Elimination of *Bean yellow mosaic virus* from infected cormels of three cultivars of gladiolus using thermo-, electro- and chemotherapy

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Abstract

Bean yellow mosaic virus (BYMV) is a prevalent virus and major threat to gladiolus cultivation the world over. In the gladiolus repository at CSIR-NBRI, Lucknow, several plants (82–88%) of three economically important cultivars were found infected by BYMV showing severe mosaic and stripe symptoms. Affected plants exhibit diminished quality and quantity of florets and corms, thus reducing their value. Attempts were made to eliminate BYMV from the infected gladiolus cormel explants in vitro through thermotherapy (37 °C for 30 days), chemotherapy (30 mg/L ribavirin for 30 days), and electrotherapy (30 mA for 20 min), either alone and in different combinations. The in vitro regenerated plants were free from BYMV infection when checked by RT-PCR using BYMV-specific primers. The combination of electro- and chemotherapies has given the best response as compared to other treatments. Among the individual therapies, electrotherapy (30 mA/20 min) was found to be the best for and production of BYMV-free gladiolus plants (44–46%) with moderate regeneration efficiency (54–58%) followed by chemotherapy and thermotherapy. However, the cormels obtained from a combination of electro- and chemotherapy treatment (30 mA/20 min + 30 mg/L) has given highest virus free (46–52%) and highest therapy efficiency indices (56%) as compared to other treatments. Further, these cormels showed better developed root systems and produced more cormels which were larger in size as compared to the other treatments and control when grown in tissue culture media.

Keywords Gladiolus · BYMV · Chemotherapy · Electrotherapy · Cormels · Virus free

Charanjeet Kaur and Rashmi Raj contributed equally to this work.

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Introduction

Gladiolus (family Iridaceae), a genus of cormous flowering plants, is native to South Africa and one of the economically important cut flowers in Asia, Mediterranean Europe, South Africa, and tropical regions in Africa. A survey of world flower bulb production reveals that six genera including gladiolus occupy 90% of the reported production acreage (Le Nard and De Hertogh 2002). It is valued both as an ornamental garden plant and as elite cut flower crop (De Groot 1999). Due to its vast popularity, the new varieties/species are being developed the world over, of which 276 species have been accepted by the World Checklist of Selected Plant Families (Anonymous 2014). It ranks second in production, area and volume of cut flowers grown in India. Uttar Pradesh is the primary gladiolus-producing state in India (http://www.nhb.gov.in/area-pro/NHB_Database_2015.pdf).

The propagation of gladiolus by vegetative means using cormels increased the probability of pathogen infection. They suffer from a variety of viral diseases that affects their

quality and yield (Stein 1995). Amongst them, BYMV is the most prevalent virus causing mosaic symptoms on leaves, color breaking in flowers, and reducing the plant's vigor (Bridgmon and Walker 1952; Fry 1953; Srivastava et al. 1983; Park et al. 1998; Zaidi et al. 1993; Dubey et al. 2009; Duraisamy et al. 2011). The serological and molecular identification lead to the characterization of BYMV in corms. The variability among three isolates of BYMV from Czech Republic originating from gladiolus was examined based on their biological and molecular characteristics (Ganesh et al. 2009). In India, Srivastava et al. (1983) detected the BYMV infection from every part of gladiolus plant showing mosaic symptoms on leaves and color breaking in flowers and recorded a gradual deterioration in the size of plant and spike over the course of time. Spreading of BYMV infection in the gladiolus field has also been reported earlier (Singh et al. 2007) that resulted in infection by the virus in all gladiolus plants grown. For development of virus-free plants, in vitro propagation of meristematic tissue coupled with thermotherapy, chemotherapy and a combination of both the therapies have been applied (Kumar et al. 2009). The meristematic tissue is preferred over others as the virus failed to invade meristem as high concentrations of growth regulators, active metabolic process, and inhibitors (phenolamines) restrict virus multiplication, immense competition for cellular enzymes for virus survival, metabolic disruption of enzymes necessary for viral replication and RNA degradation (Vanzaayen et al. 1992). In vitro production of virus-free plants using chemotherapy has been demonstrated as a successful method for eliminating many viruses in vegetatively propagated plants, such as orchids, lily, chrysanthemum, begonia, and gladiolus (Panattoni et al. 2013). Anti-viral compounds like virazole (1-beta-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide), a nucleoside inhibitor, suppress replication as well as multiplication of virus in infected cells (De Fazio et al. 1978). In gladiolus, elimination of virus from infected cormel explants through in vitro chemotherapy coupled with thermotherapy has been suggested previously (Selvarajan et al. 1999; Singh et al. 2007). A putative reason for getting a high number of virus-free plants during thermotherapy was that the viral RNA silencing is significantly enhanced at high temperatures (Chellappan et al. 2005). Electrotherapy which utilizes electrical pulses to eliminate viruses from the infected plant tissue has also received attention. Electrotherapy is a simple method of virus eradication where an electric current is applied to plant tissues that disrupt or degrade viral nucleoprotein, thus eliminating its virulence (Hormozi-Nejad et al. 2010). This method was first comprehensively employed for the elimination of Potato virus X (PVX) from potato plants in which an electric current of 15 mA for 5 min resulted in 60–100% elimination of PVX in different potato cultivars (Lozoya et al. 1996). Electrotherapy was also used for elimination of Potato virus

