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# Sanitation of Autochthonous Grapevine Varieties from Algeria by Chemotherapy

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Several grapevine viruses were reported in Algeria and especially in grapevine germplasm collection, therefore it is a great challenge to free these varieties from virus infection before any breeding programs. Our study focused on the development of chemotherapy on autochthonous varieties collected in the grapevine germplasm collection of ITAFV. All these varieties were tested by DAS-ELISA and the presence of GLRaV-3 and GFLV was confirmed in all used samples for the sanitation. After 8 weeks of shoot tips *in vitro* culture in a modified M S medium containing ribavirin, DAS-ELISA test revealed that GLRaV-3 was completely eliminated and GFLV to a significant rate.

**Keywords:** GLRaV-3, GFLV, chemotherapy, micropropagation.

Grapevine was reported to be infected approximately by 70 distinct virus species that belong to a wide range of taxonomic groups (17 families and 27 genera), usually infected by several ones (Meng et al., 2017). Some of these viruses were widely distributed in all grapevine growing regions over the world. Grapevine leafroll viruses were reported as widely distributed (Martelli and Boudon-Padieu, 2006; Mahfoudhi et al., 2008; Almeida et al., 2013; De Moura et al., 2018). These viruses were detected in several autochthonous germplasm collections in association with GFLV, GFKV (Mahfoudhi et al., 2014; Lehad et al., 2015). These collections were infected by several viruses and it is important to develop new methods for virus sanitation in order to protect this human patrimony. Different methods were developed. GLRaV-3, GVA, and GRSPaV were eliminated from infected grapes using somatic embryogenesis (Bouamama Gzara et al., 2017).

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The thermotherapy allowed to eliminate virus from 72,2% GFLV infected grapevine and 24,7% for GLRaV-3 (Panattoni and Triolo, 2003). The cryotherapy revealed 97% of elimination for GVA (Wang et al., 2003). Studies revealed the efficiency of chemotherapy for different grapevine viruses. Guţă et al. (2014) revealed a total elimination of GFKV from infected grapevine with chemotherapy. Some drugs were reported to have antiviral activity on grapevine virus, ribavirin, oseltamivir, mycophenolic acid, 6-mercaptopurine, 6-thioguanine, amantadine, 2,4-dioxo-hexahydro-1,3,5-triazine, (S)-9-(2,3-dihydroxypropyl)-adenine and tiazofurine (Weiland et al., 2004; Panattoni et al., 2007, 2011; Luvisi et al., 2012; Guţă et al., 2014).

Ribavirin is an antiviral agent that may be toxic. Guta et al. (2014) reported the toxicity of ribavirin on grapevine tissue culture. Thus the virus elimination process often involved the evaluation of phytotoxicity of virucides in various concentrations and periods of exposure (Guţă et al., 2009).

The grapevine germplasm collection of ITAFV was found to be infected by distinct viruses generally in mixed infection; it was previously reported as infected by GLRaV-3 (Lehad et al., 2015).

Our study focused on the elimination of GLRaV-3 and GFLV from autochthonous varieties provided by the autochthonous germplasm collection of ITAFV using a micropropagation associated with the antiviral agent ribavirin.

The main objective of this study was the elimination of two different viruses belonging to two families, GLRaV-3 (*Closteroviridae*) and GFLV (*Nepovirus*). The advantages of this technic in grapevine micropropagation and nursery are enormous, due to the set-up of a multiplication method which presents antiviral activity and eliminate viruses from propagating material. Thus, the use of this technique may reduce significantly the dissemination of plant viruses through the use of virus free propagating material.

## Materials and Methods

*Virus source*
*Infected material:*

Samples were collected from varieties infected by GLRaV-3 and GFLV maintained in greenhouse provided from the grapevine germplasm collection of ITAFV. The samples were tested by DAS ELISA and confirmed the infection by GLRaV-3 and GFLV (Table 1).

