

# INFLUENCE OF RIBAVIRIN ON *PRUNUS DOMESTICA* L. REGENERATION, GENOME STABILITY AND VIRUS ERADICATION *IN VITRO*

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*Prunus necrotic ring spot ilarvirus (PNRSV) and Apple chlorotic leaf spot trichovirus (ACLSV) are common in plum orchards. The aim of the study was to obtain virus-free planting material of Prunus domestica L. by chemotherapy in vitro. Ribavirin at concentrations of 10 to 50 mg·l<sup>-1</sup> was added to Murashige–Skoog (MS) nutrition medium for virus eradication from microshoots. After a two-week period of chemotherapy, meristems were subcultured monthly on MS medium and proliferation index of shoots was estimated. Microshoots were retested by reverse transcription polymerase chain reaction for presence of virus. At lowest concentrations of 10 mg·l<sup>-1</sup> ribavirin was entirely ineffective for ACLSV and 10 to 30 mg·l<sup>-1</sup> was ineffective for PNRSV elimination. Ribavirin concentrations of 40 and 50 mg·l<sup>-1</sup> destroyed both pathogens. However, at higher concentrations of 40 and 50 mg·l<sup>-1</sup> ribavirin exhibited some signs of phytotoxicity on microshoots in the first subcultivation period. In order to test the genetic stability of the microplants after chemotherapy the amplified fragment length polymorphism (AFLP) method was applied. Plant genome stability in ‘Magna Glauca’ at concentrations of 40 mg·l<sup>-1</sup> was damaged, as the presence of polymorphic AFLP markers were observed.*

**Key words:** ACLSV, chemotherapy, PNRSV, virus free plant.

## INTRODUCTION

Vegetative propagation of plums, climate change and the increasing pest population (viral disease vectors) will facilitate the spread of the virus in orchards (Whitfield *et al.*, 2015). However, there are no effective protective measures against viral diseases, which could allow plums to grow normally and develop for a long time in the garden if intensive control of fungal diseases and pests is not undertaken. Common virus infections in *Prunus* spp. include *PNRSV* (*Prunus necrotic ringspot virus*), *ACLSV* (*Apple chlorotic leafspot virus*), *PPV* (*Plum pox virus*) and *PDV* (*Prune dwarf virus*) (Nemeth, 1986; Cieszlinska and Malinowski, 2002; Cieszlinska and Morgas, 2010). *PNRSV* and *ACLSV* are common virus diseases in plum trees in the genetic resource orchard in the Horticulture Institute of the Lithuanian Research Centre for Agriculture and Forestry (LAMMC) (16.8% and 4.8%, respectively). Low infection rates (1.6%) of mixed infections involving *PNRSV* and

*ACLSV* were investigated in plum trees. Trees damaged by mixed infections were asymptomatic (Mažeikienė *et al.*, 2016).

*PNRSV* belongs to the *Bromoviridae* family, *Iilarvirus* genus (Paduch-Cichal *et al.*, 2011) and infect the plants of *Prunus* and *Rosa* genus: plums, cherries, peaches, apricots, and almonds (Moury *et al.*, 2001). Virus vectors are mites, nematodes and trips (Smith *et al.*, 1988; Scott, 2014). The virus can also spread through infected pollen and seeds (Scott, 2014). Virus-induced damage is significant for plum harvests (Nemeth, 1986; Cieszlinska and Malinowski, 2002). *PNRSV* can reduce bud counts in nurseries, reduce fruit growth (10 to 30%), fruit yield (20 to 60%), delay fruit maturity and increase winter injury susceptibility in orchards (Saubier, 1972).

*ACLSV* belongs to the *Flexiviridae* family and *Trichovirus* genus (Martelli *et al.*, 1994) and infects most plants of the

*Rosaceae* family. It is more commonly found in pome fruits like apple and pear (Pupola *et al.* 2011; Mazeikiene *et al.* 2018) and less often in stone fruits — cherries, plums, apricots, peaches (Nemeth, 1986; Ulubas *et al.*, 2004; Niu *et al.*, 2012; Gospodaryk *et al.*, 2013). *ACLSV* is easily spread through grafting and mechanical inoculation; there are no biological vectors. *ACLSV* is latent in most cultivars of plums (infected trees do not manifest observable symptoms). *ACLSV* is often detected in co-infections and can have a devastating effect on plum tree growth and productivity (Nemeth, 1986).

