68	0.26	12.23	0
	+ $BPPCT034_{222}$	0.65	0.42	0.02	66.21	1.65	-0.26	-11.7	0
	+ $BPPCT034_{230}$	0.66	0.44	0.02	71.49	1.62	-0.44	-16.47	0
	+ $BPPCT034_{226}$	0.68	0.46	0.02	55.1	1.6	-0.18	-8.29	0
	+ $BPPCT034_{237}$	0.69	0.48	0.02	62.64	1.57	-0.35	-12.63	0
	+ $BPPCT034_{204}$	0.7	0.5	0.02	72.33	1.54	0.29	11.22	0
	+ $CPSCT038_{185}$	0.72	0.52	0.02	82.97	1.5	0.17	8.04	0
	+ $BPPCT034_{216}$	0.72	0.52	0	13.9	1.5	0.08	3.73	0.0002
Thickness	$BPPCT034_{208}$	0.43	0.19	0.19	398.23	2.04	-0.13	-3.85	0
	+ $BPPCT034_{218}$	0.5	0.25	0.06	151.09	1.96	-0.29	-12.64	0
	+ $BPPCT034_{251}$	0.58	0.33	0.08	215.8	1.85	-0.23	-10.83	0
	+ $CPSCT038_{204}$	0.59	0.35	0.02	55.2	1.82	0.27	11.87	0
	+ $BPPCT034_{222}$	0.61	0.37	0.02	56.19	1.79	-0.21	-8.82	0
	+ $BPPCT034_{230}$	0.62	0.38	0.01	23.13	1.78	-0.37	-12.57	0
	+ $BPPCT034_{237}$	0.63	0.4	0.02	47.29	1.76	-0.36	-11.83	0
	+ $BPPCT034_{226}$	0.64	0.41	0.01	43.74	1.74	-0.12	-4.66	0
	+ $BPPCT034_{204}$	0.66	0.43	0.02	53.06	1.71	0.28	9.75	0
	+ $CPSCT038_{190}$	0.67	0.45	0.02	56.36	1.68	-0.15	-6.31	0
	+ $BPPCT034_{216}$	0.67	0.45	0	8.54	1.68	0.09	3.49	0
	+ $BPPCT034_{224}$	0.67	0.45	0	5.94	1.68	0.06	2.44	0.015
Ellipsoid area (cm^2)	$BPPCT034_{208}$	0.11	0.01	0.01	48.43	18.43	-0.09	-5.11	0
	+ $CPSCT038_{185}$	0.15	0.02	0.01	30.68	18.36	0.09	5.43	0
	+ $CPSCT038_{204}$	0.16	0.02	0	14.68	18.32	0.06	3.83	0
Weight	$BPPCT034_{208}$	0.44	0.19	0.19	409.28	1.3	-0.16	-5.8	0
	+ $BPPCT034_{218}$	0.54	0.29	0.1	257.04	1.22	-0.38	-19.17	0
	+ $BPPCT034_{251}$	0.65	0.42	0.13	379.74	1.1	-0.31	-16.72	0
	+ $CPSCT038_{204}$	0.66	0.43	0.01	39.72	1.09	0.25	12.54	0
	+ $BPPCT034_{222}$	0.67	0.45	0.02	48.39	1.08	-0.23	-11.05	0
	+ $BPPCT034_{226}$	0.69	0.47	0.02	69.5	1.06	-0.22	-10.62	0
	+ $BPPCT034_{230}$	0.7	0.49	0.02	65.94	1.04	-0.42	-16.41	0
	+ $BPPCT034_{237}$	0.72	0.52	0.03	99.38	1.01	-0.4	-15	0
	+ $CPSCT038_{185}$	0.73	0.53	0.02	64.01	0.99	0.21	10.11	0
	+ $BPPCT034_{204}$	0.75	0.56	0.03	108.24	0.96	0.25	10.27	0
	+ $BPPCT034_{216}$	0.75	0.57	0	18.8	0.96	0.09	4.34	0
Seed weight	$CPSCT038_{204}$	0.15	0.02	0.02	41.09	0.02	0.2	6.78	0
	+ $CPSCT038_{185}$	0.2	0.04	0.01	27.05	0.01	0.17	6.09	0
	+ $BPPCT034_{204}$	0.22	0.05	0.01	18.06	0.01	0.29	7.96	0
	+ $BPPCT034_{208}$	0.23	0.05	0.01	11.12	0.01	0.3	7.71	0
	+ $BPPCT034_{230}$	0.25	0.06	0.01	18.95	0.01	-0.18	-5.63	0
	+ $BPPCT034_{224}$	0.27	0.07	0.01	14.04	0.01	0.14	5.17	0
	+ $BPPCT034_{222}$	0.28	0.08	0.01	14.47	0.01	-0.09	-3.39	0.001
	+ $BPPCT034_{237}$	0.29	0.08	0.01	9.71	0.01	-0.12	-3.34	0.001
	+ $BPPCT034_{251}$	0.3	0.09	0	8.92	0	0.08	2.99	0.003
Pulp weight	$BPPCT034_{208}$	0.13	0.02	0.02	61.28	2.31	-0.12	-6.77	0
	+ $CPSCT038_{185}$	0.17	0.03	0.01	51.76	2.3	0.08	4.5	0
	+ $CPSCT038_{204}$	0.19	0.04	0.01	20.94	2.29	0.06	3.15	0.002
	+ $BPPCT034_{218}$	0.19	0.04	0	7.21	2.29	-0.06	-3.41	0.001
	+ $BPPCT034_{251}$	0.2	0.04	0	9.86	2.28	-0.06	-3.14	0.002

Table 22 Linear regression sour cherry colour Pav-Rf-SSR. Individual alleles are compared to single phenotype characteristics and alleles with $p < 0.05$ are shown.

