

PATHOGEN ELIMINATION USING MERISTEMS *IN VITRO* CULTURE – THE STRATEGY OF HOP PROTECTION AGAINST VIRUSES AND VIROIDS

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INTRODUCTION

Hop plants in Poland are commonly infected by *Hop mosaic virus* (HpMV), *Apple mosaic virus* (ApMV) and *Hop latent viroid* (HLVd). Infection is usually symptomless but in many cases it can have a deleterious effect on cone yield, bitter acids content and composition of hop essential oils. The response of individual hop cultivars to infection is different. One of the most effective methods of hop protection against viruses and viroids is the use of planting material free from these pathogens for establishing hop gardens. This material has to be propagated in suitable greenhouse conditions starting from virus- and viroid-free mother plants obtained by elimination of these pathogens using apical meristems *in vitro* cultures.

MATERIAL AND METHODS

Hop plants of four hop cultivars, i.e. 'Magnum', 'Magnat', 'Marynka' and 'Puławski' growing in a greenhouse conditions were used as a donor material for pathogens elimination. Apical meristems about 0.1 – 0.2 mm long were excised from suitable donor plants and transferred to three different regeneration media for growing, shoots development and rooting. Regenerated plantlets were tested for the presence of viruses and viroid using ELISA and RT-PCR methods, respectively.

RESULTS

Meristems culture have been successful in eliminating both viruses, but it was less effective against viroid. All plantlets regenerated from meristems were free of the HpMV and ApMV, whereas only from 2.2% to 36.4%, depending of hop cultivar, were free of HLVd (Tab.1). The highest percentage of completely healthy plants was obtained for the 'Puławski' cultivar, while the lowest for the 'Magnat' cultivar.

Healthy plantlets were multiplied *in vitro* to the requirement number, then transferred into the pots and placed in the greenhouse where they adapted to the outside growing conditions. These mother plants, after retesting for HpMV, ApMV and HLVd, were propagated by single-node cuttings. Currently, the healthy mother plants of seven most important hop cultivars grown in Poland, i.e. 'Iunga', 'Lubelski', 'Magnat', 'Magnum', 'Marynka', 'Puławski', 'Sybilla', are maintained and propagated.

Table 1. Effectiveness of viruses and viroid elimination from hop plants using apical meristems *in vitro* cultures

Hop cultivar	Number of <i>in vitro</i> plantlets tested for HpMV, ApMV and HLVd	Number of infected plantlets			Number of completely healthy plantlets
		HpMV	ApMV	HLVd	
Magnat	89	0	0	87	2
Magnum	45	0	0	39	6
Marynka	29	0	0	23	6
Puławski	22	0	0	14	8
Total	185	0	0	163	22



Plantlets regenerated from meristems



Healthy mother plants and their multiplication by single-node cuttings



Distribution to hop growers

Elimination of viruses and viroids doesn't protect hop plants against reinfection. The frequency of reinfection with HpMV, ApMV and HLVd was studied in 26 hop plantations five years after establishing them using healthy planting material (Tab.2.). From 10 to 20 plants were tested from each plantation, depending on its size. Reinfection with HLVd, ApMV and HpMV was observed in 2.8%, 1.4% and 0.8% of tested plants, respectively. On the average, 5.0% of samples were positive for at least one pathogen. The highest rate of reinfection was found in cultivar 'Marynka' and the lowest in cultivar 'Lubelski'.

Table 2. The frequency of reinfection in five years old hop gardens established using healthy planting material

Hop cultivar	Number of hop plantation studied	Number of hop plants tested	Number of infected plants			% of infected plants
			HpMV	ApMV	HLVd	
Lubelski	5	43	0	1	0	1,4
Magnat	10	135	0	1	5	4,4
Marynka	2	35	0	0	4	11,4
Puławski	3	40	1	1	1	7,5
Sybilla	6	77	2	2	0	5,2
Total	26	360	3	5	10	5,0

HpMV, ApMV and HLVd were successfully eliminated from hop plants using apical meristems *in vitro* cultures. Elimination effectiveness depended on pathogen and was much greater in case of viruses. Plants free of viruses and viroid were propagated to produce high-quality hop planting material. The rate of reinfection during first five years after planting was low, thus the hop gardens established using healthy planting material remained for long time with high health status.