Y (PVY), Potato virus A (PVA), Potato virus S (PVS) and Potato leaf roll virus (PLRV) from potato (Pazhouhande and Mozafari 2001), Onion yellow dwarf virus and leek yellow stripe virus from garlic (Hernández et al. 1997); Banana streak virus from banana (Hernández et al. 2002), Dasheen mosaic virus and Grapevine leaf roll virus from cocoyam (*Xanthosoma sagittifolia*) and grapevine (*Vitis vinifera*), respectively (Igarza-Castro et al. 2001; Guta et al. 2010). However, the use of electrotherapy and combination of various therapies for elimination of BYMV from gladiolus has not been attempted. The present study on BYMV elimination from the infected gladiolus cormels was undertaken to establish an effective and facile virus management strategy for quality improvement of gladiolus.

Materials and methods

Plant material

The cormels from 30 out of 200 plants of 3 gladiolus cultivars: Aldebaran, Tiger Flame and Vink's Glory exhibiting severe mosaic and stripe symptoms were collected from the experimental plots of CSIR-NBRI, Lucknow. The apparently healthy plants of these cultivars were used as control for comparison. The cormel explants were first indexed for the presence of BYMV by RT-PCR using BYMV-coated protein (CP) gene-specific primers (forward primer: 5'-TGA CTTGCCCCCTAACTGCTG-3' and reverse primer: 5'-TGA CTTGCCCCCTAACTGCTG-3') and cormels found positive for the virus were used for further experimentation. Total RNA was extracted from 100 mg cormel tissues from the selected plants of each cultivars using Spectrum™ plant total RNA kit (Sigma-Aldrich Co., MO, USA). The CP gene of BYMV was amplified from extracted RNA employing MMuLV Reverse Transcriptase enzyme (MBI Fermentas Inc., MD, USA) and *Taq* polymerase (MBI, Fermentas, Inc., Hanover, MD, USA) by BYMV-For/Rev primer pair in two-step RT-PCR. The size of the amplicon was assessed by 1% agarose gel electrophoresis with 1 kb DNA ladder marker (MBI Fermentas Inc., MD, USA). Cormels of the infected gladiolus plants were stored for subsequent experiments at 4 °C until use.

Regeneration of virus-free gladiolus cormels

Indexed cormels (ten cormels/replication of each cultivar) of 0.3–0.5 cm³ size were surface sterilized. Briefly, the husk of the cormel was removed and washed thoroughly under running tap water for 30 min in a beaker covered with muslin cloth as suggested by Aminuddin and Singh (1993). The explants were then treated with 0.4% (w/v) carbendazime (Bavestin, BASF India Ltd., India) for 10 min with