Phenotype	SSR markers (alleles)	r	R^2	R^2 change	F change	Standard error	Standardized beta coefficients	*t* value	*P* value
L	Pav-Rf-SSR$_{349}$	0.09	0.01	0.01	30.99	4.6	0.07	4.32	0
	+ Pav-Rf-SSR$_{347}$	0.11	0.01	0	15.52	4.59	0.08	4.33	0
	+ Pav-Rf-SSR$_{351}$	0.12	0.01	0	5.3	4.59	-0.04	-2.3	0.021
a	Pav-Rf-SSR$_{343}$	0.12	0.01	0.01	50.23	9.46	-0.13	-7.47	0
	+ Pav-Rf-SSR$_{351}$	0.13	0.02	0	8.99	9.45	-0.06	-3.28	0.001
	+ Pav-Rf-SSR$_{347}$	0.13	0.02	0	4.15	9.45	0.03	2.04	0.042
b	Pav-Rf-SSR$_{343}$	0.12	0.01	0.01	49.13	5.28	-0.1	-5.56	0
	+ Pav-Rf-SSR$_{347}$	0.15	0.02	0.01	34.28	5.26	0.09	5.4	0
	+ Pav-Rf-SSR$_{349}$	0.16	0.02	0	3.93	5.26	0.03	1.98	0.047
C	Pav-Rf-SSR$_{343}$	0.12	0.01	0.01	51.77	10.67	-0.13	-7.32	0
	+ Pav-Rf-SSR$_{347}$	0.13	0.02	0	5.85	10.67	0.05	2.82	0.005
	+ Pav-Rf-SSR$_{351}$	0.14	0.02	0	7.68	10.66	-0.05	-2.77	0.006
h$^{\pm 33°}$	Pav-Rf-SSR$_{351}$	0.05	0	0	8.23	10.55	0.05	2.87	0.004
Ctifl	Pav-Rf-SSR$_{343}$	0.08	0.01	0.01	11.11	2.34	0.07	2.98	0.003
	+ Pav-Rf-SSR$_{347}$	0.1	0.01	0	5.52	2.34	-0.06	-2.35	0.019
Flesh colour	Pav-Rf-SSR$_{343}$	0.41	0.17	0.17	353.55	0.88	0.37	15.29	0
	+ Pav-Rf-SSR$_{349}$	0.42	0.18	0.01	15.36	0.88	-0.1	-4.32	0
	+ Pav-Rf-SSR$_{351}$	0.42	0.18	0	7.2	0.87	-0.06	-2.68	0.007
Juice colour	Pav-Rf-SSR$_{343}$	0.33	0.11	0.11	216.52	0.89	0.28	12.27	0
	+ Pav-Rf-SSR$_{347}$	0.37	0.14	0.03	56.72	0.88	-0.15	-6.52	0
	+ Pav-Rf-SSR$_{349}$	0.39	0.15	0.01	28.36	0.87	-0.12	-5.33	0
Soluble solid content	Pav-Rf-SSR$_{343}$	0.17	0.03	0.03	52.12	3.66	0.17	7.03	0
	+ Pav-Rf-SSR$_{347}$	0.19	0.04	0.01	15.86	3.65	-0.11	-4.37	0
	+ Pav-Rf-SSR$_{349}$	0.2	0.04	0	6.27	3.64	0.06	2.5	0.012

