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Elimination of *Citrus psorosis virus* using shoot tip grafting and electrotherapy techniques

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ABSTRACT

During the spring of 2020, *Citrus psorosis virus* isolate was identified in citrus orchards in Qalyubia Governorate, displaying typical virus symptoms such as bark scaling lesions and ringspot on the leaves and fruits. Using chip buds from the donor plant grafted into Madam Vinous and Navelina sweet orange as indicator plants, the isolated virus was identified using biological indexing. DAS-ELISA and RT-PCR were used to test the plants that yielded positive results. RT-PCR employing virus-specific primers revealed single amplified fragments of the appropriate size (434 bp) in infected plants, while no bands in healthy plants were detected. Electrotherapy was used to eliminate the infection. Stem cuttings from infected plants were subjected to electric currents of 10, 20, and 40 mA for 10 and 20 minutes, respectively, before being grafted on the indicator plants. The treatment of 40 mA for 20 minutes was shown to be more effective in developing virus-free plants (85%). Also, for the elimination of *Citrus psorosis virus*, small shoot tip explants (0.1-0.2 mm) from virus-infected shoots were grafted onto a young rootstock of troyer citrange, growing *in vitro* on Murashige and Skoog medium with a success rate of 3%, after which the grafted plants were transferred to the greenhouse for acclimatization. The micrografted plants were successfully regrafted on potted sour orange seedlings in the greenhouse. DAS-ELISA was used to test all plants which survived for *Citrus psorosis virus*.

Keywords: *Citrus psorosis virus*, biological indexing, RT-PCR, shoot tip, electrotherapy.

INTRODUCTION

Citrus psoriasis virus (CPsV) is one of the most important and damaging viruses infecting citrus (*citrus* sp.), sweet orange, mandarin and grapefruit producing bark scaling lesions, but not regularly showing ringspots on leaves and fruits. CPsV is the type of member of the genus *Ophiovirus*, family *Aspividae* (Garcia *et al*., 2017). Its genome is consisting of three single-stranded RNA segments of negative sense (Milne *et al*., 2000). Bark scaling on infected branches and trunks is often accompanied by wood staining. Sour oranges, lemon, pummelo and rough lemon usually are not showing external symptoms (Roistacher, 1991). Two types of symptoms have been suggested, Psorosis A (PsA) and Psorosis B (PsB) (Fawcett and Klotz, 1939). PsA protects against a challenge from the more severe bark lesions induced by the more aggressive psorosis (PsB) (Wallace, 1957). Citrus psorosis disease is worldwide in many parts of the world, including South America and the Mediterranean areas (Roistacher, 1993). In Egypt, some investigators isolated and identified CPsV (Nour-Eldin, 1956; Nour-Eldin, 1957; Fahmy *et al*., 2002). Citrus crop losses of graft-transmissible diseases were estimated as much as 15-25% (Moreno *et al*., 2015). These low losses may be due to the limited spread of the disease in Tucuman (Fawcett and Klotz, 1939). CPsV has been detected by several methods (Martin *et al.,* 2004) including DAS-ELISA (Garcia *et al*., 1997), TAS-ELISA-AP (AP, alkaline phosphatase) and TAS-ELISA-HP (HP, Horseradish peroxidase) (Alioto *et al*., 1999). These methods were developed and applied for field detection. In addition, RT-PCR, the alternative detection method was applied by using several primers (Martin *et al*., 2004). Biological indexing could be performed for CPsV detection by graft-inoculating citrus in sensitive indicator plants such as sweet orange and then testing for cross-protection with severe isolate (PsB) (Roistacher and Calavan, 1965; Roistacher, 1993). A new method of biological indexing was organized for diagnosis of Citrus viruses and viroids by using indicator cutting as an alternative to seedlings (ElBacki *et al*., 2005; El Sayed, 2005; D'nghia *et al*., 2009).