continuous stirring. Then, the cormels were washed in 5% Teepol solution for 10 min followed by four rinses in distilled water. The explants were dipped in 70% (v/v) alcohol for 30 s, dipped in 1% (w/v) HgCl_2 treatment for 10 min, and then rinsed in sterile distilled water six times. Three different media were used separately for germination (MSg), proliferation (MSp) and multiplication (MSc) of gladiolus plantlets. MSg medium was prepared using MS basal medium (Murashige and Skoog 1962) supplemented with 1.0 mg/L BAP, 0.5 mg/L IAA and 2.0 mg/L 2, 4-D with 3% sucrose. MSp medium was prepared using MS basal medium supplemented with 0.2 mg/L BAP, 0.2 mg/L NAA and 20 mg/L AdSO_4 with 3% sucrose. MSc medium contained MS basal medium supplemented with 0.2 mg/L NAA and 30 mg/L AdSO_4 with 6% sucrose. All these media were solidified by addition of 0.25% (w/v) CleriGel (Himedia Labs Pvt Ltd., Mumbai, India).

For the elimination of BYMV from infected cormel explants, 30 cormel explants from each of three cultivars in three replications were used for thermotherapy, chemotherapy and electrotherapy either alone or in combination. For thermotherapy, the infected cormel explants were first surface sterilized, kept on MSg medium and incubated at $37 \pm 2^\circ\text{C}$ for 30 days as suggested earlier (Selvarajan et al. 1999; Nesi et al. 2009; Nezamabad et al. 2015). For chemotherapy, a gradient of 30, 40, 50 and 60 mg/L of ribavirin (Duchefa Biochemie, the Netherlands) was used. Ribavirin was added to the MSg medium and explants were allowed to grow in the culture room. For electrotherapy, the cormels were immersed in 1X TAE buffer (Tris:glacial acetic acid:EDTA, 40:20:1, pH 8.0) in an electrophoresis tank (Midi sub system 03-02; Genei Pvt. Ltd., Bangalore, India) and exposed to electric currents of 10, 20 and 30 mA for 20 min using a power supply (BGPS 300/400; Genei Pvt. Ltd., Bangalore, India). Immediately after treatment, the explants were surface sterilized and then kept on MSg medium. For a combination of chemotherapy and electrotherapy, after electrotherapy, the explants were placed on ribavirin-added MSg medium. All explants culture of all treatments along with untreated control explants were maintained in a culture room at $23\text{--}25^\circ\text{C}$ with 16 h light.

Regenerated plantlets, obtained from different treatments, were tested for the presence or absence of BYMV in three stages using RT-PCR. In initial testing, total RNA was extracted from 50 mg leaf tissue of germinated gladiolus plantlets and tested by RT-PCR using BYMV-specific primers (as described above). The plantlets in which the presence of BYMV was not detected were multiplied in MSp medium and used for second stage virus testing for the presence of BYMV by RT-PCR. The plantlets that remained virus free were then allowed to induce cormels in MSc medium. After 60 days, the plantlets were subjected for the third stage testing by RT-PCR using leaf tissue.

Regeneration efficiency

To measure the percentage of virus-free plantlets (regeneration efficiency), the regenerated plantlets were tested by RT-PCR, 30 days after the start of the in vitro culture and the regeneration efficiency (RE) was estimated for each treatment as:

$$\text{RE (\%)} = (\text{regenerated plantlets/total explants}) \times 100.$$

The percentage of BYMV-free plantlets was calculated as:

BYMV-free (%)

$$= (\text{BYMV-free plantlets/total regenerated plantlets}) \times 100.$$

To find a treatment leading to high rates of both virus elimination and plant regeneration, the therapy efficiency index (TEI) defined by Lozoya et al. (1996) was estimated for each treatment as:

$$\text{TEI} = (\text{percentage of regenerated plantlets} \times \text{percentage of virus-free samples})/100.$$

Plantlets obtained from thermotherapy, chemotherapy, electrotherapy and combinations of therapies, as well as their respective controls, were removed from the culture medium and excess medium was washed off. Plants were acclimatized in 4 inch pots containing a sterilized mixture of soil, vermiculite and organic matter (2:2:1), kept under a transparent polystyrene cover, and irrigated daily with Hoagland's nutrient solution (Hoagland and Arnon 1950) for 2 weeks. After 2 weeks, the cover was removed and plants were kept in glasshouse conditions. The number and size of BYMV-free cormels were increased by sub-culturing BYMV-free plantlets in MSc medium for 60 days and the performance was recorded by comparing with respective controls.