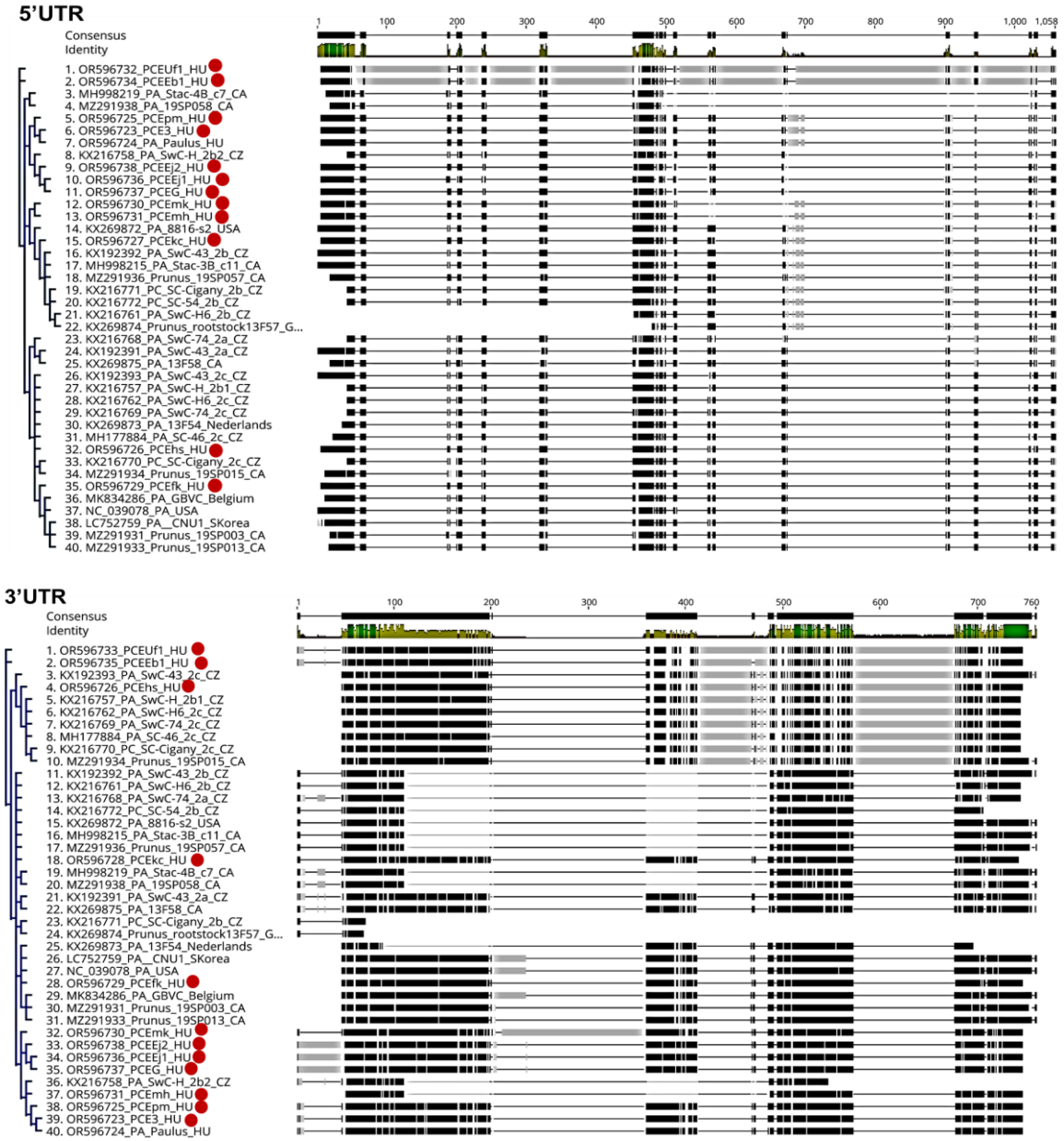
Table 23. Linear regression sour cherry colour Ma039a. Individual alleles are compared to single phenotype characteristics and alleles with $p < 0.05$ are shown

Phenotype	SSR markers (alleles)	r	R^2	R^2 change	F change	Standard error	Standardized beta coefficients	*t* value	*P* value
L	$Ma039a_{175}$	0.14	0.02	0.02	70.99	4.57	0.13	7.83	0
	+ $Ma039a_{157}$	0.17	0.03	0.01	31.55	4.55	-0.11	-6.55	0
	+ $Ma039a_{205}$	0.19	0.04	0.01	33.87	4.53	0.1	5.82	0
a	$Ma039a_{175}$	0.13	0.02	0.02	62.13	9.45	0.14	8.18	0
	+ $Ma039a_{205}$	0.15	0.02	0	13.48	9.43	0.06	3.67	0
b	$Ma039a_{175}$	0.12	0.01	0.01	50.59	5.28	0.12	6.86	0
	+ $Ma039a_{205}$	0.13	0.02	0	8.99	5.28	0.06	3.44	0.001
	+ $Ma039a_{157}$	0.14	0.02	0	8.15	5.27	-0.05	-2.85	0.004
C	$Ma039a_{175}$	0.13	0.02	0.02	61.21	10.66	0.13	7.73	0
	+ $Ma039a_{205}$	0.14	0.02	0.02	13.05	10.64	0.07	3.92	0
	+ $Ma039a_{157}$	0.15	0.02	0.02	4.39	10.64	-0.04	-2.1	0.036
$h^{\pm 33°}$	$Ma039a_{157}$	0.03	0	0	3.95	10.56	0.03	1.99	0.047
Ctifl	$Ma039a_{175}$	0.14	0.02	0.02	36.32	2.32	-0.15	-6.42	0
	+ $Ma039a_{205}$	0.19	0.03	0.01	25.05	2.31	-0.12	-5.01	0
Flesh colour	$Ma039a_{175}$	0.4	0.16	0.16	333.35	0.88	-0.38	-17.26	0
	+ $Ma039a_{157}$	0.42	0.17	0.01	30.88	0.88	0.12	5.56	0
Juice colour	$Ma039a_{175}$	0.31	0.1	0.1	184.32	0.9	-0.29	-12.95	0
	+ $Ma039a_{157}$	0.33	0.11	0.02	32.03	0.89	0.15	6.51	0
	+ $Ma039a_{205}$	0.36	0.13	0.02	30.53	0.89	-0.13	-5.53	0
Soluble solid content	$Ma039a_{175}$	0.06	0	0	5.46	3.71	-0.06	-2.48	0.013
	+ $Ma039a_{205}$	0.07	0.01	0	4.19	3.71	-0.05	-2.05	0.041

Table 24. Linear regression sour cherry colour LG3_13.146. Individual alleles are compared to single phenotype characteristics the allele with $p < 0.05$ is shown