For the elimination of plant viruses, several methods are used, these including meristem culture, chemo and thermotherapy. But these methods are required experts and much time to perform, on the other hand, Electrotherapy is a simple method without the need to use a special or expensive instrument. In this method, the electric current is performed to disrupt the viral coat protein leading to inhibiting viral replication and thus, eliminating or inactivating the viral activity. It was found that the use of low electric current (3-15 A plant -1) encourages growth and stimulates the ion concentration in tomato plants (Black, 1971). Moreover, Blanchard

(1974) by using a direct electric current of 1-4 A pulsed to 6500 Vh^{-1} after 2-3 days, got virus-free plant tissues. These investigations set up a basis of electric current as a method to eliminate plant RNA viruses. Production of virus-free plants by the applied electric current was used in many crop plants since the 1990'-s. Ninety percent of the viruses in Cactanucia tree stakes, which showed mosaic symptoms, were eliminated by applying 500 V for 5-10 minutes, as described by Quacquarell *et al*., (1980). To get rid of PVX (60-100%) in several varieties of potatoes, Lozoya *et al*., (1996) applied an electric current of 15 m A for 5 minutes. Tomato yellow leaf curl virus (DNA virus) from tomatoes; Onion yellow dwarf virus and Leek yellow stripe virus from garlic (Hernandez *et al*., 1997); Banana streak virus from bananas (Hernandez *et al*., 2002); Dasheen mosaic virus from cocoyam (*Xanthosomas agittifolia*); Grapevine leaf roll virus from grapevines (*Vitis vinifera*); and (Fallah *et al*., 2007).

In addition to chemotherapy, electrotherapy was also effective in eliminating PVY (Mahmoud *et al*., 2009). Guta *et al*., (2010), on the other hand, reported that morphological and molecular evaluations of plants regenerated using electrotherapy revealed no compromises in genetic stability, fidelity, or uniformity. Navarro et al. (1975)'s usual method of shoot-tip grafting was followed. Seedlings of Troyer citrange were grafted when they were between 14 and 17 days old. Trees cultivated outdoors or plants in potted greenhouse conditions were harvested for their shoot tips. According to previous findings by Ioannou *et al*. (1991) during the sanitation of the Cyprus local lemon, the average success rate for micrografting was 17% after 448 attempts, and sweet orange and Carrizo citrange appeared slightly better than sour orange, Troyer citrange, and Swingle citrumelo general scion material from the field gave higher grafting success than the glasshouse-grown material. However, the field materials were only available during certain times of the year. The best scion material was collected between May and October, and there was nothing available for grafting during the winter. Hence, the objectives of this study were to determine the causative virus and evaluate the efficacy of electrotherapy in eradicating CPsV and generating virus-free plants.

MATERIALS AND METHODS

Virus source:

Samples were collected from symptomatic citrus trees grown in orchards at Qalyubia Governorate (Moshtohor). Samples collected during spring of 2020 showed a typical Citrus psorosis virus symptom Fig. (1). The collected samples were indexed biologically according to Roistacher (1991) and Roistacher *et al.,* (2000) on indicator seedlings of Madam Vinous and Navelina sweet orange budded on rough lemon and kept under greenhouse conditions.

Fig. 1. Typical bark scaling lesions (A & B) on sweet orange, green color under stem bark (C & D) produced by CPsV.

Biological indexing:

Rooted cutting:

About 50 fresh, semi-hard wood and hard wood cuttings for each indicator plant with 4-6 nodes were inoculated by chip budding using the bark tissue collected from CPsV-infected plants Fig. (2). Grafting cuttings was always maintained with the basal part in the water. Grafts were sealed with parafilm, and the inoculated cuttings were,

dipped in the IBA solution (500 ppm) and placed in the peat moss and perlite media inside a plastic bag. Negative control was also included in the trial using ten cuttings/indicators which were chip budded with healthy tissue. Inoculated cuttings were grown in the indexing greenhouse at warm temperatures (34-36°C). Four days after inoculation, the plastic bag was opened from the top and 10 days later completely removed for acclimatization. Inoculated cuttings were examined after 10 days for graft viability and after 2 weeks for rooting. Symptom observations were routinely carried out since shoots flushing. Plants to be further investigated (*i.e*., bark scaling symptoms) were maintained longer (more than 3 months) in the same pots. After the 1st month of growth, Nutrition with macro and microelements was used as foliar spraying every 2 weeks. Symptoms were observed after 5 weeks for CPsV. Nevertheless, most of the symptoms developed after 25-30 days.

Fig. 2. Grafting of treated cuttings with electrotherapy on healthy indicator cutting of citrus.

Indicator seedling:

Seedlings of Madam Vinous and Navelina sweet orange, budded on rough lemon, were employed as indicator plants. Non-inoculated plants were used as the negative controls. All grafted plants were kept under cool temperatures (24-27°C maximum during the daytime and 18-21°C minimum at night).