Results

Screening of BYMV in gladiolus cultivars

An experimental plot of CSIR-NBRI having 200 plants of each gladiolus cultivar, Aldebaran, Tiger Flame and Vink's Glory was thoroughly surveyed for symptoms like severe mosaic, leaf stripe and color breaking on florets (Fig. 1a) for BYMV infection. Based on morphological symptoms, Aldebaran (176/200), Tiger Flame (165/200) and Vink's Glory (185/200) were found infected with a high degree of disease incidence (Supplementary table-1). The infection caused 10% reduction in the average plant height as compared to healthy plants, whereas the average number

Fig. 1 **a** Leaf and corm samples of symptomatic showing mild to severe virus-like mosaic symptoms on leaflets and color breaking on florets. **b** A representative image of RT-PCR with CP-specific primers to check BYMV infection in one plant of Aldebaran. **c** Morphology of healthy and infected plants

of tillers in infected plants was reduced by 40–60% in three cultivars. The average number of spikes per plant and florets per spikes was also reduced by 5–9% and 10–25% respectively. The average number of cormels was also reduced by 25–40% while the fresh weight of cormels was reduced by 31–34% in infected plants as compared to healthy plants (supplementary table 1). The RT-PCR of 30 selected plants from each cultivar using CP gene-specific primers revealed the presence of BYMV infection in all tested gladiolus plants as shown in a representative gel image of one plant of Aldebaran cultivar with all plant parts (Fig. 1b). The average of height, number of tillers, length of spikes, number of florets in a spike, cormels and fresh weight of corms were also reduced in all three cultivars as compared to healthy plants (Supplemental table 1; Fig. 1c). Cormels of the infected gladiolus plants were collected for subsequent experiments and stored at 4 °C until use.

Production of BYMV-free gladiolus plants

The infected cormels of three gladiolus cultivars were subjected to thermotherapy, chemotherapy, electrotherapy and combined chemo- and electrotherapy. The treated cormels were transferred to MSg, MSp and MSc medium for germination, proliferation and multiplication, respectively

ers to check BYMV infection in one plant of Aldebaran. **c** Morphology of healthy and infected plants

(Fig. 2). The percentage of RE, BYMV-free plants and TEI was assessed in comparison to untreated (control) explants (Fig. 3). The average REs of Aldebaran, Tiger Flame and Vink's Glory in control experiments were 86, 82 and 84%, respectively (Fig. 3; Table 1). The RE was observed as 26, 16 and 22% in three gladiolus cultivars treated with thermotherapy, respectively (Table 1; Fig. 3). In case of chemotherapy treatment with ribavirin at concentrations of 30 mg/L, 40 mg/L and 50 mg/L, the maximum RE was observed with 30 mg/L ribavirin as 48, 46 and 46% in Aldebaran, Tiger Flame and Vink's Glory, respectively (Table 1; Fig. 3). RE was reduced with increasing concentration of ribavirin (Table 1) and the cormels did survive at 40 mg/L ribavirin but subsequent germination was inhibited. The treatments at concentrations > 40 mg/L were lethal in all three gladiolus cultivars. Electrotherapy was applied at three different electric current strengths (10, 20 and 30 mA for 20 min). The average RE was 76, 74 and 74% in three gladiolus cultivars which was observed with electrotherapy (10 mA for 20 min). While the RE of the same cultivars in control experiments, at 20 mA for 20 min electric currents was 75.0–85.0% and at 30 mA for 20 min was 70.0–75.0% (Table 1; Fig. 3a). The combinations of thermotherapy (37 ± 2 °C) with chemotherapy (30 mg/L ribavirin) and electrotherapy (30 mA for 20 min) resulted in the death of all explants (data not

Fig. 2 Different stages of production of BYMV-free gladiolus plants by combination of chemotherapy (30 mg/L) with electrotherapy (30 mA for 20 min). **a** Infected gladiolus mother corm bearing cormels (shown by arrows); **b** cormels getting electrotherapy in elec-

trophoretic tank; **c** cormels explants in MSc media amended with ribavirin (30 mg/L); **d** germination after 30 days; **e** proliferation in MSP medium; **f** harvesting of cormels after drying; and **g** acclimatization of gladiolus plantlets

