Electrotherapy; a promising therapy for virus eradication from olive shoot tip cultures cv. Meshkat

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Abstract

Olive tree harbors several viruses affecting the yield and quality of fruit worldwide. Application of virus-free planting materials is one of the main strategies to counteract virus diseases. The elimination of *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Cucumber mosaic virus* (CMV) and *Strawberry latent ringspot virus* (SLRSV) by different electrotherapies in combination with shoot tip culture was investigated in a native Iranian olive cultivar Meshkat to find out the most efficient virus elimination procedure. The results showed that survival, regrowth and proliferation rates were dependent on the therapy and meristem type. In addition, the regrowth rate in the virus mixed-infected cv. Meshkat was notably increased by doubling the electrotherapy duration. ArMV, CLRV, CMV and SLRSV were completely eradicated by electrotherapy treatment (35 mA, 100 V, 30 min) and the apical shoot tip culture proved to be the most compatible technique. The therapy efficiency index was 54 for all studied viruses. The results concluded that the combination of electrotherapy with shoot tip culture can be successfully used as a virus elimination method for producing virus-free olive plants.

Key Message

ArMV, CLRV, CMV, and SLRSV have been for the first time eradicated from olive cv. Meshkat shoot tip cultures using electrotherapy.

Introduction

Olive (*Olea europaea* L.), known as a symbol of Mediterranean basin, is considered a holy tree for Middle East countries (Motamedifar et al. 2007). With the origin in Middle East (Besnard et al. 2018; Besnard et al. 2013), it might be originated from Iran, where it has old trees of wild and domesticated genotypes distributed across the country (Hosseini-Mazinani et al. 2014; Hosseini-Mazinani et al. 2013; Owen et al. 2005). Iranian western provinces are the main olive planting areas (Hosseini-Mazinani et al. 2014). Olive trees are susceptible to several pathogens including viruses (Montilon et al. 2023; Martelli 2013; Godena et al. 2012; Roschetti et al. 2009; Martelli 1999; Triolo et al. 1996; Henriques et al. 1992). Eighteen viruses belonging to ten genera, five phytoplasmas and *Xylella fastidiosa* have been described for olive so far (Montilon et al. 2023; Alabi et al. 2021; Morelli et al. 2021; Xylogianni et al. 2021; Chiumenti et al. 2021; Campos et al. 2019; Naderpour et al. 2013; Loconsole et al. 2010; Alabdullah et al. 2010; Bertolini et al. 2001; Martelli 1999). Most of these viruses cause latent infections and may become apparent in susceptible cultivars by manifestation of disease symptoms such as ‘bumpy fruits’, ‘leaf yellowing complex’, ‘vein banding’, and ‘vein clearing’. Olive trees infected with viruses exhibit reduced vigor, productivity, rooting ability and oil quality (Martelli 2013; Godena et al. 2012; Roschetti et al. 2009; Martelli 1999; Henriques et al. 1992) but the symptoms are not simply recognizable in the field (Martelli et al. 2002). Several Nepoviruses including *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), and *Olive latent ring spot virus* (OLRSV), *Strawberry latent ring spot Sadwavirus* (SLRSV), *Cucumber mosaic Cucumovirus* (CMV), *Olive leaf yellowing-associated Closterovirus* (OLYaV), *Olive latent Alphanecrovirus 1* (OLV-1), and *Olive latent Oleavirus 2* (OLV-2) are frequently found in Tunisia (75%), Syria (51%), Italy
(33%), Lebanon (31%), Croatia (25%), and Portugal (31% of Necroviruses) (Zellama et al. 2019; Luigi et al. 2011; Varanda et al. 2010; Faggioli et al. 2005; Fadel et al. 2005; Al Abdullah et al. 2005). A virus infection survey in the ten most important Greek cultivars showed SLRSV as the predominant virus (55%), followed by CLRV, OLYaV and ArMV (Mathioudakis et al. 2020), and newly reported *Olive Tepovirus T* (OLV-T) (4.4%) (Xylogianni et al. 2021). However, other graft-transmissible agents including newly reported Geminivirus and prokaryotes including *Xylella fastidiosa* and *Candidatus* Phytoplasma sp. are being reported in some olive trees (Morelli et al. 2021; Alabi et al. 2021). Olive viruses are transmitted in nature by biological vectors including plant nematodes and mites (Nepoviruses), aphids (Cucumovirus, Closterovirus), chytrid fungi (Necrovirus), and leafhopper (Maravivirus). However, strains of Nepoviruses and Alphanecroviruses are also readily transmitted by infected pollens and seeds (Herrbach and Chesnais 2021), and by grafting in several host species. SLRSV infection caused bumpy fruit (fruit and kernel deformation, misshapen leaves) in cv. Ascolanatenera (Marte et al. 1986) and morphological and agronomical dissimilarities (smaller flowers and leaves) in cv. Raggiola compared to cv. Frantoio while they were genetically similar (Faggioli et al. 2005). OLYaV infection also resulted in leaf yellowing complex disease consisting of poor fruit set, bright yellow discoloration of the foliage, mottling, necrosis, extensive defoliation and dieback in cv. Carolea (Albanese et al. 2012).