Scientists have been investigating the possibility of removing virus from plant material for several decades. The positive result depends on the culture of the plant, structural and biological characteristics of the virus, the interaction among the virus and the host plant and the chosen method of virus contamination. Virus eradication by chemotherapy and grafting at the tip of the shoots is limited in stone fruits, due to low percentage of plant survival (Deogratias *et al.*, 1989). Chemotherapy *in vitro* for obtaining PPV-free plants was investigated by Paunovic with colleagues (2007). Eradication of some viral pathogens from herbaceous and woody plants by chemotherapy method has been successfully used (Pūpola *et al.*, 2009; Panattoni *et al.*, 2013; Hu *et al.*, 2015; Singh, 2015). Chemical antiviral agents are being used to obtain virus free plants. The antiviral agent ribavirin (Virasol) 1-β-ribofuranosyl-1,2,4-triazole-3-carboxamide is guanosine (ribonucleic) analogue that is used to stop viral RNA synthesis and viral mRNA capping. Ribavirin's antiviral effect is by inducing hyper mutations in RNA viruses, which is lethal for virus replication (Crotty *et al.*, 2001; Graci *et al.*, 2005).

Manipulation *in vitro* leads to genetic instability of plants. Genome mutations were observed in different plants *in vitro* (Hashmi *et al.*, 1997; Zucchi *et al.*, 2002; Ngezahayo *et al.*, 2006; Tiwari *et al.*, 2013). The amplified fragment length polymorphism (AFLP) presents the most suitable and established method for plant genome investigation. AFLP markers have the potential to resolve genetic differences of DNA fingerprints (Ulrich *et al.*, 1999). AFLP studies have shown an insufficient genetic stability in some plant populations using AFLP (Wen *et al.* 2005; Tiwari *et al.* 2013).

The aim of this study was to identify the most effective way of eliminating *ACLSV* and *PNRSV* pathogens from *P. domestica* shoots using chemotherapy and *in vitro* methods to obtain virus-free and genetically stable planting material.

## MATERIAL AND METHODS

**Plant material and nutrition medium *in vitro*.** *P. domestica* cultivars that were maintained in the fields of the Institute of Horticulture LAMMC formed the source plant material for breeding and propagation. 'Magna Glauca' and 'Cacanska Rana' shoots with mixed infection (*PNRSV* and *ACLSV*) were chosen for chemotherapy. The nodal explants were sterilised with 0.2% solution of Chapion WP and Gra-

nozant (mix solution) for 15 minutes, rinsed twice with sterile water, immersed in 0.1% solution of HgCl<sub>2</sub> for 7 minutes, and rinsed twice with sterile distillate water. After sterilisation the nodal explants were inoculated on Murashige–Skoog (MS) (Murashige and Skoog, 1962) medium (pH 5.8) supplemented with 0.75 mg·l<sup>-1</sup> 6-Benzylamino-purine (BAP) and 0.14 mg·l<sup>-1</sup> Indole-3-butyric acid (IBA) in combination with sucrose at 30 g·l<sup>-1</sup> and phytoagar 7 g·l<sup>-1</sup> and were maintained in cultivation room at 21 ± 2 °C and photoperiod 16-h under cool white fluorescent light (50 μmol m<sup>-2</sup>s<sup>-1</sup> PPF).

**Chemotherapy for virus eradication.** Multiplied cultures (*PNRSV* and *ACLSV* infected plums shoots 0.8 ± 0.2 cm) were treated on MS medium with ribavirin for two weeks. Five different concentrations of ribavirin were used: 10, 20, 30, 40, and 50 mg·l<sup>-1</sup>. Shoots were maintained in the cultivation room at 21 ± 2 °C and photoperiod 16-h under cool white fluorescent light (50 μmol m<sup>-2</sup>s<sup>-1</sup> PPF). After chemotherapy treatment, meristems (0.2 cm) were subcultivated monthly on MS medium. The shoot proliferation index was evaluated after 4 and 8 weeks.

