Elimination of *Grapevine fanleaf virus* in grapevine by *in vivo* and *in vitro* thermotherapy

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ABSTRACT: In this paper, results of the *in vitro* treatment are compared with those of *in vivo* therapy (i.e. treatment of plants in a peat substrate) when eliminating GFLV (*Grapevine fanleaf virus*) from three grapevine rootstocks. Therapy took 45 days under the temperature of 37°C in both cases. As far as the health condition of treated plants was concerned, no differences were found between the two methods. The differences were manifested in numbers of plants dying during the therapy and in the course of cultivation of apical segments treated with thermotherapy. Based on these results, it can be recommended to apply the thermotherapy *in vivo*, which – as compared with the *in vitro* method – enables us to obtain a greater number of apical segments from treated plants and to establish a smaller number of *in vitro* cultures in the course of the treatment. A shorter period of *in vitro* cultivation of plants is another advantage of this method because it helps to reduce the risk of somaclonal variability.

Keywords: GFLV; thermotherapy; *Vitis*; virus elimination; rootstocks

Grapevine fanleaf virus (GFLV) is responsible for fanleaf degradation, which is one of the most severe diseases of grapevine worldwide (Andret-link et al. 2004). To eliminate this virus and to regenerate virus-free plants, the thermotherapy under *in vitro* conditions is a frequently used method of treatment (Blazina et al. 1991; Leonhardt et al. 1998; Laimer et al. 2005).

The method of elimination of viral diseases, which consists of a combination of thermotherapy and subsequent sampling of apical segments (greater than meristems) has been used since 1960s (Galzy 1961). The obtained apical segments are then used for the regeneration of virus-free grapevine plants. This method is based on the effects of a decreasing concentration of viruses towards the apex on the one hand and of inhibition of viral propagation and diffusion due to increased temperatures on the other.

Both recent and older studies (Galzy 1961; Leonhardt et al. 1998; Laimer et al. 2005) dealt with thermotherapy of *in vitro* cultivated grapevine plants when eliminating the GFLV. However, the thermotherapy *in vivo* (i.e. heat treatment of plants directly in the substrate combined with a subsequent rooting of sampled segments under sterile conditions) has also a number of advantages. In this paper, the *in vivo* thermotherapy (proposed method) is compared with *in vitro* thermotherapy, which is frequently used. Comparison was done from the viewpoint of the effect on the health condition and mortality of plants in the course of the therapy.

MATERIAL AND METHODS

Plant material

For experiments the following rootstocks originating from the crossing of *Vitis berlandieri* Planch. × *Vitis riparia* Michx. were used: Teleki 5C, SO4 and 125 AA. Among all rootstocks one maternal plant was selected, which – when tested by means of RT-PCR method described by Holleinová et al. (2006) – showed a positive reaction to the presence of GFLV.

The selected plants were propagated vegetatively as cuttings; these cuttings were used for the establishment of *in vitro* cultures and for the process of *in vivo* thermotherapy.

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Table 1. A survey of cultivation media

Medium	Composition
A	MS; 0.3 mg/l BA; 0.1 mg/l IAA; 30 g/l saccharose; agar 0.5%
B	MS; 0.2 mg/l IBA; 20 g/l saccharose; gerlite 0.25%
C	MS; 0.1 mg/l NAA; 0.1 mg/l IAA; 30 g/l saccharose; agar 0.5%

MS – medium (Murashige, Skoog 1962), BA – 6-benzylaminopurine, IAA – indole-3-acetic acid, IBA – indole-3-butyric acid, NAA – 1-naphtalene acetic acid

In vivo thermotherapy

Plants propagated as softwood cuttings were cut behind the fifth leaf, transferred into containers with peat substrate (size 9 × 9 × 12 cm), and placed into a thermobox with the photoperiod 16 (light)/8 (darkness) hours and the temperature of 37°C. Relative air humidity and light intensity were set up at 80% and 22 μmol/m^2/s, respectively. After 45 days of thermotherapy, apical segments (5 mm long) were sampled, their surface was disinfected and they were placed on the cultivation medium C (Table 1).

In vitro thermotherapy

In vitro cultures were established using nodal segments. Cultivation was performed on MS (Murashige, Skoog 1962) medium containing 0.3 mg/l BA (6-benzylaminopurine) and 0.1 mg/l IAA (indole-3-acetic acid). Experimental plants were transferred to a fresh medium every four weeks. After four months, young plants were passed through thermotherapy on three different cultivation media marked A, B, C (Table 1). Each plant was placed into a separate test tube. Conditions and duration of the therapy were the same as described in the section *In vivo* thermotherapy.

Fig.1. Taking of apical segment (5 mm long) from *in vitro* thermotherapy treated plant

Final cultivation of young plants and testing

After both (i.e. *in vitro* and *in vivo*) types of therapy, the apical segments (5 mm long) were transferred on the C medium (Table 1) to enable their elongation growth and rooting. Rooted plants were then placed into the peat substrate with added Agroperlite (Fig. 1).