High quality germplasm collections are essential for developing new cultivars, which are dependent on the supply of healthy and true-to-type plant materials (Byrne et al. 2018; Wang et al. 2018). Due to the vegetative propagation in olive trees and the widespread occurrence of latent infections (Loconsole et al. 2010; Alabdullah et al. 2010; Martelli 1999), viral diseases are easily transmitted from stock plants to the progenies (Touranget al. 2016; Bayati et al. 2011), therefore the selection of healthy plants by visual examination is not sufficient (Mathioudakis et al. 2020; Campos et al. 2019; Martelli 1999). However, the use of healthy plant materials for propagation is the most effective method to combat plant viral infections (Albanese et al. 2012). Indeed virus elimination techniques play important roles in the sanitation of planting materials for establishing virus-free nuclear stocks for subsequent propagation purposes (Bettoni et al. 2019; Zhao et al. 2018dărău et al. 2014; Emami et al. 2011), particularly in olive that have cultivar-dependent micropropagation (Mirzaei et al. 2021; Rugini et al. 2016).

Meristem or shoot tip culture is a traditional method for virus elimination where the virus-free undifferentiated areas is mechanically separated from the virus-infected differentiated tissues (Bettoni et al. 2016). Meristem culture is extensively used as a virus eradication procedure in infected plants (Zare et al. 2024; Wu et al. 2020; Kazemi et al. 2020; Bettoni et al. 2019; Wang et al. 2018; Zhao et al. 2018dărău et al. 2014; Wang and Valkonen 2009), which can synergistically increase the efficiency of other virus elimination methods (Bhat and Rao 2020; Bettoni et al. 2019; Zhao et al. 2018; Wang and Valkonen 2009; Martelli 1999). Until now there is some limited reports of *in vivo* thermotherapy and cryotherapy for the elimination of viruses and cryopreservation in olive cultivars (Martelli 1999; Rugini 1984; Walkey 1980), however investigating other sanitization methods is a necessity in this valuable plant.

Electrotherapy is an effective, less demanding, and low-cost method for virus elimination (Maliogka et al. 2015dărău et al. 2014). It applies a continuous electric current in plant tissues to decrease virulence
through degrading the nucleoprotein of virus (Sastry and Zitter 2014). However, several mechanisms for virus elimination in electrotherapy have already been suggested including the denaturation of viral components due to the increased temperature by electric pulses, inhibition of virus replication by changing cell pH, and finally, induction of systemic resistance or systemic acquired resistance in plants by increasing the apoplastic space temperature during virus movement in plasmodesmata (Singh and Kaur 2016; Asadi and Torkaman 2014; Henry et al. 2012; Zhao 2009; Pazhouhande 2001; Hernández et al. 1997; Lozoya-Saldaña et al. 1996; Wazanan et al. 1994; Goldsworthy 1987). In terms of virus elimination rate and shootlet regrowth, electrotherapy was identified as the most effective method in comparison with the typical techniques of meristem culture and thermotherapy (Lozoya-Saldaña et al. 1996). Another study also showed that the efficiency of electrotherapy technique depends on the virus structure, plant genotype, the electric current intensity and duration (Emami et al. 2011). Electrotherapy (15–35 mA) has been widely used for elimination of several viruses in different plant species including potato (Solanum tuberosum L.), garlic (Allium sativum), cocoyam (Colocasia esculenta), banana (Musa sp.) and grapevine (Vitis vinifera L.) (Guta et al. 2010; Igarza-Castro et al. 2001; Pazhouhande 2001; Hernández et al. 1997; Lozoya-Saldaña et al. 1996).