Serological detection (DAS-ELISA):

DAS-ELISA assay (Garcia *et al.,* 1997) was used to confirm the results of biological indexing: commercial kits were used for the detection of CPsV (Agritest SRL, Italy). Plates were coated with antibodies in coating buffer, diluted 1:1000 and incubated for 2 h at 37°C, then washed 3 times using PBS Tween at 3 min intervals and dried by blotting on paper. Samples were extracted at 1/10 concentration with extraction buffer, using cortical scraping, 200 µL of the extract was added per each well of the plate, then incubated overnight at 4°C. Plates were washed 3 times and then incubated for 2 h at 37°C with labeled antibodies with alkaline phosphatase. Washing was repeated, and then a substrate (p-nitrophenyl phosphate) in a substrate buffer (1 mg/mL) was added and incubated at room temperature, till yellow color developed. Absorbance was read at 1 h and 2 h using an ELISA reader at 405 nm. Reading greater than twice the A405 value of healthy control was considered positive.

Molecular detection:

RNA extraction:

Citrus leaf samples of Navelina sweet orange were used for RNA extraction using RNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions.

Primers for the partial coat protein gene of CPsV

The forward primer (consF: 5'-ACAAAGAAATTCCCTGCAAGGG-3') and the reverse primer (consR: 5'-AAGTTTCTATCATTCTGAAACCC-3') were used to amplify part of the coat protein gene of CPsV according to Roy *et al*., (2005).

One-step RT-PCR:

Using the "iScript One Step qRT-PCR Kit" (BIOMATIK) for a 25 L reaction volume, we performed a one-step RT-PCR reaction. The following were added to each reaction: 1 µL of RNA extract (40 ng of total RNA), 12.5 µL iGreen Mastermix, 1.5 µL of 10 M of each primer, 0.5 µL of qRT-PCR Enzyme Mix, and 25 µL of nuclease-free water. The cDNA synthesis conditions were 50°C for 15 minutes, 94°C for denaturation, 30°C for 30 seconds, 48°C for 30 seconds, 72°C for 45 seconds, and 72°C for 10 minutes. Under UV light, images were captured with the digital imaging system gel doc after 5 µL of PCR products were placed onto 1.5% agarose gels with a 100 bp DNA ladder (BIOMATIK) (Syngene Bio Imagins, IN Genius).

Electrotherapy treatments and grafting of virus-free plants:

Before applying the electrotherapy treatments, the inoculated citrus cvs. of Navelina and Madam Vinous sweet orange seedlings were tested by DAS-ELISA against virus infection. Infected plants were used to obtain stem segments containing axillary buds for electrotherapy treatment (Fig. 3). Each infected plant got approximately 20 nodal cuttings that were subsequently used for electrotherapy treatment. Stem segments were immersed in TAE buffer in an electrophoresis tank and exposed to electric currents of 10, 20 and 40 mA for 10 and 20 min using a power supply (Bio-Rad 1000/500, Fig. 3). Immediately after treatment, the stems were grafted on healthy as rootstock cuttings. The treated grafted cuttings were kept in the greenhouse as described above. All grafted plants were observed daily for CPsV symptoms and checked by DAS-ELISA, thirty days after grafting. To evaluate the electrotherapy treatment, the Therapy Efficiency Index (TEI) was estimated (Lozoya *et al*., 1996). The TEI was estimated as follows:

TEI = percentage of regenerated plantlets × percentage of virus-free samples

The experiment was repeated twice for each electrotherapy treatment.

Fig. 3. Electrotherapy equipment used to produce virus-free plants in *Citrus psorosis virus* (CPsV). Stem segments with 3-5 buds used for therapy), single node explants prepared for grafting (A), Bio-Rad power supply and electrophoresis tank used for producing electric currents (B).

Shoot tip grafting technique *in vitr:*

Most research facilities are regularly employed by Navarro *et al*., (1975), a standard protocol for shoot tip grafting (STG) *in vitro*. The procedure entails preparing the rootstock, the scion, the graft, the *in vitro* culture of the grafted plants, and finally the transfer to soil.