Phenotype	SSR markers (alleles)	r	R^2	R^2 change	F change	Standard error	Standardized beta coefficients	*t* value	*P* value
L	$LG3\_13.146_{218}$	0.14	0.02	0.02	66.65	4.58	-0.14	-8.16	0
a	$LG3\_13.146_{218}$	0.18	0.03	0.03	121.11	9.37	-0.18	-11	0
b	$LG3\_13.146_{218}$	0.17	0.03	0.03	103.44	5.24	-0.17	-10.17	0
C	$LG3\_13.146_{218}$	0.18	0.03	0.03	118.96	10.58	-0.18	-10.91	0
Ctifl	$LG3\_13.146_{218}$	0.11	0.01	0.01	20.95	2.33	0.11	4.58	0
Flesh colour	$LG3\_13.146_{218}$	0.06	0	0	0.96	6	0.06	2.45	0.014
Juice colour	$LG3\_13.146_{218}$	0.11	0.01	0.01	21.27	0.94	0.11	4.61	0
Soluble solid content	$LG3\_13.146_{218}$	0.05	0	0	4.87	3.71	-0.05	-2.21	0.027

Figure 21. Consensus identity of 5' UTR and 3' UTR for PrVF. In red: sour cherry infected with PrVF in Hungary



A3 Appendix: List of viruses found in sweet and sour cherry

Family	Genus	Virus/Viroid	Abbreviation	HTS	ELISA	Sanger/Other	RT-PCR	Accession number	Host Plant		Symptoms	Transmission	Indicator plants	Resistance	Reference
Pospiviroidae	*Apscaviroid*	**Apple scar skin viroid**	ASSVd	-	-	Yes	Yes	NC_001340.1	-	Sweet Cherry	Mosaic symptoms on leaves, white spots on fruits	Grafting, infected plant material	n.a.	-	Messmer et al., 2017, Di Serio et al., 2018
	Hostuviroid	**Hop stunt viroid**	HSVd	Yes	-	-	-	NC_001351.1	Sour Cherry	Sweet Cherry	n.a.	Infected plant material	n.a.	-	Messmer et al., 2017., Kaponi et al., 2024
Avsunviroidae	*Pelamoviroid*	**Peach latent mosaic viroid**	PLMVd	-	-	Yes	Yes	NC_003636.1	-	Sweet Cherry	leaf mosaic, botches and extreme chlorosis	Infected plant material	Peach 'GF305'	-	Messmer et al., 2017, Lin et al., 2013
Betaflexiviridae	*Capillovirus*	**Cherry virus A**	CVA	Yes	Yes	Yes	Yes	NC_003689.1	Sour Cherry	Sweet Cherry	Leaf vein necrosis, Necrotic lesions, Chlorotic rings, greasy blotches, bud blight, symptoms as LCHV.	Infected plant material	n.a.	-	Jelkmann 1995, Sabanadzovic et al., 2005, Isogai et al., 2004 Marais et al., 2008
	Closteovirus	**Apple chlorotic leaf spot virus**	ACLSV	Yes	Yes	-	Yes	NC_001409.1	Sour Cherry	Sweet Cherry	Similar to PPV, latent infection is generally observed with possible severe leaf deformations.	Infected plant material	*Malus platycarpa*	-	Přibylová et al., 2020
	Robigovirus	**Cherry virus turkey**	CVTR	Yes	-	-	Yes	MK600387.1	Sour Cherry	Sweet Cherry	Chlorotic spots on the leaf, similar to CGMV, CRMV, CTLaV and CNRMV	n.a.	n.a.	-	Çağlayan et al., 2019
		Cherry green ring mottle virus	CGRMV	Yes	Yes	Yes	Yes	NC_001946.1	Sour Cherry	Sweet Cherry	Marked fruit, bud blight, yellow and green mottle of mature leaves Epinasty, bending downwards.	Infected plant material	Flowering cherry 'Kwanzan', Sweet cherry 'Bing', 'Black Republican', 'Deacon', 'Lambert' and 'Napoleon'	-	Přibylová et al., 2020, Sabanadzovic et al., 2005, Isogai et al., 2004, Quero-Garica et al., 2017
		Cherry necrotic rusty mottle virus	CNRMV	Yes	Yes	Yes	Yes	NC_002468.1	Sour Cherry	Sweet Cherry	Necrotic rusty mottle, bud blight, brown angular necrotic spots on leaves, which turn to shot-holes.	Infected plant material	Sweet cherry 'Sam', 'Bing'	-	Sabanadzovic et al., 2005 Isogai et al., 2004
	Tepovirus	**Prunus virus T**	PrVT	Yes	-	-	Yes	NC_024686.1	-	Sweet Cherry	n.a.	Infected plant material	n.a.	-	Messsmer et al., 2017, Marais et al., 2015
	Trichovirus	**Cherry mottle leaf virus**	ChMLV	Yes	Yes	-	Yes	NC_002500.1	Sour Cherry	Sweet Cherry	Irregular chlorotic mottling, leaf distortion of terminal leaves, puckering and tattering, shot holes and a reduction of leaf size.	Grafting, mite, infected plant material	Sweet cherry 'Bing'	-	Messmer et al., 2017, James et al., 1996