**PCR for virus detection.** Shoots 0.8 ± 0.2 cm in length were retested by polymerase chain reaction (PCR) method for virus detection after eight weeks (two subcultivations). The total RNA was extracted from fresh tissues of microshoots using the GeneJET Plant RNA Purification Mini Kit (Thermo Scientific Ltd.) according to the supplied protocol. The RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific Ltd.) was used for the synthesis of the complementary DNA strand (cDNA) according to the manufacturer's protocol. Oligonucleotide primer pairs *PNRSV*f 5'GAACCTCCTCCGATTTAG3' / *PNRSV*r 5'GCTTCCCTAACGGGGCATCCAC3' by Sánchez-Navarro *et al.* (2005) and *ACLSV*f 5'TTCATGGAAAGACAGGGGCAA3' / *ACLSV*r 5'AAGTCTACAGGCTATTTATTATAAGTCTAA3' by Menzel *et al.* (2002) were used for amplification of virus coat protein (CP) gene fragments 346 bp and 667 bp, respectively. The amplification reaction (20 μl) was composed of 10 × *Taq* DNA polymerase buffer 2.5 μl, 2.5 mM MgCl<sub>2</sub> 2 μl, 0.2 mM dNTP mix 2 μl, 0.1 μM forward and reverse primer 0.2 μl, 0.1 units *Taq* DNA polymerase and 200 ng of cDNA. The amplification reaction was performed in a Master-cycler gradient S thermal cycler (Eppendorf Ltd.) under the following conditions: 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 40 s at 59 °C (for *PNRSV*) and 61 °C (for *ACLSV*), 40 s at 72 °C and the final elongation step of 10 min at 72 °C. The amplification products were analysed in 1.5% (w/v) agarose gel using electrophoresis.

**AFLP for genome stability.** Genomic DNA was extracted from 0.1 mg fresh leaf tissue of microshoots using the CTAB-based extraction protocol by Doyle and Doyle (1990). The amplified fragment length polymorphism (AFLP) method was applied for genetic stability testing of the plum shoots obtained after chemotherapy *in vitro*. AFLP analysis was performed according to the Vos *et al.* (1995)

method. The AFLP Plant Fingerprinting Kit (Applied Biosystems Ltd.) was used for sample preparation. All procedures were performed according to manufacturer protocol. About 150 ng of genomic DNA was digested with restriction endonucleases *EcoRI* and *TruII* (*MseI*) (Thermo Scientific Ltd.) and corresponding adaptors were ligated. Pre-amplification was carried out with standard primers *EcoRI* A and *MseI* C (205 nM each) in a 20 µl reaction volume containing 4 µl of diluted restriction-ligation mix and 15 µl of AFLP Amplification Core Mix (Applied Biosystems Ltd.). Pre-amplification conditions were as follows: 94 °C for 2 min followed by 20 cycles of 20 s at 94 °C, 30 s at 56 °C and 120 s at 72 °C, followed by a final step of 2 min at 72 °C and 30 min at 60 °C. Selective PCR amplification was carried out under the same reaction conditions, except that 2 µl of diluted pre-amplification template was used and *MseI/EcoRI* primers sets (CG/ACC, CTC/ACC, CAA/ACC, CTA/ACC, CG/ACT, CTC/ACT, CAA/ACT, CTA/ACT) were used at 50 nM and 250 nM concentration, respectively. Selective amplification was performed using the following programme: an initial cycle of 30 s at 94 °C, 30 s at 65 °C and 80 s at 72 °C, followed by 10 cycles of 30 s at 94 °C 30 s at 65 to 56 °C, 1 °C per cycle, 80 s at 72 °C; followed by 23 cycles of 30 s at 94 °C, 30 s at 55 °C and 80 s at 72 °C, and a final step of 5 min at 72 °C. Samples were prepared for capillary electrophoresis by mixing 1 µl of the PCR product with 8.88 µl of highly deionised (Hi-Di) formamide and 0.12 µl of Gene Scan 500 LIZ ladder (Ap-

plied Biosystems, Ltd.), and analysed using the Genetic Analyzer 3130 (Applied Biosystems Ltd.).

**Statistical analysis.** The data of the experiment were analysed by ANOVA. The treatment effects were tested by the least significant differences  $LSD_{05}$  and  $p$  tests by SigmaStat software.

## RESULTS

Co-infection in shoots of ‘Magna Glauca’ and ‘Cacanska Rana’ was confirmed by PCR. Conditions for micropropagation of plums shoots *in vitro* were investigated in our laboratory in previous studies. After establishment of aseptic culture and multiplication of shoots (Fig. 1), mixed infections of *PNRSV* and *ACLSV* in shoots were re-confirmed by PCR (positive control in Fig. 3).