The combined application of electrotherapy with shoot tip culture for virus elimination in olive has not been reported so far (Albanese et al. 2012; Reed 2008). Therefore, in the present study we applied this combination for virus elimination in the local cv. Meshkat that was infected with several viruses including ArMV, CLRV, CMV and SLRSV. To the best of our knowledge, this is the first study on the evaluation of electrotherapy in olive.

**Methods**

**Plant materials and general procedure**

Plant materials of olive cv. Meshkat were collected from Tarom Olive Research Station, Iran. Dr. Ali Asghar Zeinanloo undertook the formal identification of the cultivar planted in this research station collection used in this study. Samples were collected from old and young shoots of four sides of 7-year-old trees and transferred onto liquid nitrogen. Leaf and bark tissues were grinded in fine powders for RNA extraction and downstream applications. Permissions were obtained from concerned authority to collect the plant sample. All local, national and international guidelines and legislations were adhered in the production of virus-free plant materials. All chemicals were of analytical grades.

Young nodal explants (2–4 cm) of actively growing *in vivo* shoots were surface sterilized in ethanol 70% (v/v) (AR, CHN) for 60–75 s followed by treatment with an aqueous solution containing sodium hypochlorite 2.5% (CLOROX, USA) and Tween-20 1% (v/v) (RPI, USA) for 13 min and rinsed in sterile distilled water for 5 min. Then, nodal segments were placed in Rugini Olive Medium (Rugini 1984) supplemented with sucrose 3% (w/v) (Duchefa Biochemie, NLD), 2iP 0.5 mg L⁻¹ (2.15 µmol/L) (Sigma-Aldrich, CA), BAP 0.23 mg L⁻¹ (0.99 µmol/L) (Sigma-Aldrich, CA), IBA 0.04 mg L⁻¹ (0.17 µmol/L) (Merck, USA), calcium gluconate 540 mg L⁻¹ (2327.39 µmol/L) (RPI, USA), FeEDDHA 50 mg L⁻¹ (215.49 µmol/L)
(Duchefa Biochemie, NLD), and boric acid (H$_3$BO$_3$) 4 mg L$^{-1}$ (17.24 µmol/L) (Duchefa Biochemie, NLD). The pH of culture medium was adjusted to 5.8 ± 0.2 and solidified with 0.7% (w/v) plant agar (Duchefa Biochemie, NLD) (Mirzaei et al. 2021). Cultures were grown in phytotrones at 24 ± 2 °C with a photoperiod of 16 h of light supplemented by high-pressure metal halide lamps (37 µmol m$^{-2}$ s$^{-1}$). Subcultures were performed every 6 weeks and 12-month in vitro cultures were used for virus eradication experiments.

**Virus detection assays**

All the field and in vitro samples were checked for ArMV, CLRV, CMV and SLRSV using molecular detection approaches according to Grieco et al (2000) (CMV and SLRSV), and Naderpour et al (2013) (ArMV, CLRV). Briefly, total RNA was extracted using TRIzol™ Reagent (Thermo Fisher Scientific, USA). RNAs were subjected to cDNA synthesis using random hexamer primer, M-MuLV reverse transcriptase (Promega, USA) and 1 µg of total RNA according to manufacturer’s instruction. PCRs were done in 25 µL reaction mixture consisting of 2.5 µL PCR buffer (10X), 1.1 µL MgCl$_2$ (50 mM), 2 µL dNTPs (2 mM), 1 µL of each forward and reverse primers (10 µM) and 0.2 µL Taq DNA polymerase (Promega, USA). PCRs were run in ThermoCycler (Applied Biosystems, USA) using the cycling profile for all primer pairs comprised of 3 min incubation at 94°C, followed by 35 cycles of denaturation (30 s at 94°C), annealing (45 s at primer specific annealing temperature) and extension (45 s at 72°C), with a final extension step at 72°C for 5 min. To confirm virus infection, the amplified PCR products were sequenced directly at Bioneer (South Korea) and the retrieved sequences were blast analyzed in NCBI (https://blast.ncbi.nlm.nih.gov/).