		Cherry latent virus 1	CLV-1	Yes	-	-	Yes	MK770441	-	Sweet Cherry	n.a.	n.a.	n.a.	-	Brewer et al., 2020
		Apricot pseudo-chlorotic leaf spot virus	APCLSV	Yes	-	-	Yes	NC_006946.1	-	Sweet Cherry	Chlorosis	Infected plant material	n.a.	-	Liberti et al., 2005
Bromoviridae	*Cucumovirus*	**Cucumber mosaic virus**	CMV	-	-	-	Yes	AB188233.1 (RNA3)	-	Sweet Cherry	Chlorotic mottling and deformation of leaves	Infected plant material	n.a.	-	Messmer et al., 2017, Cao et al., 2017
	Ilarvirus	**Apple mosaic virus**	ApMV	Yes	Yes	-	Yes	NC_003480.1 (RNA3)	Sour Cherry	Sweet Cherry	Brown, dead spots on leaves, turning into holes. Yellowing on leaves. Enation, or outgrowths.	Infected plant material	n.a.	-	Přibylová et al., 2020
		Prune dwarf virus	PDV	Yes	Yes	Yes	Yes	NC_008038.1 (RNA3)	Sour Cherry	Sweet Cherry	Necrotic lesions, Leaf vein necrosis, chlorotic rings, mottle on young expanding leaves and shot holes. Fruits show chlorotic ring.	Pollen, infected plant material, Thrips	n.a.	-	Messmer et al., 2017, Kelley et al., 1983
		Prunus necrotic ringspot virus	PNRSV	Yes	Yes	Yes	Yes	NC_004364.1 (RNA3)	Sour Cherry	Sweet Cherry	Marked fruit, chlorotic to yellow line pattern mosaic and shot holes in leaves, a rugose mosaic, bud death.	Seed, pollen, infected plant material	n.a.	RNAi silencing	Přibylová et al., 2020. Sabanadzovic et al., 2005, Messmen et al., 2017, Kelley et al., 1983
		American plum line pattern virus	APLPV	Yes	Yes	-	Yes	NC_003453.1 (RNA3)	-	Sweet Cherry	oak-leaf pattern and yellow or white lines on the leaves, discoloured areas may appear in shades of white, yellow or pink, sometimes with large rings.	Infected plant material	Peach 'GF305', Flowering cherry 'Shirofugen'	-	Katsiani et al., 2018, Myrta et al., 2003
Closteroviridae	*Ampelovirus*	**Little cherry virus 2**	LCHV2	Yes	Yes	Yes	Yes	NC_005065.1	Sour Cherry	Sweet Cherry	Bud blight, reduction in yield, fruit weight, fruit size and trunk circumference. Small angular and pointed fruit that do not fully ripen and are imperfectly coloured.	Mealybugs, grafting, infected plant material	Sweet cherry 'Sam', 'Canindex I'	-	Přibylová et al., 2020, Isogai et al., 2004, Schröder et al., 2010
		Plum bark necrosis and stem pitting asssociated virus	PBNSPaV	Yes	Yes	Yes	Yes	EF546442.1	Sour Cherry	Sweet Cherry	Chlorotic rings, greasy blotches. Stem pitting, thickened corky bark, trunk deformation, bark cracking, clorisis or clorotic spots on leaves.	Infected plant material	n.a.	-	Sabanadzovic et al., 2005, Myrta et al., 2003
	Velavirus	**Little cherry virus 1**	LCHV1	Yes	Yes	Yes	Yes	NC_001836.1	Sour Cherry	Sweet Cherry	Necrotic leaf, yellowish oak-leaf pattern symptoms, reddening of leaves, bud blight. Small angular and pointed fruit that do not fully ripen and are imperfectly coloured. Red colour of leaves and bronzing in autumn.	Mealybugs, grafting, infected plant material	Sweet cherry 'Sam', 'Canindex I'	-	Sabanadzovic et al., 2005, Katsiani et al., 2018, Messmer et al., 2017. Jelkmann et al., 2006
Luteoviridae	*Luteovirus*	**Cherry associated luteovirs**	ChaLV	Yes	-	-	Yes	NC_031800	Sour Cherry	Sweet Cherry	n.a.	Infected plant material	n.a.	-	Lenz et al., 2017
Orthornavirae	*Robigovirus*	**Cherry rusty mottle-associated virus**	CRMaV	Yes	-	Yes	Yes	KF030870.2	-	Sweet Cherry	n.a.	n.a.	n.a.	-	Poudel et al., 2017
		Cherry twisted leaf associated virus	CTLav	-	-	-	Yes	NC_024449.1	-	Sweet Cherry	Abrupt bending of the midrib of the leaf, twisting of the leaf. Curling, necrotic leaf. Stunted trees, and spurs may appear bunchy, fruits are deformed and pedicel necrosis.	Infected plant material	Sweet cherry 'Bing'	Immune varieties of cherry cv.	Messmer et al., 2017, James et al., 2014