Rootstock preparation:

In vitro seed germination produces rootstocks. Peeled seeds of Troyer citrange rootstock [*Poncirus trifoliata* (L.) Raft. x *Citrus sinensis* (L.) Os b.] are surface sterilized by immersion for 10 minutes in a 0.1% sodium hypochlorite solution with 0.1% Tween-20 wetting agent and rinsed three times with sterile distilled water. Individual seeds are planted in 25 × 150-mm culture tubes containing 25 mL of Murashige and Skoog (1962) plant cell culture salt solution solidified with 1% Bacto Agar). For two weeks, culture tubes are incubated at a constant 27°C in complete darkness. Light has a considerable influence on grafting success during seed germination. Using Troyer citrange seedlings grown in continuous darkness, a high frequency of successful grafts was produced (Navarro *et al*., 1975).

Fig. 4. Mature fruits used for seed extraction collected from virus-free trees of *Troyer citrange* (A, B & C), seeds germination for rootstocks of *T. citrange* (D, E & F).

Scion Preparation:

The infected citrus trees (Navelina sweet orange) that were growing in pots within the greenhouse had all of their leaves removed by hand, and then they were moved to either a warm greenhouse or a growth chamber (Fig. 5). In a period of eight to fifteen days, depending on the temperature (Navarro et al., 1980), several buds will begin to sprout, producing flushes that will later be harvested for their shoot tip content. To prevent the abscission of degenerating shoot tips, flushes no longer than three centimetres in length are utilized. They are then cleaned by being submerged in a solution of 0.25 percent sodium hypochlorite containing 0.1 percent Tween-20 wetting agent and then rinsed three times in sterile distilled water. After this, they are prepared for surface sterilization by being cut to a length of approximately one centimetre and having their larger leaves removed. Flushes that have been produced by budwood that has been cultured *in vitro* in a culture medium that contains the plant cell culture salt solution of Murashige and Skoog (1962), which has been solidified with 1.2 percent Bacto Agar, and which has been maintained at a temperature of 32 degrees Celsius while being illuminated for 16 hours each day for 45 days are also an excellent source of shoot tips for STG.

Fig. 5. Micrografting steps, source of shot tips (A & B), new flashes (C & D).

Grafting:

A sterile environment was used to remove the rootstock seedling from the test tube before it was decapitated, with around 1.5 centimetres of the epicotyl being left intact. Following the removal of the cotyledons and their axillary buds, the root is trimmed to a length of 4-6 centimetres. Grafting at the top of the epicotyl that has been beheaded, placing the tip of the shoot in contact with the vascular ring, or making an incision in the shape of an upside-down "T" (Navarro *et al*., 1975). This is accomplished by making a horizontal cut that is 1-2 millimetres broad and a vertical incision that is 1 millimetre long, beginning at the spot where the head was severed. The incisions are made through the cortex, and then the flaps of the incision are lifted very little so that the cortex can be seen. With the use of dissecting instruments and a microscope, the remaining leaves on the plant are removed, except for the three leaf primordia that are the youngest. After measuring between 0.1 and 0.2 millimetres, the shoot tips were cut off using a razor blade sliver that was linked to a surgical handle. These shoot tips consisted of the apical meristem as well as three-leaf primordia. The shoot tip is either placed at the top of the decapitated seedling in touch with the vascular ring or it is inserted inside the incision that was made in the rootstock with its cut surface making contact with the cortex that was exposed by the horizontal cut that was made in the incision.

Culture *in vitro* of grafted plants:

Grafted plants are cultivated in a liquid nutrient medium containing Murashige and Skoog's (1962) plant cell culture salt solution, modified White's vitamins, and 75 mg sucrose (Navarro *et al*., 1975). In 25 mL aliquots, the nutritional medium is placed into 25 x 150 mm test tubes. In the nutrient solution is a folded paper platform with a hole in the center for inserting the root section of the rootstock. For 45 days, the cultures are kept at a constant 27°C and exposed to illumination for 16 hours every day.

Transfer to soil:

Typically, this stage is attained 3 to 6 weeks following grafting. Before being transplanted to soil, scions of successful micrografted plants have at least two enlarged leaves and are placed in containers with an artificial soil mixture ideal for growing citrus that has been steam sterilized. In a shaded region of a greenhouse that has its temperature managed to be between 18-25°C, pots are encased in polyethylene bags that are fastened with rubber bands. The bags are opened after 8 to 10 days, and after an additional 8 to 10 days, they are removed, allowing the plants to grow normally in a greenhouse.