**In vitro virus eradication therapies of olive cultivar**

**Shoot tip culture**

_in vitro_ apical and axillary buds (1 mm) of Meshkat were excised from 12-month-old in vitro stocks using dissecting microscope SZM-2 (Seimens, DEU) under aseptic conditions and cultured on optimized OM as previously reported (Mirzaei et al. 2021) unless otherwise stated.

**Electrotherapy**

The 12-month old _in vitro_ shoots (4–5 cm) containing apical and axillary buds were harvested from the cultivar Meshkat and were cut into 2–3 cm single node segments. They were immersed directly in 1 M NaCl in vertical electrophoresis tank (WAVESYS VS20, UK) and exposed to electric current intensity of 35 mA with 100 V for 15 (electrotherapy #1) and 30 min (electrotherapy #2) using power supply Enduro 300 V (Labnet, USA). To prevent recontamination, after treatments the stems were surface sterilized using ethanol 70% (v/v) for 1 min followed by sodium hypochlorite 0.1% (v/v) for 1 min and rinsed three times with double distilled water. Then, shoot tips along with some lower layers (1 mm) in both apical and axillary types were cut using dissecting microscope SZM-2 (Seimens, DEU) under aseptic conditions and cultured on OM (Mirzaei et al. 2021). Non-treated apical and axillary shoot tips (1 mm) of the cultivar were excised and cultured on OM as controls.

**Statistical analysis**
The experiments were conducted under a completely randomized design. Survival and regrowth indices consisting of quantitative and qualitative data were measured 4 and 8 weeks after treatments, respectively. Quantitative traits were the proliferation rate (number of axillary shoots at 8 weeks), main shoot length and the total length of shoot tips. Regrowth is the ability to grow after treatments and proliferation is the number of shoots proliferated after therapies. Qualitative data of callus production, vitrification and growth quality (health conditions like color, etc) were visually scored (0 and 5 were the minimum and maximum scores, respectively). At least 5 replications, each with 3 explants, were used for all experiments. The replication number for the calculation of therapy efficiency index (TEI) of cv. Meshkat was 8 according to the number of in vitro plantlets regenerated from shoot tip culture after electrotherapy #2. Data were analyzed by General Linear Model and Duncan’s Multiple Range Test using SPSS Statistics 22 (IBM, USA) and scored as significant if $P \leq 0.05$.

Therapy efficiency index (TEI) was calculated according to Badarau et al (2014):

$$\text{TEI} = \frac{\text{percentage of regenerated plantlets} \times \text{percentage of virus-free samples}}{100}$$

Based on the results of survival, regrowth and proliferation rates, the sanitary status of the in vitro mixed-infected cv. Meshkat treated with electrotherapy was assessed after 21 months of therapy (Fig. 3).

Results

Virus diagnosis

Agarose gel electrophoresis of Triazol-based extracted RNA from field samples of olive cv. Meshkat and subsequent amplification of two housekeeping genes, i.e. Nad5 and Rbc 1 (Mirzaei et al. 2022), revealed the suitability of RNAs for RT-PCR diagnosis of viruses. All target genes of SLRSV, ArMV, CLRV and CMV (Grieco et al. 2000; Naderpour et al. 2013) were amplified as monomorphic sharp fragments. Further sanger sequencing and blast analyses in NCBI showed that the amplified targets belong to respected viruses and the cv. Meshkat was mixed infected with all those viruses.

Applying the same RNA extraction and RT-PCR protocols on regenerated in vitro cultures by electrotherapy #2 showed complete eradication of viruses. Moreover, 50% and 62.5% of plantlets treated with electrotherapy #1 have been free of CLRV and ArMV, respectively (Table 2).