The multiplication index before chemotherapy *in vitro* is labelled as Control R\_0 in Figure 2. 4.6 to 6.3 microshoots of ‘Magna Glauca’ and 4.1 to 4.3 of ‘Cacanska Rana’ developed per initial individual during propagation. The multiplication indexes of plum shoots differed after ribavirin treatments (Fig. 2). The antivirus agent had a significant phytotoxic effect on shoot proliferation of ‘Magna Glauca’ and ‘Cacanska Rana’ in the first subcultivation period (four weeks after chemotherapy). The lowest proliferation was observed for variant R\_40 for both cultivars — multioliga-

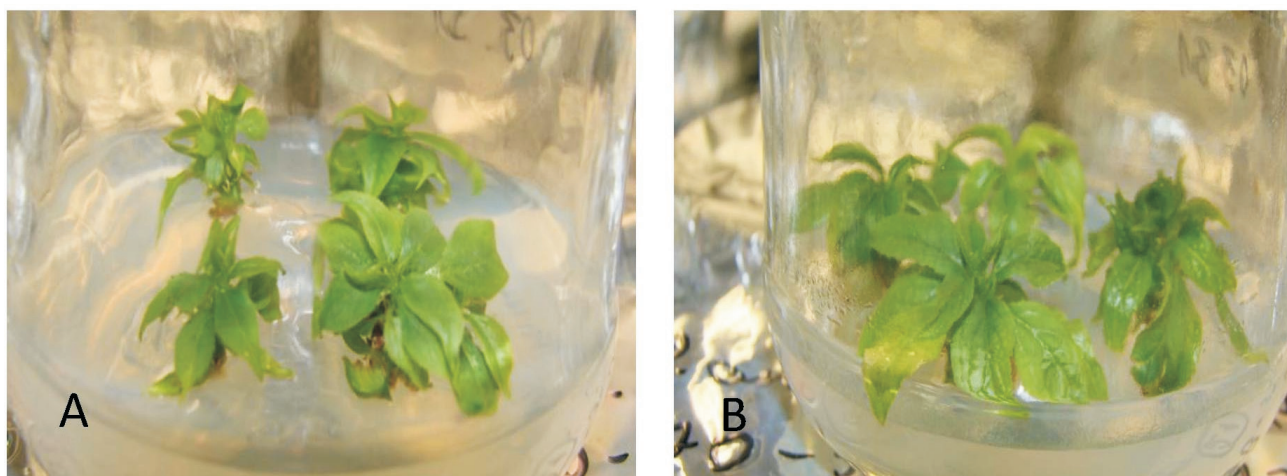


Fig. 1. ‘Magna Glauca’ (A) and ‘Cacanska Rana’ (B) shoots with mixed infection (*PNRSV* and *ACLSV*) were multiplied on modified MS medium.

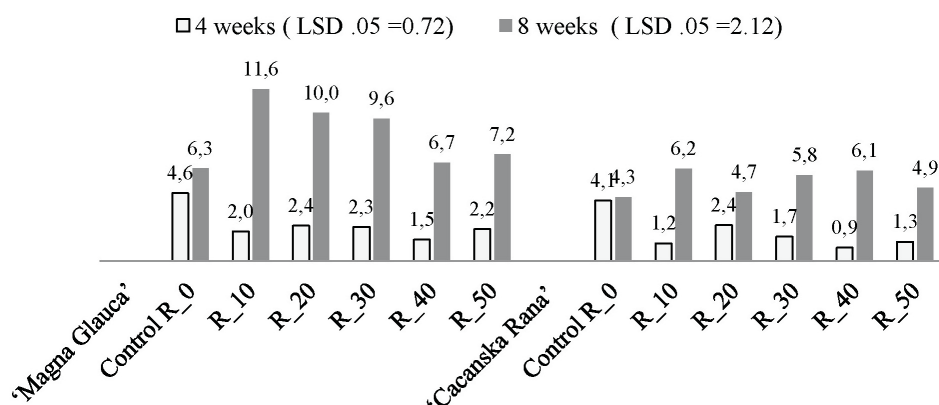


Fig. 2. Plum shoots multiplication index after chemotherapy with ribavirin.