RESULTS

Virus source:

Samples collected from symptomatic citrus orchards obtained from Qalyubia Governorate (Moshtohor) during the spring of 2020 showing a typical *Citrus psorosis virus* symptom as clear in Fig. (1) were tested by DAS-ELISA, and the positive samples showed yellow color, while the negative or healthy samples showed no color.

Biological indexing detection:

Symptoms expression on rooted inoculated cuttings of the indicator plants and traditional indexing on the indicator seedlings used in this experiment are recorded in Table (1) and Fig. (6) including chock and leaf drops. All used rootstock citrus plants (Madam Vinous and Navelina) which grafted on the rough lemon as rootstock were indexed by graft inoculation with two blind buds from CPsV-infected citrus plants. The rootstock tested differed in response to CPsV isolate. Madam Vinous appeared hypersensitive which gave shock symptoms while Navelina appeared higher response which gave shock and drop-leaf symptoms. Control inoculations with tissue showing no symptoms gave negative results, and none of the tested plants showed systemic symptoms. These tested plants that gave positive results were also tested serologically by DAS-ELISA against specific CPsV antiserum.

Table 1. Symptoms and incubation period of CPsV on inoculated rooted cuttings and seedlings.

Inoculated cuttings	Rooting		Seedlings		DAS-ELISA
	Symptoms	The incubation period (days)	Symptoms	The incubation period (days)	
Madam Vinous	Chock	28	Chock	35	+
Navelina orange	Chock	25 days	Leaf drops	35	+

Results obtained in Table (1) indicate that; CPsV produced shock symptoms on rooted inoculated cuttings and seedlings of Madam vinous and Navelina sweet orange. Whereas it exhibited chock asymptomatic on rooted inoculated cuttings and leaf drops on seedlings of Navelina sweet orange.

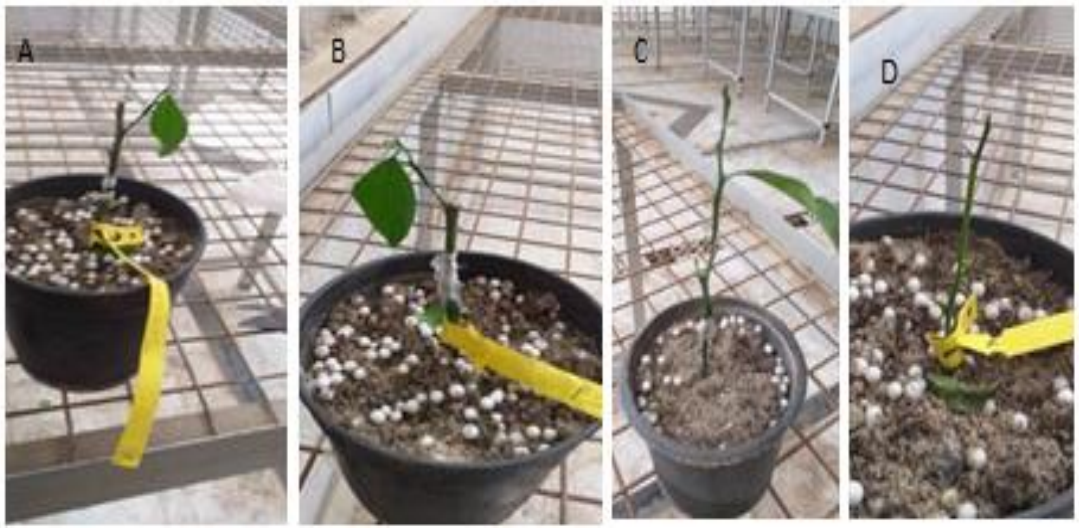

Fig. 6. Typical chock symptoms (A & B), drop leaf and chock on seedlings (C & D) on Navelina and Madam Vinous.

Serological detection (DAS-ELISA):

Results of biological indexing were confirmed by ELISA using plant tissue from symptomatic and symptomless indicators after 5 weeks of growth. All inoculated indicator stem cuttings were ELISA-positive to CPsV.

Molecular detection:

One step RT-PCR:

Orange leaf samples collected from growing areas were subjected to the RT-PCR assay. RT-PCR was carried out to amplify the partially coat protein gene of CPsV using a set of primers consF and consR, as described above. This set of primers succeeded to amplify the expected size bands for the partial coat protein gene of CPsV at 411 bp (Fig. 7). The samples were selected from previous positive ELISA tests.