Effect of therapies on survival rate

The regrowth rate of survived mixed-infected cv. Meshkat is shown in Fig. 1. The results presented in Fig. 1 shows that although electrotherapy treatments reduced survival rate of both axillary and apical meristems of cv. Meshkat; electrotherapy #2 in apical meristem showed the highest survival between treated shoot tips while no significant differences with electrotherapy #1 was seen. Figure 1 also displays that the survival rate is to some extent dependent on meristem type. It shows that the increment of current duration resulted in higher ($P < 0.05$) regrowth rates in both meristem types approximately 3 to 4 times
more. In the rest of study, the apical meristems were applied due to the ease of cutting and more acceptable growth qualities in cv. Meshkat (Fig. 1).

**Effect of therapies on regrowth parameters**

Analyses of therapy impact on regrowth parameters revealed that although electric pulses were considered a stress to plant materials, applying 30 min of electric current in apical meristems ameliorate proliferation rate compared to 15 minutes of therapy (Table 1).

### Table 1

The effect of therapy regimes on the proliferation rate of *in vitro*-cultured virus-infected shootlets of olive cv. Meshkat.

<table>
<thead>
<tr>
<th>Meristem</th>
<th>Cultivar</th>
<th>Control</th>
<th>Electrotherapy#1</th>
<th>Electrotherapy#2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axillary</td>
<td>Meshkat</td>
<td>0.61 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.37 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Apical</td>
<td>Meshkat</td>
<td>0.56 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.59 ± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented after 8 weeks of therapy as mean ± standard error; Different lowercase letters denote significant differences among the treatments at *P* ≤ 0.05 by Duncan's test.

Figure 2 shows that therapy method was the important factor for vitrification and growth quality. Cv. Meshkat had the lowest (*P* < 0.05) amount of callus production and vitrification in electrotherapy#2 compared to electrotherapy#1. Moreover, electrotherapy#2 had higher growth quality in cv. Meshkat (Fig. 2).

**Effect of electrotherapy and shoot tip culture on virus eradication in cv. Meshkat**

The virus eradication rate in electrotherapy methods is shown in Table 2. Electrotherapy #1 combined with shoot tip culture showed less therapy efficiency index. Shoot tips treated with electrotherapy #1 could only eradicate 62.5 and 50 percent of ArMV and CLRV, respectively (Table 2).
the effects of electrotherapy combined with shoot tip culture on virus elimination and therapy efficiency index in cv. Meshkat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variable</th>
<th>SLRSV (0/3)</th>
<th>ArMV (0/3)</th>
<th>CLRV (0/3)</th>
<th>CMV (0/3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Shoot tip culture)</td>
<td>Virus elimination</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Electrotherapy #1 + Shoot tip culture</td>
<td>Virus elimination</td>
<td>0 (0/8)</td>
<td>62.5 (5/8)</td>
<td>50 (4/8)</td>
<td>0 (0/8)</td>
</tr>
<tr>
<td></td>
<td>TEI</td>
<td>0</td>
<td>31.25</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Electrotherapy #2 + Shoot tip culture</td>
<td>Virus elimination</td>
<td>100 (8/8)</td>
<td>100 (8/8)</td>
<td>100 (8/8)</td>
<td>100 (8/8)</td>
</tr>
<tr>
<td></td>
<td>TEI</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
</tbody>
</table>

Sanitary status of the *in vitro* mixed-infected cv. Meshkat treated with electrotherapy was assessed after 21 months of therapy.

The virus eradication rates for the apical meristem of *in vitro* virus infected Meshkat plantlets treated with electrotherapy #2 along with apical shoot tip culture were 100% for all four viruses (Table 2). Moreover, TEI was 54 for SLRSV, ArMV, CLRV, CMV (Table 2).