**Table 1**

Infected and no infected samples after sanitation

| Variety | Nbr GFLV positive samples | Nbr GLRaV-3 positive samples | Mother vine GLRaV-3 test | Mother vine GFLV test | GLRav-3 negative | GFLV negative | |
|---|---|---|---|---|---|---|---|
| Aberkane | 8 | 8 | 2 | 2 | 8/8 (100%) | 6/8 | 75% |
| Bezzoul El Khadem | 8 | | | 2 | | 7/8 | 87.5% |
| Muscat de Fandouk | 8 | 8 | 2 | 2 | 8/8 (100%) | 5/8 | 62,5% |
| Ferrana | | 8 | 2 | | 8/8 (100%) | | |

In vitro *chemotherapy:*

Grapevine apices (0.2–0.3 cm) and axillary buds, collected from infected mature plants over the growing season were grown on modified MS (Murashige and Skoog, 1962). Ribavirin (20 ml/L) acting as the antiviral agent was added to the proliferating medium for eight weeks. The vitroplant obtained were transferred on virucid-free multiplication medium, for 1–3 subcultures. As the microshoots differentiated, they were cultivated on rooting medium. A positive control from infected mother plant for each variety was *in vitro* regenerated on a free-drug medium. The explants were maintained under controlled condition (24 ± 1 °C, 16 h photoperiod).

*Assessment of ribavirin effect and phytotoxicity:*

Mortality of shoot tip was assessed. The Tukey's HSD test at $p = 0.05$ was used to compare the effect of the ribavirin on the number of shoot formation using the software Statistica version 12.

*Virus detection by ELISA:*

DAS-ELISA test was performed for GLRaV-3 and GFLV using commercial reagents produced by BIOREBA according to the method described by Clark and Adams (1977).

## Results

After eight weeks of growth on MS medium with ribavirin, the explants were tested by DAS-ELISA for the two viruses GLRaV-3 and GFLV found in mixed infection. Results revealed 100% eradication of all tested samples for GLRaV-3. Thus, the autochthonous varieties Aberkane, Muscat de Fandouk and Ferrana revealed 100% eradication after eight weeks of micropropagation on medium containing ribavirin compared to the positive control regenerated in a drug-free medium collected from the mother plant that showed a positive reaction.

For the GFLV sanitation, results revealed 75% eradication for the variety Aberkane, 87,5% for the variety Bezoul El Khadem, and 62,5% for the variety Muscat de Fandouk. The positive control revealed a positive reaction (Fig.1).

Several authors determined ribavirin as an antiviral agent. Thus, ribavirin and oseltamivir were used for the elimination of the virus in various horticultural species (Guţă et al., 2014). Ribavirin has been used for the sanitation of Grapevine virus A (GVA) (Panattoni et al., 2007), for the eradication/elimination of *Grapevine fanleaf virus* (GFLV) (Weiland et al., 2004), Grapevine rupestris stem pitting-associated virus associated (GRSPaV) (Skiada et al., 2013; Hu et al., 2018).

The assessment of the shoot growth revealed the formation of adventitious buds from which many microshoots differenciate (Figs 1, 2). Same results were observed by Guţă et al. (2014). The Tukey HSD test revealed significant effects between the number of microshoot cultivated under the drug medium and the drug-free medium for all varie-

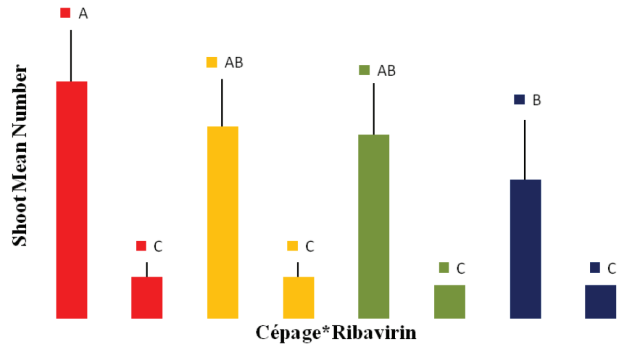


Fig. 1. Effect of treatments on shoot means number, WHO, without ribavirin; WH, with ribavirin; ABK, Aberkane; BEK, Bezoul El Khadem; Mf, Muscat de Fandouk: F, Farrana

Fig. 2. Shoot tip culture in M and S medium containing ribavirin and differentiation of new shoot tips

ties tested. The same test also revealed significant effects for the interaction between the number of shoots regenerated and the varieties. Observation conducted during the *in vitro* culture revealed the formation of call followed by the formation of new microshoot from the callus.