Potyviridae	*Potyvirus*	**Plum pox virus**	PPV	Yes	Yes	Yes	Yes	NC_001445.1	Sour Cherry	Sweet Cherry	Diffuse branch necrosis, leaf deformity, chlorotic and necrotic rigspots or notches on fruit and fruit drop. On sour cherry may include chlorotic ringspot symptoms on leaves, depression, necrosis and rings on fruit, with rings disappearing during maturity. Chlorotic and necrotic spots along the veins.	Aphid, Grafting, Infected plant material	Peach 'GF305', 'Monclar', Sweet cherry 'Jaspi', 'Edabriz', *Prunus mahaleb*, *P. avium*	RNAi silencing	Přibylová et al., 2020, Navrátil et al., 2003, Bodin et al., 2003, Fanigliulo et al., 2003
Secoviridae	*Cheravirus*	**Cherry rasp leaf virus**	CRLV	Yes	-	-	Yes	NC_006272.1 (RNA2)	Sour Cherry	Sweet Cherry	Rasp leaf, raised protuberances, spurs and branches on the lower part of the tree may die. Fruit deformation, reduced production and poor taste, reduced tree vigour and life expectancy.	Nematode (*Xiphinema americanum*), grafting	Sweet cherry 'Bing'	-	Messmer et al., 2017, James et al., 2003
	Fabavirus	**Prunus virus F**	PrFV	Yes	-	-	Yes	NC_039078.1 (RNA2)	-	Sweet Cherry	n.a.	Infected plant material	n.a.	-	James, 2019, Tahzima et al., 2019, Villamor 2017
	Nepovirus	**Cherry leaf roll virus**	CLRV	Yes	-	-	Yes	NC_015415.1 (RNA2)	Sour Cherry	Sweet Cherry	Yellowing, chlorosis, leaf rolling and bunching on shoots	Seed, pollen, grafting, infected plant material	n.a.	-	Přibylová et al., 2020, Quero-García et al., 2017
		Tomato ringspot virus	ToRSV	Yes	-	-	Yes	NC_003839.2 (RNA2)	Sour Cherry	Sweet Cherry	Die back, chlorotic spots, enation on lower surface of the leaf, leaves appear droopy turn yellow or red prematurely	Nematode, grafting	Peach 'Lovell', 'Elberta', 'GF305', Prunus tomentosa 'IR473/1', 'IR474/1'	-	Messmer et al., 2017, Mandic et al., 2007
Tymoviridae	*Marafivirus*	**grapevine Syrah virus 1-like virus**	not yet assigned	-	-	-	-	n.a.	*Sour Cherry cv. 'Rannaja' (Cz)*	-	n.a.	n.a.	-	-	Koloniuk et al., 2018
Unassigned	*Ourmiavirus*	**Epirus cherry virus**	EpCV	Yes	Yes	-	Yes	NC_011067.1 (RNA3)	Sour Cherry	Sweet Cherry	Rasp leaf, stunting and deformed leaves of reduced size	Seed, infected plant material	n.a.	-	Messmer et al., 2017, Avgelis and Barba, 2011
Virgaviridae	*Tobamovirus*	**Tobacco mosaic virus**	TMV	-	-	-	-	NC_001367.1	Sour Cherry	Sweet Cherry	n.a.	Infected plant material	Sweet Cherry 'Tardiva di Roccamonfina'	-	Messmer et al., 2017, Nemeth, 1986
	Tombusvirus	**Petunia asteroid mosaic virus**	PAMV	-	Yes	-	-	NC_038692	Sour Cherry	Sweet Cherry	Shoot necrosis, severe stunting of the tree. Sharp twisting of leaf blades resulting from the necrosis of the midrib and main veins, one-sided shoot shoot dieback with replacement by lateral buds resulting in a zig-zag growth of shoots.	n.a.	Sweet cherry 'Lambert', 'Sam', 'Van'	-	Mandic et al.2007, Pfeilstetter et al 1996
		Carnation Italian ringspot virus	CIRV	-	-	-	-	NC_003500.3	-	Sweet Cherry	Shoot necrosis similar to Canker disease, however limited to shoot tips, stunting. Sharp twisting of leaf blades resulting from the necrosis of the midrib and main veins. Stones of the fruit are malformed and spotted with seeds that are aborted.	n.a.	Sweet cherry 'Lambert', 'Sam', 'Van'	-	Messmer et al., 2017, Nemeth, 1986
		Tomato bushy stunt virus	TBSV	Yes	-	-	Yes	NC_017824.1	-	Sweet Cherry	Shoot necrosis is similar to Canker disease, however limited to shoot tips, and stunting. Sharp twisting of leaf blades resulting from the necrosis of the midrib and main veins, brittle shoots and branches and bark canker with a strong flow of gum.	n.a.	Sweet cherry 'Lambert', 'Sam', 'Van'	-	Messmer et al., 2017, Nawaz et al., 2014