**Discussion**

**Effect of the type of meristem on survival rate**

Different studies have shown that the survival rate depends on several factors including host genotype, meristem size, therapy method and duration (Farhadi-Tooli et al. 2021; Mathew et al. 2021; Kazemi et al. 2020; Bettoni et al. 2019). The results showed that the survival rate in different therapies was highly influenced by the meristem type (Fig. 1). Kazemi et al. (2020) reported that the survival rates in the thermo-treated pear meristems (56–95%) depended on cultivar, therapy duration, the size of dissected meristems and their interactions. As shown in Fig. 1, the apical meristems had higher survival rates compared with the axillary ones in electrotherapy #2, which can be attributed to the higher contents of endogenous auxins and cytokinins (Watanabe et al. 2006) in the actively growing apical meristems (Vivek and Modgil 2018).

**Effect of therapies on survival rate and regrowth parameters**

In the present study, increasing the duration of electric current showed no significant differences in axillary and apical meristems’ survival rate (Fig. 1). Bădărău et al. (2014) reported that increasing the duration of electrotherapy resulted in significant decline in the survival rate of potato which is not consistent with the findings of this study. Wang et al. (2018) also reported an inverse relationship between the regeneration percentage and virus-free plant production rate. Moreover, Bayati et al. (2011) reported
the lower survival rate of grapevine (*Vitis vinifera* L. cv Black) in cryotherapy (59%) compared with electrotherapy (62%, 30 mA, 15 min).

The results showed that 35 mA current intensity with 100 V for 15 min reduced proliferation rate of both types of meristems. However, proliferation rate for the apical meristem of cv. Meshkat in the electrotherapy#2 was ameliorated, which can be related to the expression of stress tolerance proteins as well as the increase in the cell activity by enhancing the ionic flow of sodium and chloride ions (Zhao 2009). The electric current can act as an abiotic elicitor in plants influencing their photosynthesis, antioxidant defense systems and metabolites synthesizing (Walkey et al. 1972). The exposure of several herbaceous plants to electric current and magnetic fields remarkably enhanced the growth yield (30–57%) (Walkey et al. 1972). Several studies reported the stimulation effect of electrotherapy on plant growth indices including increasing the number of leaves in potato (Rakosy-Tican et al. 2005) and mineral uptake in tomato (Ward 1996). Padilla et al (2009) reported that in Spanish cultivars including 'Arbequina', 'Manzanilla de Sevilla' and 'Gordal Sevillana', electroporation in indole-butyric acid (IBA) not only had a cultivar-dependent influence on rooting ability of shoots but also on shoot growth and development, with longer shoots and higher axillary shoot sprouting and growth after some of the treatments.

Although callus induction was remarkably cultivar-dependent (data are not shown), vitrification and growth quality were significantly associated with therapies (Fig. 2). It was previously reported that callus induction in olive genotypes varies amongst cultivars (Mirzaei et al. 2021; Rugini 1984). Callus production is induced when explants are exposed to the stress where there is a scope for more deeply documentation. Vitrification is another negative characteristic in plant tissue culture and less vitrified green shootlets are considered more qualified.

The results showed that the regrowth rate of cv. Meshkat in the electrotherapy treatment enhanced with increasing current duration. Different studies have shown that the regrowth rate differs based on the cultivar, therapies combination, source and position of the explants (Kazemi et al. 2020; Bettoni et al. 2019; Zhao et al. 2018) and it varied from 19–64% in pome fruits cultivars (Farhadi-Tooli et al. 2021; Bettoni et al. 2019; Zhao et al. 2018).

**Effect of electrotherapy and shoot tip culture on virus eradication in cv. Meshkat**

The results showed that the eradication rate and TEI were highly dependent on the virus type. Electrotherapy#2 eliminated 100% of studied viruses from cv. Meshkat (Table 2) while Electrotherapy#1 only eliminated 50 to 62.5% of two viruses. SLRSV and CMV were more resistant in 15 minutes of therapy, which can be attributed to the possible latency phase of the virus as it is more recalcitrant for detection and eradication. The diagnosis of virus infected olive trees is difficult due to the symptomless virus infection and highly concentrated inhibitors (Faggioli et al. 2005). The present study demonstrated the asymptomatic mixed-infection in cv. Meshkat (Fig. 3).
Bădărău et al (2014) reported that around 90% of virus elimination occurred in 100 mA for 10 min for *Potato virus X Potexvirus* (PVX) and *Potato virus Y Potyvirus* (PVY) having similar TEI (63.35). Electrotherapy treatment combined with meristem culture resulted in completely sanitized plantlets (100% free from *Dahlia mosaic Caulimovirus*, DMV) of dahlia (*Dahlia pinnata*), while only electrotherapy (35 mA for 20 min) showed the highest eradication rate (85%) for DMV (Nerway et al. 2020). It is reported that treatment duration and the size of *in vitro* shoots affect the eradication rate (Zhao et al. 2018). Therapy efficiency significantly depends upon cultivar, antiviral agents, virus type and treatment duration (Bettoni et al. 2019; Gong et al. 2019).