The phytotoxicity effect of ribavirin after 8 weeks of treatment was 60% for the variety Bezoul El Khaddem, 52,94% for the varieties Aberkane and Farrana, 46,66% for Muscat de Fandouk (Table 2).

**Table 2**

Effect of ribavirin on choot tips growth

| Variety | Nbre of budding | Phytotoxicity | Survival shoot tip | Medium without ribavirin | Medium with ribavirin |
|---|---|---|---|---|---|
| Aberkane | 17 | 09 | 8 | 1 | 6 |
| | | | | 2 | 6 |
| | | | | 1 | 8 |
| | | | | 1 | 10 |
| | | | | 1 | 7 |
| | | | | 2 | 8 |
| | | | | 1 | 8 |
| | | | | 1 | 5 |
| Bezoul El Khadem | 20 | 12 | 8 | 1 | 6 |
| | | | | 1 | 7 |
| | | | | 2 | 4 |
| | | | | 1 | 8 |
| | | | | 2 | 6 |
| | | | | 1 | 7 |
| | | | | 1 | 4 |
| | | | | 1 | 5 |
| Muscat de Fandouk | 15 | 07 | 08 | 1 | 5 |
| | | | | 1 | 4 |
| | | | | 1 | 8 |
| | | | | 1 | 5 |
| | | | | 1 | 4 |
| | | | | 1 | 8 |
| | | | | 1 | 5 |
| | | | | 1 | 6 |
| Ferrana | 17 | 09 | 08 | 1 | 2 |
| | | | | 1 | 3 |
| | | | | 1 | 4 |
| | | | | 1 | 2 |
| | | | | 1 | 6 |
| | | | | 1 | 5 |
| | | | | 1 | 7 |
| | | | | 1 | 5 |

# Discussion

In Algeria GLRaV-3 represents a prevalence of 44% (Lehad et al., 2015). The spread of this virus may be explained by vector transmission but also by the use of infected propagating material. For this reason, the use of virus-free propagating material plays a great role in plant virus control. The major technique used to control plant virus is thermotherapy. Several technique were developed in order to get more efficiency. The grapevine germplasm collection was found to be infected by several viruses in general in mixed infection; it was previously reported infected by GLRaV-3 (Lehad et al., 2015). In our study, we found that this germplasm collection was also infected by GFLV. The chemotherapy technique was used in order to assess the efficiency of this technique and clean this grapevine collection in purpose to integrate these genetic resources in breeding programs. Thus, several autochthones varieties and wild species were found to have interesting traits for breeding but the infection by viruses constitutes a great problem for their valorization.

The studies on the use of antiviral agent for plant virus sanitation are increasing and permit to find several antiviral agents. Ribavirin was successfully used for chemotherapy sanitation of several grapevine varieties and several virus species (Hu et al., 2018; Weiland et al., 2004; Skiada et al., 2013).

Different studies revealed that the treatment with ribavirin allow to eliminate 100% of GFKV and GRSPaV after 8 weeks of treatment with 20mg/ml of ribavirin (Komínek et al., 2016) and GLRaV-1-3 ( Skiada et al., 2013; Panattoni et al., 2007)

Results obtained revealed that GLRaV-3 was eliminated from all samples in comparison to GFLV that was found in some samples with lower rate. Several authors observed differences in the rate of grapevine cleaning by ribavirin for different viruses (Hu et al., 2018; Komínek et al., 2016). However, the combined use of different antiviral agents may give more interesting results. The GFKV has been completely eliminated both from simple and mixed infections with GVA by the simultaneous use of ribavirin and oseltamivir (Guţă et al., 2014).

Also, the presence of some samples infected by GFLV may be due to the high level of concentration of the GFLV or due to the virus-plant interaction. For this, it is important to carry out experiments on different viruses in order to validate this technique.

In addition, the technique allows developing a new protocol of grapevine micropropagation. Thus, the developed medium allows the differentiation of several microshoots in comparison with the free drug medium in which we find only one microshoot. Guţă et al. (2009 and 2014) reported the same observation. Thus, we can conclude that ribavirin induces the differentiation of a new microshoot directly from the callus playing role as growth regulatory compound. This observation may constitute an important advancement in the micropropagation of grapevine by increasing the number of microshoots. Other studies are still needed in order to understand this phenomenon and improve this method in micropropagation of grapevine and other cultures.

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