Appendix A4:
R script written for this analysis.

Rstudio version 4.0
Last access: 2024.06.01

Script 1 for Frequency analysis

```
# Install the writexl package if not already installed
install.packages("writexl")
# Load the writexl package
library(writexl)
# Write the data frame to an Excel file
write_xlsx(result_table, "C:/Users/Dataset_cherry_file.xlsx")
# Install the dplyr package if not already installed
install.packages("dplyr")
# Load the dplyr package
library(dplyr)
View(example_data)
library(readxl)
R_studio_dataset_Accession_SSR_marker_size <- read_excel("C:/Users/Dataset_cherry_file.xlsx")
View(R_studio_dataset_Accession_SSR_marker_size)
example_data<-R_studio_dataset_Accession_SSR_marker_size
View(example_data)
#INSERT SCRIPT HERE
example_data <- data[,2:5]
colnames(example_data) <- paste0("Allele ",1:4)
# Assuming your data is in a data frame named excel_data and the marker column is named
"Marker"
example_data$`Allele 1`[ example_data$`Allele 1` == "-"] <- NA
example_data$`Allele 2`[ example_data$`Allele 2` == "-"] <- NA
example_data$`Allele 3`[ example_data$`Allele 3` == "-"] <- NA
example_data$`Allele 4`[ example_data$`Allele 4` == "-"] <- NA
View(example_data)
# Convert relevant columns to numeric
numeric_columns <- c('Allele 1', 'Allele 2', 'Allele 3', 'Allele 4')
example_data <- apply(example_data,2,as.numeric)
View(example_data)

# Calculate allele frequencies
allele_frequencies <- colMeans(excel_data[, numeric_columns], na.rm = TRUE)
View(excel_data)
excel_data$AlleleFreq1 <- allele_frequencies[1]
excel_data$AlleleFreq2 <- allele_frequencies[2]
excel_data$AlleleFreq3 <- allele_frequencies[3]
excel_data$AlleleFreq4 <- allele_frequencies[4]
# Calculate expected heterozygosity (H) for tetraploid organisms
excel_data$Heterozygosity <- 1 - rowSums((excel_data[, c('Allele 1', 'Allele 2', 'Allele 3',
'Allele 4')]/rowSums(excel_data[, c('Allele 1', 'Allele 2', 'Allele 3', 'Allele 4')]))^2)
# Calculate polymorphism information content (PIC) for tetraploid organisms
excel_data$PIC <- 1 - rowSums((excel_data[, c('Allele 1', 'Allele 2', 'Allele 3', 'Allele
4')]/rowSums(excel_data[, c('Allele 1', 'Allele 2', 'Allele 3', 'Allele 4')]))^2) - 2 *
rowSums((excel_data[, c('Allele 1', 'Allele 2', 'Allele 3', 'Allele 4')]/rowSums(excel_data[,
c('Allele 1', 'Allele 2', 'Allele 3', 'Allele 4')]))^2)
# Placeholder values for polymorphic and nonpolymorphic fractions (replace with your actual
values)
p <- 0.6
np <- 0.4
# Calculate effective multiplex ratio (E) for tetraploid organisms
excel_data$EMR <- n * p / (n * p + n * np)
# Calculate mean heterozygosity for polymorphic markers (Havp) for tetraploid organisms
excel_data$Havp <- rowSums(excel_data$Heterozygosity[excel_data$EMR > 0]) / sum(excel_data$EMR
> 0)
# Calculate marker index (MI) for tetraploid organisms
excel_data$MI <- excel_data$EMR * excel_data$Havp
# Placeholder values for discriminating power and resolving power (replace with your actual
values)
C <- 0.2
Ib <- 0.8
# Calculate discriminating power (D) for tetraploid organisms
excel_data$DiscriminatingPower <- 1 - C
# Calculate resolving power (R) for tetraploid organisms
excel_data$ResolvingPower <- sum(Ib)
# Select relevant columns for the final table
```

```
result_table <- excel_data[, c('Sample', 'Marker', 'Heterozygosity', 'PIC', 'EMR', 'Havp',
'MI', 'DiscriminatingPower', 'ResolvingPower')]
View(result_table)
# Print the result table
print(result_table)
# Display the column names of your data frame
print(names(result_table))
# Group by Sample and calculate mean for each column
sample_summary <- result_table %>%
group_by(Marker) %>%
summarize(
Heterozygosity = mean(Heterozygosity, na.rm = TRUE),  # Adjust the case if needed
PIC = mean(PIC, na.rm = TRUE),  # Adjust the case if needed
EMR = mean(EMR, na.rm = TRUE),  # Adjust the case if needed
Havp = mean(Havp, na.rm = TRUE),  # Adjust the case if needed
MI = mean(MI, na.rm = TRUE),  # Adjust the case if needed
DiscriminatingPower = mean(DiscriminatingPower, na.rm = TRUE),  # Adjust the case if needed
ResolvingPower = mean(ResolvingPower, na.rm = TRUE)  # Adjust the case if needed)
# Display or use the sample_summary as needed
print(sample_summary)
View(sample_summary)
```