Twelve months after the transfer to non-sterile conditions the experimental plants were tested for the presence of GFLV using the DAS-ELISA method (leaf samples were analyzed according to the methodology of the Bioreba Company) and the RT-PCR (reverse transcription-polymerase chain reaction) method. For the RT-PCR tests, the method published by Holleinová et al. (2006) was used.

RESULTS AND DISCUSSION

Virus-free plants were found after both, *in vitro* and *in vivo* therapy. Six and seven positive plants were detected by the ELISA method and the RT-PCR tests, respectively. In total 122 tests were done. As shown in Table 2, where the data of both *in vitro* and *in vivo* therapy are presented, plants of all treated rootstock cultivars were recovered. As far as the growth of plants in the course of thermotherapy is concerned, the best results were obtained on the A medium, where the length of new growth increments ranged from 37 to 55 mm and the number of dead plants was the lowest. All plants pro-

Table 2. Thermotherapy of grape rootstocks

Therapy	Used medium	Plant	Plants in therapy	Collected segments	Number of acclimatized plants	Plant tested	Healthy	Infected
In vitro	A	Teleki 5C	40	35	22	10	10	0
	B	Teleki 5C	40	2	0	0	0	0
	C	Teleki 5C	40	5	4	4	3	1
	A	SO4	40	32	24	10	9	1
	B	SO4	40	0	0	0	0	0
	C	SO4	40	3	3	3	3	0
	A	125 AA	35	28	17	10	7	3
	B	125 AA	35	0	0	0	0	0
	C	125 AA	35	0	0	0	0	0
In vivo		Teleki 5C	4	18	17	10	9	1
		SO4	4	23	20	10	10	0
		125 AA	4	12	10	4	3	1

All segments were rooted on media medium (Murashige, Skoog 1962) with 0.1 mg/l NAA (1-naphtalene acetic acid), 0.1 mg/l IAA (indole-3-acetic acid), and agar 0.5%

duced always only one shoot and no multiplication and/or callus formation was observed. The lowest growth increments were recorded on medium C.

On the medium B, all plants died within the 45 days of thermotherapy; however, this is in contradiction to the results obtained by Leonhardt et al. (1998), who successfully treated grapevine plants on this rooting medium. However, these authors used the temperature of 35°C in the course of thermotherapy, i.e. a temperature that was lower by two degrees than that used in our study, and this could support the survival of plants. For *in vitro* thermotherapy process, we recommend to use the medium A. Rooting media with addition of auxin (media B and C) are, according to the obtained results, unsuitable because of high mortality of plants.

As far as the health status of plants was concerned, no differences were found between the *in vitro* and the *in vivo* therapy. The *in vitro* therapy of plant material is recommended by many authors (e.g. Leonhardt et al. 1998; Laimer et al. 2005), mainly for the possibility to save the space inside the thermobox. However, the *in vivo* thermotherapy of plants in the substrate has several advantages such as a possibility to obtain a higher number of apical segments from one treated plant and a reduced requirement of *in vitro* cultures in the course of therapy. Due to this fact the cost of cultivation media is lower and the numbers of surface-disinfected segments are reduced as well. A shorter period of sterile cultivation (max. 3 weeks) is another advantage of the *in vivo* therapy method because it enables to reduce the risk of somaclonal variability, which increases in dependence on the number of passages (Harding et al. 1996). The length of cultivation period can be further reduced, for example when the method of micrografting on rooted healthy rootstocks is used.

CONCLUSION

The *in vivo* (i.e. performed under non-sterile conditions) thermotherapy of grapevine rootstocks appears as successful as the sterile *in vitro* treatment but it is less laborious and more friendly not only to treated plants but also to the environment. As compared with the *in vitro* method, its main advantage consists above all in a shorter period of cultivation of plant under sterile conditions. All treated plants will be further observed to reveal all possible morphological changes.

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Srovnání účinku termoterapie *in vitro* a *in vivo* na ozdravení podnoží révy od viru roncetu révy vinné (GFLV)

ABSTRAKT: K ozdravování rostlin od viru roncetu révy vinné GFLV (*Grapevine fanleaf virus*) se používá tepelná terapie v podmínkách *in vitro*. V práci je srovnávána *in vitro* terapie s metodou *in vivo* terapie (tj. ošetření rostlin v rašelinovém substrátu) z hlediska ozdravení a přežití tří podnoží révy. Terapie probíhala 45 dní při 37°C. Při srovnání z hlediska ozdravení rostlin nebyl nalezen rozdíl mezi těmito metodami. Rozdíl se projevil v počtu rostlin uhynulých během terapie a během kultivace termoterapií ošetřených apikálních segmentů. Podle výsledků práce lze doporučit aplikaci termoterapie révy metodou *in vivo*, která při stejném počtu ozdravených rostlin, ve srovnání s *in vitro* metodou, umožňuje odebrat větší počet vrcholových segmentů z terapií ošetřené rostliny a založit menší počet *in vitro* kultur v průběhu ozdravování. Další výhodou metody je kratší doba kultivace rostlin *in vitro*; tím lze snížit riziko somaklonální variability.

Klíčová slova: GFLV; termoterapie; *Vitis*; ozdravování; podnože

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