Influence of ribavirin on *Prunus domestica* L. regeneration, genome stability and virus eradication *in vitro*

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- *Prunus necrotic ring spot (Ilarvirus)* and *Apple chlorotic leaf spot (Trichovirus*) are common virus diseases in the plum trees genetic resource orchard in Horticulture Institute of LAMMC (16.8 % and 4.8 % respectively).
- Low infection rate (1.6 %) of mixed infections involving PNRSV-ACLSV in plum trees ('Magna Glauca' and 'Cacanska Rana') were determined by PCR.

✓ **Aim of study was to get virus free planting material of *Prunus domestica* L.**

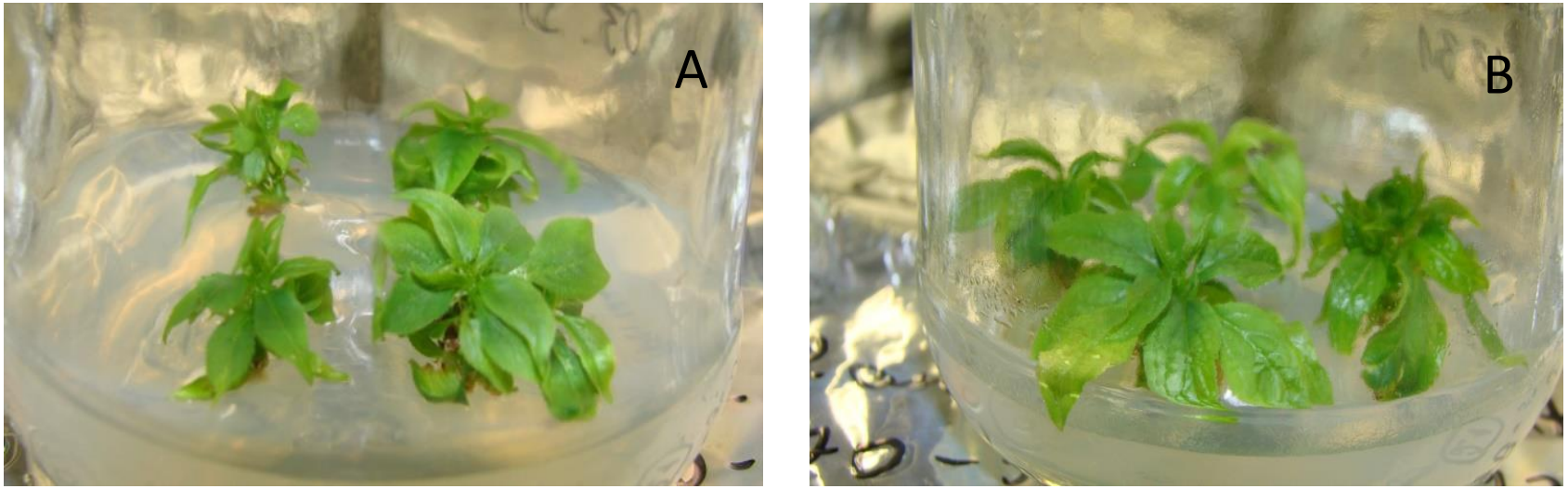

Fig 1. ‘Magna Glauca’ (A) and ‘Cacanska Rana’ (B) shoots with mixed infection (PNRSV and ACLSV) were multiplied on modified MS medium.

Virus eradication

- Multiplied cultures (PNRSV and ACLSV infected shoots) were treated on MS medium with ribavirin for 2 weeks period.
- Five different concentrations of ribavirin (Virasol) were used: 10, 20, 30, 40, 50 mg/l.
- After chemotherapy, meristems were subcultivated monthly on MS medium.
- Shoots proliferation index were evaluated after 4 and 8 weeks.
- Shoots were retested by polymerase chain reaction (PCR) method for virus detection after 8 weeks.
- The amplified fragment length polymorphism (AFLP) method was applied for the genetic stability testing of the plum shoots obtained after chemotherapy *in vitro*.