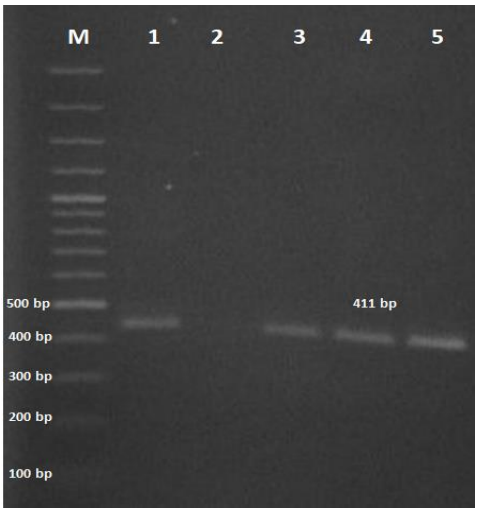

Fig. 7. Agarose gel electrophoresis of RT-PCR amplified products. M: 100 bp DNA ladder; 1: positive control; 2: negative control; 3-5: three different CPsV infected samples.

Electrotherapy treatments and grafting of virus-free plants:

It is obvious from Table (2) that, treatment E6 (40 mA for 20 min) scored the highest elimination of CPsV from infected plants (85%) (Fig. 8). It had Therapy Efficiency Index (TEI) of 4080. Whereas; treatment E3 (40 mA for 10 min) produced only 50% virus-free plants and its TEI was 3100. These results were confirmed by RT-PCR.

Table 2. Grafting rates of virus-free plantlets and the efficiency of electrotherapy treatments for the elimination of CPsV.

Treatment	Electric currents (mA)	Duration (min)	Replicates	Regenerated plants (%)	Virus-free plants (%)	Electrotherapy efficiency
N	0	0	10	90	0	0
E1	10	10	10	70	0	0
E2	20	10	10	65	0	0
E3	40	10	10	62	50	3100
E4	10	20	10	60	0	0
E5	20	20	10	62	0	0
E6	40	20	10	48	85	4080

Fig. 8. Virus-free seedlings of citrus (Navelina sweet orange) treated with electrotherapy.

Shoot tip grafting technique *in vitro:*

The shoot tip grafting technique has been effective to recover plants free of CPsV. Six CPsV-free Navelina sweet orange trees were obtained out of 200 infected shoots by shoot tip grafting *in vitro* on the troyer citrange rootstock. Micrografted plants were successfully transferred directly to the soil and grafted on Volkameriana Lemon rootstock with excellent growth and most plants displayed a normal phenotype. More than 95% of

transferred scions grew well and stayed alive. DAS-ELISA test was used to confirm the results of CPsV free plants by shoot tip grafting (Fig. 9).

Fig. 9. Micrografted plants growing *in vitro*, shoot tip (A), grafting inverted T-budding in MS medium with sterilized filter paper (B), meristem and two leaves primordial (C), micrografted plant with healthy roots (D), transplant in sterilized soil in the greenhouse (E).

