ONE-STEP RT-PCR DETECTION OF APMV, ASGV, ACLSV AND ASPV AT *IN VIVO* AND *IN VITRO* APPLE PLANT MATERIAL FROM ALBANIA

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MATERIALS AND METHODS

- *In vivo and In vitro* propagated plant material
- from cultivars *Golden delicious* and *Starking*,
- grown in three collections (Hocisht, Bitincke and Cangonj) was used to extract total RNA.
- For each category of *in vitro* plants were sampled 50 individuals, and RNA mixes were prepared (Table no1).
- For each cultivar of *in vivo* plants were sampled 50 indivuals and prepared 5 RNA Mixes for *Starking* (named S1-S5) and 5 for *Golden delicious* (named G1-G5).

Table 1. In vitro propagated plant categories.

Sample No.	Cultivar	Plantlets origine	Collection
1	*Starking*	Seeds	Hocisht
2	*Starking*	Seeds	Cangonj
3	*Golden delicious*	Seeds	Hocisht
4	*Golden delicious*	Seeds	Cangonj
5	*Golden delicious*	Buds	Hocisht
6	*Golden delicious*	Buds	Cangonj
7	*Golden delicious*	Buds	Bitincke
8	Starking	Buds	Bitincke
9	Starking	Buds	Cangonj
10	Control		

Total RNA was used as template for One-step RT-PCR performed according to HS-RT-PCR Kit of SIGMA, using virus specific primers for ASPV, ASGV, ACLSV and ApMV, and cycling conditions according to Menzel *et al*., 2002.

CONCLUSIONS

• Either plantlets propagated by seeds or by buds, or mother plants were infected.
• The amplicons concentrations varied from collection to collection and between cultivars;
• The plantlets originated from the collection of Bitincka in most of the cases gave amplicons of higher concentrations than the ones of collections of Cangonj and Hocisht;
• In few cases plants originated from buds failed to give amplicons, while the other category (originated from buds) was infected;
• Use of two categories of explants served to double check the sanitary status of mother plants in the field.
• The rate of mixed infections at three collections, measured as the number of categories of viral infections per 100 plants, shows that mixed infections are more prevalent in Bitincke, Hocisht, and less in Cangonj.
• Considering that reactions conditions and template concentrations were equal, we believe that the concentration of amplicons is proportionate to the level of infection, and can be used to monitor the sanitary situation in collections even in the absence or unclear field symptoms.

RESULTS

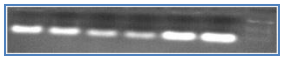


Figure 1. Amplicons of ApMV gene fragment originated from *in vitro* propagated plantlets from seeds of cultivar *Starking* and *Gold*. From right to left: Molecular marker 100bp, samples 1-6; from all of them is amplified a fragment of expected size 450bp.

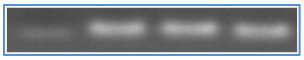


Figure 2. Amplicons of ApMV gene fragment. From right to left: *in vitro* samples 6-9; sample; from all of them is amplified a fragment of expected size ~ 450 bp.

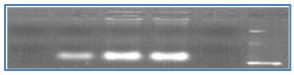


Figure 4. Amplicons of ACLSV gene fragment. From right to left: Molecular marker 1kbp, *In vitro* samples 6-9; sample 10 is control; from samples 7, 8, 9 is amplified the fragment of expected size 670 bp.

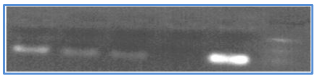


Figure 3. Amplicons of ACLSV gene fragment. From right to left: Molecular marker 1kbp, *in vitro* samples 1-5; from samples 1,3,4,5 is amplified the fragment of expected size 670 bp.

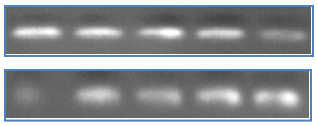


Figure 5. Amplicons of ASPV gene fragment. Above, from right to left *in vivo* samples S1-S5 *Starking*; Below samples G1-G5 *Golden delicious* from which the amplicon of expected size 370bp is produced.

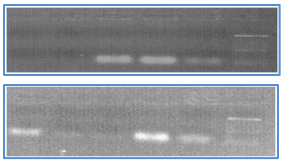
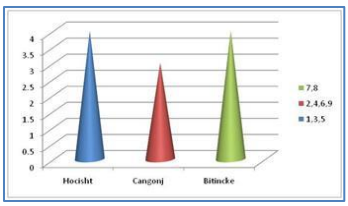


Figure 5. Amplicons of ASPV gene fragment. From *in vitro* plants. From right to left: Molecular marker 1kbp, samples 6-9; sample 10 is control; Molecular marker 1kbp- samples 1-5 from which the amplicon of expected size 370bp is produced from 1, 2, 3, 4, 5, 6, 7, 8.



Graphic No1. Comparison of the presence of mixed viral infections at collections of Hocisht, Cangonj, and Bitincke.

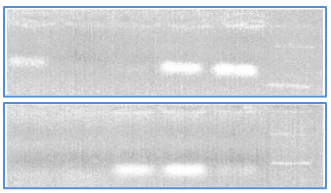
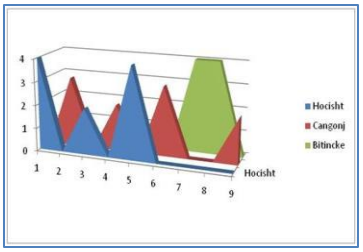


Figure 6. Amplicons of ASGV gene fragment. From right to left: Molecular marker 1kbp, *in vitro* samples 6-9; sample 10 is control; Molecular marker 1kbp- samples 1-5from which the amplicon of expected size 273 bp is produced from samples 1, 2, 5, 6, 7, 8.



Graphic No.2 Infections at each sample. It a pool of 100 plants from the collection of Hocisht are found present 2,6 out of 4 types of viruses; At the collection of Cangonj are found 1,5 out of 4 types of viruses, and at the collection of Bitincke are found 4 out of 4 viral categories.

LITERATURE

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