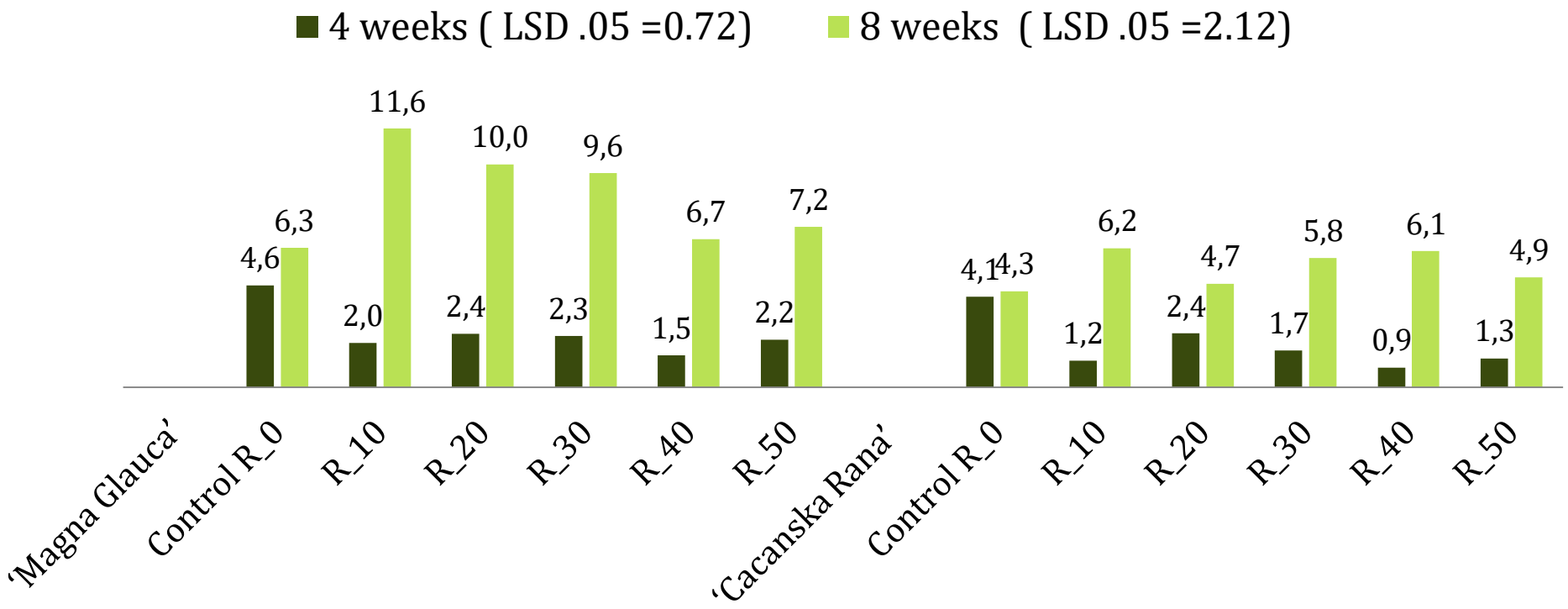

Fig. 2 Plum shoots proliferation index after ribavirin treatment

- ✓ Ribavirin in lowest concentrations (10 to 30 mg/l) influenced positively on 'Magna Glauca' shoots proliferation in first multiplication rate.
- ✓ Antivirus agent exhibited significant signs of phytotoxicity on proliferation of 'Cacanska Rana' shoots in first and second multiplication rate.