Electrotherapy is a useful procedure for producing virus-free germplasm and nucleus seed stock in crop plants infected with seed-borne viruses (Hormozi-Nejad et al. 2010). It needs the minimum period of time (2–3 months) to regenerate fully developed and healthy dahlia plants (Nerway et al. 2020).

Meristem tip culture limits the viral particles transportation due to the fast growth and lower apoplastic space of apical dome (Zhao et al. 2018; Hu et al. 2015). The molecular mechanism has recently been discovered involving WUSCHEL, a transcription factor inhibiting viral protein synthesis by repressing methyltransferases protecting undifferentiated daughter cells from biological invasion (Wu et al. 2020). This technique helps other therapies to significantly increase the elimination rate. Bădărău et al (2014) showed that using electrotherapy combined with axillary bud tip culture in PVX infected potatoes resulted in the highest rate of virus eradication and maximum therapy efficiency value.

**Conclusion**

In the present study, the combination of electrotherapy with shoot tip culture revealed satisfactory results (100%) for producing virus-free planting olive materials from several viruses belonging to different genera. SLRSV and CMV showed more resistance to eradication by electrotherapy (35 mA, 100V, 15 min) in comparison with ArMV and CLRv. Since SLRSV has a latent phase and CMV is known as a top ten harmful viruses in the world, the second electrotherapy method (35 mA, 100V, 30 min) could be considered as a promising therapy method. Because *in vitro* culture of olive fundamentally has low genotype-dependent micropropagation, the higher survival rate in electrotherapy is desirable. Electrotherapy treatment of virus infected olive plants not only eliminated viruses but also increased the proliferation rate. The application of several approaches for sanitation of planting materials is suggested as the most promising technology to achieve virus-free planting materials. However, the efficiency of each method depends mainly on plant species and relevant viruses.

**Abbreviations**

*Arabis mosaic virus* (ArMV); *Cherry leaf roll virus* (CLRv); *Cucumber mosaic virus* (CMV); *Strawberry latent ring spot virus* (SLRSV); 6-(γ, γ-Dimethylallylamino) purine (2iP); 6-benzylaminopurine (BAP); Indole-3-butyrlic acid (IBA); Liquid nitrogen (LN); Olive medium (OM); Therapy efficiency index (TEI).
Declarations

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Ethics approval and consent to participate

Licenses were obtained from concerned authority to collect the plant sample. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Authors' contributions


References


Figures

Figure 1

The effect of therapies on the survival and regrowth rate of apical and axillary meristems of olive cv. Meshkat (Mean (%)) ± SE.
Data are presented after 8 weeks of therapy as mean ± standard error; Electrotherapy #1 and Electrotherapy #2 stand for 35 mA current intensity with 100 V for 15 min and 30 min, respectively. Controls are shoot tip cultures without therapy treatments.

![Image](image1)

**Figure 2**

The effect of electrotherapy treatment (35 mA, 100 V, 15 and 30 min) of virus-infected *in vitro* apical shoot tips of olive cultivar Meshkat on callus production, vitrification and growth quality.

a and b represent *in vitro* grown shoot tips of cv. Meshkat treated with 35 mA 15 min and c represents the same sample under 30 minutes of therapy. Part d represents ongoing callus production of cv. Meshkat treated with 15 minutes of electric current after 12 weeks of *in vitro* culturing.
Figure 3

Schematic view of virus eradication process in virus-infected *in-vitro* olive plants.