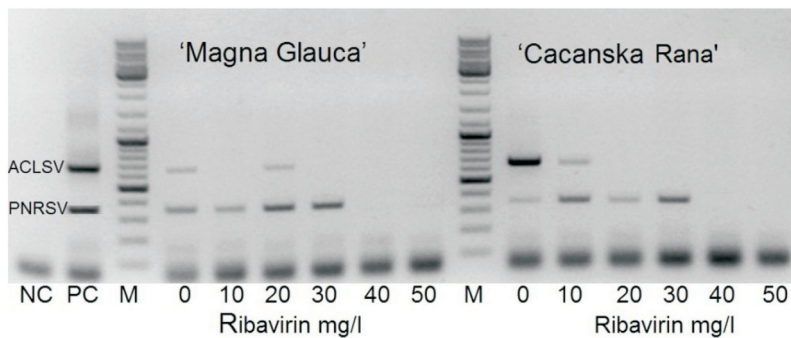


Fig. 3. Virus infection in plum shoots after chemotherapy with ribavirin. The molecular weight of PCR products was estimated by comparison with 1 kb DNA ladder SM1173 (Thermo Scientific Ltd.); NC, negative control, DNA of plum tree with virus free certificate; PC, positive control, DNA of shoots *in vitro* before chemotherapy.

tion index 1.5 for 'Magna Glauca' and 0.9 for 'Cacanska Rana'. Ribavirin had a positive effect at the lowest concentrations (10, 20, and 30 mg·l<sup>-1</sup>) on 'Magna Glauca' shoot proliferation in the second subcultivation period (eight weeks after chemotherapy); the microshoot proliferation index significantly increased from 6.3 (Control R\_0 variant) to 11.6, 10.0, or 9.6 after ribavirin treatments (R\_10, R\_20, or R\_30).

Presence of the viral infection in microshoots was retested by PCR eight weeks after chemotherapy. Specific virus RNA fragments were identified in agarose gel (Fig. 3). The lowest concentrations of ribavirin (10 to 20 mg·l<sup>-1</sup>) were entirely ineffective for *ACLSV* elimination and concentrations from 10 to 30 mg l<sup>-1</sup> were inefficient for *PNRSV* elimination. The highest ribavirin concentrations of 40 and 50 mg·l<sup>-1</sup> were caused loss of both viral pathogens. Elimination of the virus was confirmed in all microshoots of cultivars 'Magna Glauca' and 'Cacanska Rana'.

Genome mutations in plant material before and after manipulation *in vitro* were observed by AFLP method in our study. Eight combinations of primers revealed molecular markers that generated DNA fingerprints with 1075 and 1110 fragments for each cultivar (Table 1). The AFLP assays generated 117 to 152 DNA fragments per primer combination. Polymorphic fragments in DNA fingerprints of cultivars with primer pairs CG/ACC and CTC/ACC were not detected. The highest number of polymorphic DNA fragments (16) was detected with primer pair CG/ACT. The analysis of AFLP showed that the genomes of the plum cultivars 'Magna Glauca' and 'Cacanska Rana' are very similar and are mostly stable for manipulation *in vitro*. The fragments generated by eight primer pairs were confirmed to be monomorphic across tested cultivars after chemotherapy with 10, 20, 30, and 50 mg·l<sup>-1</sup> of ribavirin. Plant genome stability in cultivar 'Magna Glauca' was damaged after 40 mg·l<sup>-1</sup> ribavirin treatment. Instability of the genome was detected with primer pair CG/ACC. Five DNA fragments (56 bp, 76 bp, 131 bp, 161 bp, 245 bp) were newly presented, and absence of two monomorphic markers (134 bp, 150 bp) were found in the DNA fingerprint.