Script 2 for boxplot phenotype and genotype correlation

```
library(mpQTL)
#Custom functions -------
get_model_p <- function(model){
f <- summary(model)$fstatistic
psel <- pf(f[1],f[2],f[3],lower.tail=F)
attributes(psel) <- NULL
return(psel)
}
get_sig_hap <- function(model){
coefs <- coef(summary(model))
hap <- rownames(coefs[-1,,drop = FALSE])
eff <- coefs[-1,"Estimate"]
pv <- coefs[-1,"Pr(>|t|)"]
return(data.frame(allele = hap, effect = eff, pval = pv))
}
#Data parameters ----------
params <- list(input = c("data/raw/SSR_data_for_R_SweetCherry.xlsx",
"data/raw/SSR_data_for_R_SourCherry.xlsx"),
ploidy = c(2,4),
pheno = c("data/processed/Sweet_cherry_phenotypes.txt",
"data/processed/Sour_cherry_phenotypes.txt"),
label = c("Sweet_Cherry","Sour_Cherry"))
params <- lapply(1:length(params[[1]]),function(i) lapply(params,'[[',i))
#In this first step we test association between haplotype markers and
#different phenotypes. We obtain p-values of all alleles being significant.
#This is equivalent to an additive allele model, each SSR is tested as having an independent
#allele effect.
for(p in params){
pheno <- data.table::fread(p$pheno)
pheno <- as.data.frame(pheno)
geno <- readxl::read_excel(p$input)
inds <- gsub("_[0-9]","",geno$chromosome)
repG <- split(geno,inds)
geno <- do.call(rbind,repG[pheno$Accession])
geno <- t(geno)
colnames(geno) <- geno[1,]
geno <- geno[-1,]
#Here we use a linear model to perform this test
res <- map.QTL(phenotypes = pheno[,-1],genotypes = geno,ploidy = p$ploidy,
map = data.frame(marker = rownames(geno), chromosome = c(1,2),position = c(1,1)),
K = NULL,no_cores = 1)
#From the results we extract the pvalue significances. Most alleles
#have association with phenotypes. This matches our idea that
#genetic structure is associated with the phenotypes
markerSig <- t(sapply(res,'[[',"pval"))
write.table(markerSig,file = paste0("results/Marker_association_",p$label,".txt"),
quote= TRUE, row.names = TRUE)
sigs <- which(markerSig < 0.05)
phIndex <- (sigs) %% (ncol(pheno)) + (sigs %/% ncol(pheno))
mIndex <- (sigs %/% ncol(pheno)) + 1
#Here I make the boxplots of phenotype vs. allele dosage.
for(i in seq(length(sigs))){
if(length(sigs) == 0) next
betas <- res[[phIndex[i]]]$beta[[mIndex[i]]]
```

```
dos <- mpQTL::dosage.X(geno[mIndex[i],],ploidy = p$ploidy, haplotype = TRUE)
png(filename = paste0("results/figures/Boxplot_",p$label,"_marker_",rownames(geno)[mIndex[i]],
"_phenotype_",colnames(pheno)[-1][phIndex[i]],".png"),
width = 250*length(betas), height = 800, res = 180, type = "cairo")
par(mfrow = c(1,length(betas)),
mar = c(4,1,3,1),
oma = c(0,3,0,0))
for(j in 1:length(betas)){
tit <- paste0("Allele ",colnames(dos)[j])
boxplot(split(pheno[,-1][,phIndex[i]],dos[,j]), main = tit, xlab = "dosage")
}
mtext(colnames(pheno)[-1][phIndex[i]], side = 2, outer = TRUE,line = 1.5)
dev.off()
}
}
#We can also test which of the alleles are significantly associated with each trait
#For this we use a forward-backward variable selection approach in a multiple linear
regression
#model. This is based on the assumption that only few alleles are of importance
#to determine the effect on a phenotype. Thus, we end up with a regression model of the type
# y = u + Ax1 + Bx2 where A is the effect of allele 1 (and x1 the dosage of that allele), and
#B is the effect of allele 2. The dosage of other alleles is inconsequential (not-significant)
for(p in params){
pheno <- data.table::fread(p$pheno)
pheno <- as.data.frame(pheno)
geno <- readxl::read_excel(p$input)
inds <- gsub("_[0-9]","",geno$chromosome)
repG <- split(geno,inds)
geno <- do.call(rbind,repG[pheno$Accession])
geno <- t(geno)
colnames(geno) <- geno[1,]
geno <- geno[-1,]
#Here we apply the linear model analysis
res <- lapply(colnames(pheno)[-1],function(trait){
res <- lapply(rownames(geno),function(marker){
dos <- dosage.X(genotypes = geno[marker,], haplotype = TRUE, ploidy = p$ploidy)
colnames(dos) <- paste0("hap",colnames(dos))
data <- cbind(pheno,dos)
refHap <- which.min(abs(cor(data[[trait]],dos)))
form <- as.formula(paste0(trait,"~",paste(colnames(dos)[-refHap],collapse = "+")))
all <- lm(form, data = data)
backward <- step(all, direction = "both",scope = formula(all), trace = 0)
res <- summary(backward)
return(list(full = all, selected = backward))
})
names(res) <- rownames(geno)
return(res)
})
names(res) <- colnames(pheno)[-1]

#Here we extract the pvalues out of the models
r <- lapply(names(res),function(trait){
r <- lapply(names(res[[trait]]),function(marker){
sapply(res[[trait]][[marker]],get_model_p)
})
names(r) <- names(res[[trait]])
dplyr::bind_rows(r,.id = "marker")
})
names(r) <- names(res)
modelPval <- dplyr::bind_rows(r,.id = "trait")
#Here we extract the allele effects out of the models
r <- lapply(names(res),function(trait){
r <- lapply(names(res[[trait]]),function(marker){
get_sig_hap(res[[trait]][[marker]]$selected)
})
names(r) <- names(res[[trait]])
dplyr::bind_rows(r,.id = "marker")
})
names(r) <- names(res)
modelEff <- dplyr::bind_rows(r,.id = "trait")
modelEff <- modelEff[order(modelEff$marker,modelEff$allele,modelEff$pval),]
writexl::write_xlsx(list(allele_effects = modelEff,
model_comparison = modelPval),
paste0("results/",p$label,"_allele_effects.xlsx"))
}
```

A5 Appendix: List of publications and presentations

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
  https://doi.org/10.3390/agriculture13071287
- 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
  https://doi.org/10.1016/j.scienta.2021.110656
- **Desiderio Francesco**, Nagyné Galbács Zsuzsanna, Demian Emese, Fákó Vivien, Czako David, Varga Tünde, Barath Daniel, Jaska-Czotter Nikoletta, Koloniuk Igor, Várallyay Éva (2024) "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 Ülésszak 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 Ülésszak 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**
- Salamon Pál; Nagyné Galbács Zsuzsanna; Demián Emese; Achs Adam; Alaxin Peter; Predajna Lucas; Agyemang Duah Evans; **Desiderio Francesco**; Takács András Péter; Menzel Wulf; Skoric Dijana; Glasa Miroslav; Várallyay Éva "Clematis vitalba is a Natural Host of the Novel Ilarvirus, Prunus virus I" Viruses (1999-4915): 15 (9) paper 1964 16p. (2023) **https://doi.org/10.3390/v15091964**
- 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)