DISCUSSION

Results of biological indexing by using indicator cuttings were confirmed by ELISA. All inoculated indicator stem cuttings were ELISA-positive against CPsV; these results were in agreement with ElBacki *et al.,* (2005); El Sayed (2005), and D'Onghia *et al.,* (2009), who found that a new system of biological indexing based on the use of indicator cuttings instead of seedlings was employed for the detection of the main citrus viruses and viroids. Considering the important factors for the rooting ability of stem cuttings as the juvenility stage of the plant source and the seasonal timing of cutting harvesting, this method was applied (Bhusal *et al*., 2001). After 5 weeks from inoculation, clear symptoms developed on the new emerging shoots of the indicators. Symptoms observed were shock and leaf crinkles. These results were in agreement with D 'Onghia *et al*., (2009). Results showed that cool treatments were better for inducing shook symptoms in young emerging shoots (rooted inoculated cuttings) in Madam Vinous and Navelina sweet orange. These results were in harmony with those obtained by Figueroa *et al.* (2009). Our results showed that electrotherapy treatment of 40 mA for 20 min was the most effective treatment in producing virus-free plants (85%) which a therapy efficiency index (TEI) reaching 4080 followed by treatment of 40 m A for 10 min (50%) and its TEI was 3100. However, the grafting rate of treated buds was affected by the intensity of electrotherapy treatment as shown by the treatment E2 (10 m A for 10 min) which gave the highest regenerated plants (70%) whereas, treatment E6 (40 mA for 20 min) gave the lowest percentage of regenerated plants (48%). These results almost agreed with that obtained by several authors (Lozoya *et al*., 1996; Pazhouhandeh, 2001). These authors mentioned that electrotherapy treatment may affect citrus genotypes differently in both elimination of virus and grafting success and also may affect the growth and may induce physiological changes such as resistance to cold and drought and produce organogenesis in some crops. On the other hand, Mozafari and Pazhouhandeh (2000) found no noticeable morphological differences among regenerated plants produced after this treatment. The electrotherapy technique compared to the other elimination techniques such as meristem culture and thermotherapy is simple, faster and economically less costly. Besides, the grafting rate is much higher (Pazhouhandeh *et al*., 2002). Nevertheless, the conditions of electrotherapy treatment may differ for each host. This needs further studies to investigate the suitable condition for each crop. RT-PCR appears to recognize Egyptian isolate of CPsV and would be adapted for large-scale application. The procedure used in our study is simple, quick, and suitable for the detection of citrus psorosis virus infection as mentioned by other investigators (Martin *et al*., 2004).

Shoot tip grafting is an effective technique to recover plants free of the citrus psorosis virus. Six CPsV *in vitro* in which seeds of the *Troyer citrange* rootstock were planted on MS medium and meristem was extracted from the infected trees and then grafted on the *T. citrange* rootstock *in vitro* under sterilized conditions. Micrografted plants were successfully transferred directly to the soil and grafted on Volkameriana lemon rootstock with excellent growth and most plants displayed a normal phenotype.

CONCLUSION

Elimination of CPsV from infected citrus trees was obtained successfully by electrotherapy technique. The final assessment revealed that treated plants were 85% CPsV-free. The results presented in this paper proved that the electrotherapy technique was efficient in the elimination of CPsV. The shoot tip grafting technique is a promising method for the production of virus-free citrus seedlings.

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Conflict of Interest: The authors declare no conflict of interest.

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التخلص من فيروس قوباء الموالح باستخدام تقنية تطعيم المرستيم القمي والمعالجة الكهربائية

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تم عزل وتعريف فيروس قوباء الموالح من أشجار الموالح المصابة بمحافظة القليوبية بالفحص البصري والأعراض الظاهرية في الحقل وتم فحص العينات بالاختبارات البيولوجية باستخدام العوائل المشخصة والاختبارات السيرولوجية والبيولوجيا الجزيئية باستخدام البادئ المتخصص وقد أسفرت النتائج عن وجود فيروس قوباء الموالح بنتائج ايجابية وكان حجم جزيئات الفيروس 434 نيوكليوتيدة بينما لم تظهر العينات الغير مصابة أي نتائج ايجابية وتم استخدام المعالجة الكهربائية للقضاء على العدوى. تعرضت عقل الساق من النباتات المصابة لتيارات كهربائية 10 و 20 و 40 ملي أمبير لمدة 10 و 20 دقيقة، على التوالي، قبل تطعيمها في النباتات المشخصة.

ثبت أن المعالجة ب 40 ملي أمبير لمدة 20 دقيقة أكثر فعالية في انتاج نباتات خالية من الفيروسات بنسبة (85٪). أيضًا، من أجل القضاء على فيروس قوباء الموالح، تم إستخدام طريقة التطعيم بالمرستيم القمي في المعمل بطول لا يزيد عن (0.1-0.2 مم) من البراعم المصابة بالفيروس على أصل سترينج تروي، وتم زراعته في المعمل علي بيئة مناسبة للنمو وكانت نسبة النجاح 3٪، وبعد ذلك تم نقل النباتات المطعمة إلى الصوبة للتأقلم. تم إعادة تطعيم النباتات الناتجة بنجاح على شتلات البرتقال في الصوبة وتأكيد خلوها من الفيروس باختبار الأليزا والبيولوجيا الجزيئية.

الكلمات المفتاحية: فيروس قوباء الموالح، الفهرسة البيولوجية، تفاعل النسخ العكسى، المرستيم القمي، المعالجة الكهربائية.