shown). Among the other combinations, chemotherapy (30 mg/L ribavirin) along with electrotherapy (30 mA for 20 min) was the best performer, in which 58, 54 and 54% REs were observed in three gladiolus cultivars (Fig. 3a; Table 1). After germination and proliferation, each cultivar was indexed by RT-PCR for the presence or absence of virus (Fig. 4). The thermotherapy at 37 °C resulted in only 14, 8 and 14% virus-free plants in three cultivars (Aldebaran, Tiger Flame and Vink's Glory cultivars), while 42, 36 and 36% virus-free plants were obtained in these cultivars after chemotherapy (30 mg/L ribavirin). Electrotherapy (30 mA for 20 min) resulted in 46, 44 and 44% virus-free plants in all the cultivars (Table 1). The highest rate of BYMV-free plants in the cultivars was obtained in combination of chemotherapy and electrotherapy where 52, 48 and 46% virus-free plants were obtained in Aldebaran, Tiger flame and Vink's Glory cultivars, respectively (Table 1; Fig. 3b). All the gladiolus plantlets in control were positive for BYMV. The most effective treatment for elimination of BYMV from infected gladiolus cormels was the combination of electrotherapy (30 mA for 20 min) with chemotherapy (30 mg/L ribavirin), as the highest TEI obtained were, in the range of 53.75, 51.84 and 56.70 in these gladiolus cultivars (Fig. 3c).

The results also showed that the BYMV-free cormels of Aldebaran, Tiger Flame and Vink's Glory cultivars,

developed through various therapies, possessed more cormels along- with a well-developed root system as compared to the control (Fig. 5). Thermotherapy resulted in a lower number of cormels as compared to plants treated with chemotherapy and electrotherapy alone and in combination. The average number of cormels produced in control experiments of Aldebaran, Tiger Flame and Vink's Glory cultivars were 1–2 per plant to that of 4–6 per plant in the BYMV-free plants (Table 2). Similarly, the average size of cormels produced in vitro was also more in BYMV-free plants (Table 2).

Discussion

For the elimination of virus from ornamental crops like gladiolus, chrysanthemum gerbera etc, the meristem culture alone and a combination of meristem culture with chemotherapy and thermotherapy have been used (Aminuddin and Singh 1993; Selvarajan et al. 1999; Singh et al. 2007; Gautam et al. 2017). In this study, plants of three commercially important gladiolus cultivars, viz., Aldebaran, Tiger Flame and Vink's Glory were used, because they are susceptible to the virus infection. Severe yield reduction and quality deterioration was observed in all the three cultivars due to BYMV infection (Supplementary table-1). The studies also

Fig. 3 Graphical representation of RE, rate of BYMV elimination and TEI of three gladiolus cultivars treated with various treatments. **a** RE (%); **b** rate of BYMV elimination (%); and **c** % TEI

affirmed the previous work of Park et al. (1998), in which they observed a clear-cut reduction in the quality of flowers and spikes and vigor of the gladiolus plant due to infection of BYMV. The electrotherapy has also been reported to be efficient for the elimination of both RNA and DNA

viruses from a number of plants like banana (Hernández et al. 2002), *Phaseolus vulgaris* (Hormozi-Nejad et al. 2010), potato (Badarau and Chiru 2014) and tomato (Fallah et al. 2007). Not only in case of plant viruses, but it has also been successfully used for the attenuation of human immunodeficiency virus (HIV) in human cell cultures (Lyman et al. 1996), and for killing pathogenic bacteria, yeast, and spores in buffer solutions (Grahl and Märkl 1996; Abram et al. 2003). These previous studies prompted us to take thermotherapy, chemotherapy and electrotherapy alone or/and in combination for the effective elimination of BYMV from gladiolus using cormels as explants for regeneration, as they have maximum regenerative efficiency as compared to other explants in gladiolus (Singh et al. 2007).