## DISCUSSION

Chemotherapy has been successful in removing viruses from several plant species where chemotherapy is applicable (Panattoni *et al.*, 2013). Also, the combination of

thermotherapy with chemotherapy has been successful (Verma *et al.*, 2005; Hu *et al.*, 2005). Ribavirin, synthesised by Sidwell *et al.* (1972), was tested as an antiviral agent in plant chemotherapy, and showed significant therapeutic potential against plant viruses. However, other synthetic chemical compounds have also been investigated (Verma *et al.*, 2005; Xia *et al.*, 2006; Zhao *et al.*, 2006). Ribavirin was previously tested for ability to eliminate viruses from more than nine families of viruses, using various herbaceous and woody plants (Panattoni *et al.*, 2013). It was found that high concentration of ribavirin is required for virus eradication, but that effects are devastating for the herbaceous plant material. Zhang *et al.* (2006) reported that strawberry mottle virus and strawberry mild yellow edge virus are not sensitive to ribavirin and were not eliminated from strawberry plants. Chemotherapy with ribavirin on sugarcane was also unsuccessful due to the sensitivity of the explants to the antiviral agent (Permesur and Saumtally, 2001). High ribavirin concentrations (30 mg·l<sup>-1</sup> and higher) were found to cause meristem necrosis in potato shoots (Danci *et al.*, 2009). We were able to eradicate the mixed infections of *ACLSV* and *PNRSV* from plum shoots *in vitro* using chemotherapy with ribavirin (Fig. 3). We did not use more than 50 mg·l<sup>-1</sup> concentrations of ribavirin, as Paunovic *et al.* (2007) found that chemotherapy with high concentrations was lethal to plum shoots of cultivar 'Cacanska Lepotica'. In our study, infected shoots of 'Magna Glauca' and 'Cacanska Rana' multiplied well on modified MS nutrition medium, and did not show signs of viral infection (Fig. 1). *ACLSV* was more sensitive to ribavirin than *PNRSV*. A concentration of 20 mg·l<sup>-1</sup> ribavirin in the MS nutrition medium had lethal effects on the *ACLSV* pathogen in plum shoots. According to other researchers (Deogratias *et al.*, 1989; Hansen and Lane, 1985), the removal of *ACLSV* from apple shoots can be achieved with a lower concentration (up to 20 mg·l<sup>-1</sup>) of antiviral agent than from stone fruit shoots (40–100 mg·l<sup>-1</sup>). In our study, ribavirin concentration of 40 mg·l<sup>-1</sup> was optimal for the eradication of *PNRSV* from plum shoots. Ribavirin concentration of 40–60 mg·l<sup>-1</sup> is also suitable for *PPV* elimination from plum shoots (Paunovic *et al.*, 2007). We noticed that during the first period of subculture, all tested concentrations of ribavirin had a negative impact on plum shoot development. However, positive effects on shoots induction *in vitro* for 'Magna Glauca' and 'Cacanska Rana' were observed in the second sub-cultivation period (eight weeks after chemotherapy). The multiplication index was significantly higher in variants where ribavirin was

used at lower concentrations (10–30 mg·l<sup>-1</sup>) (Fig. 2). Our previous work showed that 20 mg·l<sup>-1</sup> exposure of apple shoots ‘Antonovka’ *in vitro* to ribavirin had positive effects on the proliferation index (data not published). A high multiplication index is an important indicator for production of planting material. The multiplication index of ‘Cacanska Rana’ (6.1 and 4.9) and ‘Magna Glauca’ (6.7 and 7.2) obtained in virus-free variants R\_40 and R\_50 were relatively high (Fig. 2).

Based on published reports on occurrence of somaclonal variation (Zucchi *et al.*, 2002; Wen *et al.*, 2005; Ngezahayo *et al.*, 2006) among plants grown through enhanced axillary branching cultures, we estimated genetic stability of plum shoots before and after manipulations *in vitro*. AFLP analysis showed that genome instability *in vitro* can be detected in *P. domestica* cultivars (Table 1). AFLP is a sensitive method for testing for instability of the plant genome, especially when various primer pairs are used for selective amplification (Beismann *et al.*, 1997; Ulrich *et al.*, 1999). Using eight pairs of primers, we obtained only monomorphic DNA fragments with seven primer pairs in the plum shoots before and after chemotherapy. Polymorphic DNA fragments were obtained with the primer combination MseI/EcoRI – CG/ACC (3.9%) in the genome of ‘Magna Glauca’. A total of 1075 genetic makers were found and 0.5% of them showed genetic instability. That is not a high genomic polymorphism, but attention should be paid to somaclonal variation *in vitro* of individual plum cultivars. Somaclonal variation was also found at different levels in *Secale cereale* — 8.8% (de la Puente *et al.*, 2008), and *Echinacea purpurea* — from 1.6% to 20.6% (Chuang *et al.*, 2009). We did not find polymorphic AFLP markers in the ‘Cacanska Rana’ shoot’s genome *in vitro*. A positive aspect is that somaclonal variation provides an alternative tool for

breeders to obtain genetic variability in horticultural crops (Krishna *et al.*, 2016). Further research *in vivo* for the evaluation of equability of the *P. domestica* propagation material after virus eradication and micropopagation is required.