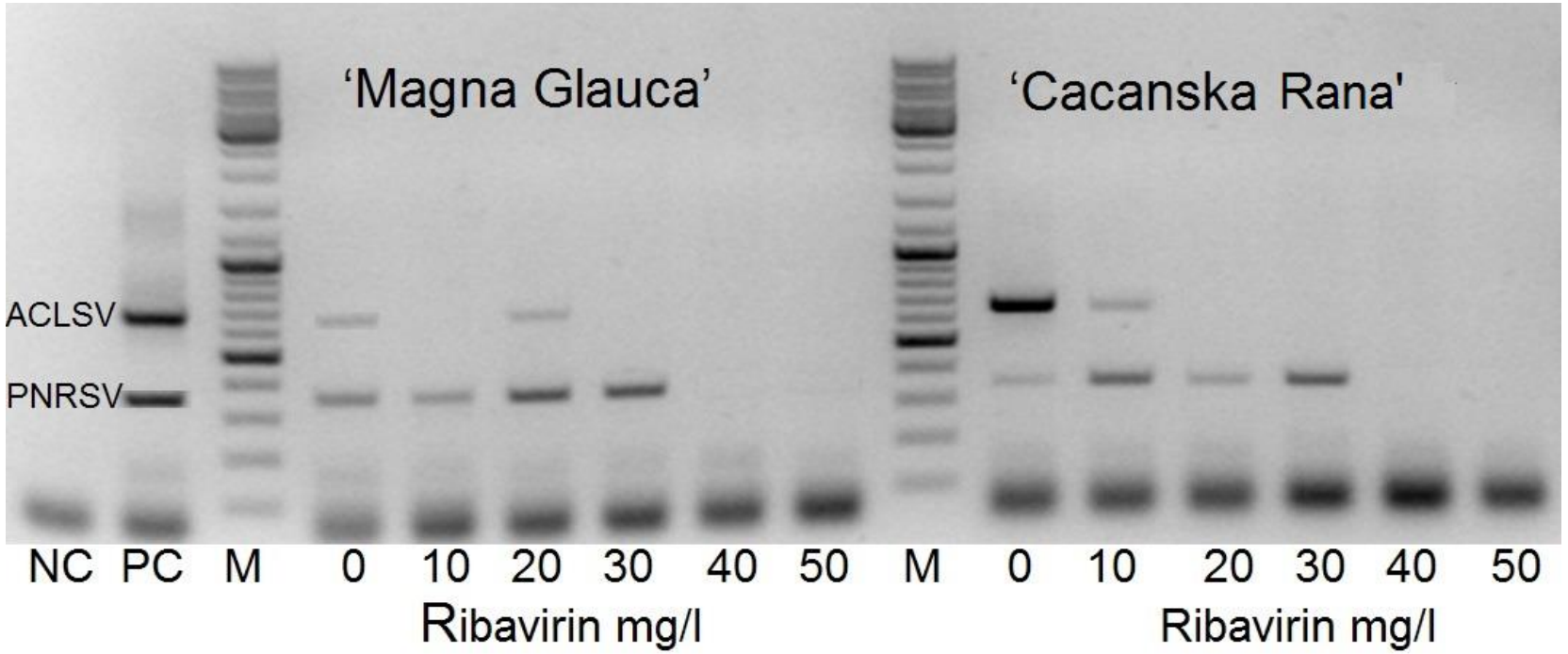

Fig. 3 Virus infection in plum shoots after chemotherapy

- ✓ Lowest concentrations of ribavirin (to 30 mg l^{-1}) were entirely ineffective for ACLSV and PNRSV elimination.
- ✓ The ribavirin concentrations 40 or 50 mg l^{-1} were disastrous for both pathogens and all shoots of plums 'Magna Glauca' and 'Cacanska Vcesna' were virus free.

Genetic stability of plum shoots after chemotherapy *in vitro*

Primer pair	AFLP fragments polymorphic/ monomorphic		
	'Cacanska Rana'	'Magna Glauca'	In treatment R_40 + new fragments; - non-amplified fragments;
CG/ACC	127/0	127/7	+56;+76; +131;-134; -150; +161;+245;
CTC/ACC	142/0	142/0	
CAA/ACC	145/0	143/0	
CTA/ACC	122/0	117/0	
CG/ACT	152/0	136/0	
CTC/ACT	143/0	138/0	
CAA/ACT	135/0	137/0	
CTA/ACT	144/0	135/0	
Viso:	1110	1075	

✓ The fragments generated by eight primer pairs proved to be monomorphic across tested plants of plums after chemotherapy with 10, 20, 30 and 50 mg l^{-1} of ribavirin. Plant genome stability in 'Magna Glauca' was damaged after impact of ribavirin 40mg l^{-1}, presence of polymorphic DNA fragments was observed.

Thank you for your attention!

‘Magna Glauca’

‘Cacanska Rana’