For thermotherapy, the increased temperature ($37 \pm 2^\circ\text{C}$) was applied to the infected gladiolus cormels for 30 days and the regeneration efficiency was found to be poor in all the gladiolus cultivars tested. Poor growth after exposure to elevated temperatures is common in some plant species, as observed in potyvirus elimination studies in potato (Luciana et al. 1993; AlMaarri et al. 2012). In chemotherapy, the infected gladiolus cormels were subjected to 30 mg/L ribavirin which showed nearly 46–48% germination and only 36–42% virus-free plants. Electrotherapy at 30 mA for 20 min showed the best result where 54–58% cormels were germinated and 44–46% plants were found to be free from virus infection in three gladiolus cultivars. Electrotherapy was first employed for the elimination of *Potato virus X* (PVX) from potato plants in which an electric current of 15 mA for 5 min led to 60–100% elimination of PVX in various cultivars (Lozoya et al. 1996). Electrotherapy has also been successfully used for elimination of *Potato leaf roll virus* (PLRV), *Potato virus A* (PVA), *Potato virus S* (PVS) PVX and *Potato virus Y* (PVY) from potato (Pazhouhande and Mozafari 2001). Hormozi-Nejad et al. (2010) observed 71.9% of *P. vulgaris* plants became *Bean common mosaic virus* (BCMV) free after application of 15 mA electricity for 10 min, but regeneration was decreased with increasing exposure to electric current. In contrast, the regeneration rate increased as compared to controls when 10 mA of current was applied for 20 min. It is speculated that electrical stimulation may induce physiological changes and cause organogenesis in gladiolus also as previously reported in some other crops (Lozoya et al. 1996; Meybodi et al. 2011). When the current was increased to 30 mA for 20 min, the regeneration rate decreased as compared to control experiments, however, this therapy was less degenerative to the cormels as compared to the thermotherapy and chemotherapy treatments.

The rate of regeneration and percentage of BYMV-free plants in all the cultivars was the highest in the combination of chemotherapy and electrotherapy as represented by the highest TEI. Similar results were observed in the

Table 1 Summary of the results obtained in various treatments using thermotherapy, chemotherapy, electrotherapy and the combination of electro- with chemotherapy in three gladiolus cultivars and their respective germination efficiencies in MSg medium and virus-free plants as (% number $\pm$ st. dev)

V*	Thermotherapy (37 $\pm$ 2 °C)			Chemotherapy (virazole mg/L)			Electrotherapy (mA/min)					
	30 mg/L			40 mg/L			10 mA/20 min		20 mA/20 min		30 mA/20 min	
	Germi-nated plants	Virus-free plants	Germi-nated plants	Germi-nated plants	Virus-free plants	Germi-nated plants	Germi-nated plants	Virus-free plants	Germi-nated plants	Virus-free plants	Germi-nated plants	Virus-free plants
A	86 $\pm$ 5.5	0.0 $\pm$ 0.0	26 $\pm$ 5.5	14 $\pm$ 5.5	42 $\pm$ 4.5	48 $\pm$ 4.5	66 $\pm$ 5.5	36 $\pm$ 5.5	66 $\pm$ 5.5	44 $\pm$ 5.5	58 $\pm$ 4.5	46 $\pm$ 5.5
T	82 $\pm$ 4.5	0.0 $\pm$ 0.0	16 $\pm$ 5.5	8.0 $\pm$ 2.5	36 $\pm$ 5.5	46 $\pm$ 5.5	18 $\pm$ 0.44	12 $\pm$ 0.45	6.0 $\pm$ 0.55	6.0 $\pm$ 0.55	54 $\pm$ 5.5	44 $\pm$ 5.5
V	84 $\pm$ 5.5	0.0 $\pm$ 0.0	22 $\pm$ 4.5	14 $\pm$ 5.5	36 $\pm$ 5.5	46 $\pm$ 5.5	14 $\pm$ 0.55	8.0 $\pm$ 0.45	8.0 $\pm$ 0.45	66 $\pm$ 5.5	56 $\pm$ 5.5	44 $\pm$ 5.5
Electrotherapy + chemotherapy												
V*	Thermotherapy (37 $\pm$ 2 °C)			30 mg/L + 10 mA/20 min			30 mg/L + 20 mA/20 min		30 mg/L + 30 mA/20 min			
	Virus-free plants			Germinated plants			Virus-free plants		Germinated plants		Virus-free plants	
	Germinated plants			Virus-free plants			Germinated plants		Virus-free plants		Germinated plants	
A	86 $\pm$ 5.5	0.0 $\pm$ 0.0	26 $\pm$ 5.5	14 $\pm$ 5.5	32 $\pm$ 4.5	54 $\pm$ 5.5	56 $\pm$ 4.5	46 $\pm$ 5.5	58 $\pm$ 5.5	52 $\pm$ 8.4		
T	82 $\pm$ 4.5	0.0 $\pm$ 0.0	16 $\pm$ 5.5	8.0 $\pm$ 2.5	44 $\pm$ 5.5	54 $\pm$ 5.5	48 $\pm$ 4.5	46 $\pm$ 5.5	54 $\pm$ 5.5	48 $\pm$ 4.5		
V	84 $\pm$ 5.5	0.0 $\pm$ 0.0	22 $\pm$ 4.5	14 $\pm$ 5.5	34 $\pm$ 5.5	54 $\pm$ 5.5	48 $\pm$ 4.5	44 $\pm$ 5.5	54 $\pm$ 5.5	46 $\pm$ 5.5		