## CONCLUSIONS

Chemotherapy *in vitro* with ribavirin concentration at 40 mg·l<sup>-1</sup> is optimal to eradicate viruses PNRSV (*Ilarvirus* genus) and ACLSV (*Trichovirus* genus) from plum shoots. The toxic effects of ribavirin on development and growth of plum shoots disappears one month after chemotherapy. Chemotherapy with ribavirin at concentrations 10–30 mg·l<sup>-1</sup> has a positive effect on the proliferation index of shoots ‘Magna Glauca’ and ‘Cacanska Rana’ *in vitro*. An AFLP analysis of genetic stability after virus eradication *in vitro* is required for plants of *P. domestica*.

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Table 1

### GENETIC STABILITY OF PLUM SHOOTS BEFORE AND AFTER CHEMOTHERAPY *IN VITRO*

Primer pair MseI/EcoRI	AFLP fragments detected in DNA of initial plant material and in DNA of shoots after chemotherapy and multiplication <i>in vitro</i>		
	‘Cacanska Rana’ initial/ <i>in vitro</i>	‘Magna Glauca’ initial/ <i>in vitro</i>	Polymorphic AFLP markers
CG/ACC	127/127	127/130*	*In treatment R_40 Presence of new fragments – 56 bp; 76 bp; 131 bp; 161 bp; 245 bp; Absence fragments – 134 bp; 150 bp
CTC/ACC	142/142	142/142	
CAA/ACC	145/145	143/143	
CTA/ACC	122/122	117/117	
CG/ACT	152/152	136/136	
CTC/ACT	143/143	138/138	
CAA/ACT	135/135	137/137	
CTA/ACT	144/144	135/135	
Total:	1110/1110	1075/1078	

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## RIBAVIRĪNA IETEKME UZ PRUNUS DOMESTICA L. REĢENERĀCIJU, GENOMA STABILITĀTI UN VĪRUSU ELIMINĀCIJU *IN VITRO*

*PNRSV* (*Prunus necrotic ring spot*) vīruss un *ACLSV* (*Apple chlorotic leaf spot*) vīruss ir bieži sastopami plūmju stādījumos. Pētījuma mērķis ir iegūt vīrusbrīvu *Prunus domestica* L. stādmateriālu, pielietojot ķīmijterapiju *in vitro*. Ribavīrīns tika pievienots Murashige–Skoog (MS) mikrosāļu barotnei 10 līdz 50 mg·l<sup>-1</sup> koncentrācijā vīrusu eliminācijai no mikrodzinumiem. Pēc divu nedēļu ķīmijterapijas perioda tika grieztas meristēmas un turpināta mikropavairošana uz MS barotnes, nosakot proliferācijas koeficientu. Vīrusu eliminācijas efektivitātes novērtēšanai mikrodzinumi tika testēti, izmantojot reversās transkripcijas polimerāzes ķēdes reakciju. Tika konstatēts, ka *ACLSV* eliminācijai zemākā ribavīrīna koncentrācija 10 mg·l<sup>-1</sup> bija neefektīva, kā arī *PNRSV* eliminācija pie koncentrācijas 10 līdz 30 mg·l<sup>-1</sup> bija neefektīva. Savukārt pie ribavīrīna koncentrācijas 40 un 50 mg·l<sup>-1</sup> abi patogēni tika sekmīgi eliminēti. Tomēr pie šīs augstās ribavīrīna koncentrācijas bija vērojams fitotoksiskums mikroaugu pirmajā pasāžā. Lai noskaidrotu metodes ietekmi uz mikroaugu ģenētisko stabilitāti, tika pielietota amplificētu fragmentu garuma polimorfisma (AFLP) metode. Šķirnei 'Magna Glauca' pie ribavīrīna koncentrācijas 40 mg·l<sup>-1</sup> tika konstatētas ģenētiskas izmaiņas, jo tika atrasti polimorfī AFLP marķieri.