V* varieties, A Aldebaran, T Tiger Flame, V Vink's Glory

Fig. 4 Detection of BYMV by RT-PCR in vitro germinated gladiolus plants developed after various therapies. *M* 1 kb DNA ladder marker, *A1–A21* Aldebaran, *VG1–VG11* Vink's glory, *TF1–TF4* Tiger flame, *P* BYMV-infected gladiolus plant

Fig. 5 Comparison of cormels and root systems of “Aldebaran” cultivar of gladiolus developed through various treatments with control. (D1–4), control (untreated BYMV-infected regenerated cormels); (T1–2), thermotherapy-treated BYMV-free cormels; (C1–2), chemo-

therapy-treated BYMV-free cormels; (E1–2), electrotherapy-treated BYMV-free cormels; and (CE1–2), chemotherapy coupled with electrotherapy-treated BYMV-free cormels

case of elimination of PVY from potato, in which the combination of chemotherapy and electrotherapy was found to be the most effective (Mahmoud et al. 2009).

However, the lowest RE, rate of BYMV-free plants and TEI was found in thermotherapy in all gladiolus cultivars. The elevated temperature ($37 \pm 2^\circ\text{C}$) for 30 days duration

Table 2 Performance of BYMV-free gladiolus corms developed through various treatments as compared to the control

Cultivars	Av. no. of cormels produced per plant		Average size of cormels produced (in cm ³)	
	Control	BYMV-free	Control	BYMV-free
Aldebaran	2.0±0.50	5.0±0.50	0.35±0.04	0.56±0.07
Tiger Flame	1.0±0.31	4.0±0.31	0.30±0.01	0.55±0.02
Vink's Glory	2.0±0.57	6.0±0.57	0.33±0.07	0.51±0.05

caused a lower regeneration rate and was found to be most unsuitable treatment for the elimination of BYMV in all the varieties of gladiolus. It has been postulated that viral nucleoproteins may be denatured by increasing temperature inside the tissue caused by exposure to electric currents (Lozoya et al. 1996; González et al. 2006). The electrotherapy works in a similar way to controlled thermotherapy, where the temperature is increased only within tissues, because they may be less conductive than the TAE buffer (González et al. 2006). Since cells are thermally isolated by the cell wall, thermal inactivation might not work when the nucleoproteins are within the cells (Hormozi-Nejad et al. 2010).

The root system and the number of gladiolus cormels treated with electrotherapy alone and/or in combination with chemotherapy were found better as compared to the control explants, which indicated the induction of some growth hormones in gladiolus plants due to electric currents. Black (1971) suggested that small currents may stimulate active ion pumps or alter the internal distribution of growth-regulating compounds in tomato plants and also found it helpful in healing wounds on trees (Blanchard 1974). Overall, the established protocol to eliminate BYMV from gladiolus may be used at a commercial level for management of virus diseases in ornamental plants and would be helpful to provide healthy planting material to gladiolus growers for better quality production in the country.

Conclusions

This study reports for the first time on the elimination of BYMV from infected explants using three different therapies like thermo-, chemo- and electrotherapies alone and in the combination of chemo- and electrotherapies. Electrotherapy coupled with chemotherapy in three commercially important gladiolus cultivars (Aldebaran, Tiger Flame and Vink's Glory cultivars) was employed successfully. This therapy was found more effective than other treatments and can be recommended for better quality production and improvement of gladiolus as well as other valuable crops.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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