Eradication of potato viruses and evaluation of different soil mixtures on minitubers production

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ABSTRACT

Recently production of virus-free potato minitubers is critical. For this purpose, infected tubers with *potato leafroll virus* (PLRV) or *potato virus x* (PVX) from two cultivars Spunta or Lady Rosette as demonstrated by serological detection were used as a source for virus-free minitubers production. This achived by subjecting tubers directly to thermotherapy treatment at 36ºC, 37ºC or 38ºC for three weeks which resulting in 50%, 70% or 80% of PLRV-free tubers and 0%, 16.6% or 33.3% of PVX-free tubers respectively. Moreover, no effects on the survival rate of tubers were detected. High percentage (93.1% or 76%) of PLRV- or PVX-free plantlets was achieved with meristem tip excision (0.1-0.2mm) from thermo-treated tubers at (38ºC) with survival rate 96.6% or 83.3% respectively. DAS-ELISA method was applied to the tubers directly, *in vitro* cultures and young acclimatized plantlets to detect these viruses. After direct transplant of *in vitro* virus-free plantlets in greenhouse. The statistical analysis showed that cultivars and ratio of bed components and their interaction had insignificant ($P>0.05$) effects on minituber number and minituber weight. However, ratio of bed components and interaction with cultivars were significantly ($P<0.05$) affected on minituber diameter. Maximum minituber diameter (13.64 mm) was recorded for Lady Rosette cultivar treated with Vermiculite + Sand (4:1), followed by Spunta cultivar (13.53 mm) applied with Vermiculite + Sand (4:1). Pots received Vermiculite + Sand (4:1), produced highest minituber diameter, while the least minituber was recorded for pots having Peat moss + Sand (4:1).

Key Words: Potato, PLRV, PVX, thermotherapy, meristem culture, minitubers, soil mixture.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the second most important vegetable crop after tomato in Egypt, Potato is not only a basic food need in Egypt, but also has an enormous impact on the economic situation of Egyptian.

Egypt imports seed potatoes directly from EU to increase potato production and produces basically two potato markets, small beautiful potatoes for export to the EU countries and larger potatoes for the local market and other countries like Russia. In most recent years the Egyptian potato exports to the EU increased from 70% to 90% and the total value of potato exports to the EU was about 63 million Euros. (EU delegation to Egypt, 2010).

Since potato is propagated vegetatively, it is exposed to phytopathogens such as fungus, bacteria, viruses, phytoplasmas, viroids and nematodes more often resulting severe yield reduction in the infected plantations. In Egypt, six viruses, *potato virus x* (PVX), *potato virus y* (PVY), *potato leafroll virus* (PLRV), *potato yellow dwarf virus* (PYDV), *tobacco rattle virus* (TRV) and *mop top virus* (MTV) are of primary concern and have received attention in potato production programs or import phytosanitary requirements and specification for importation of seed

potatoes (Ministry of agriculture and land reclamation, 2012). Among the several viruses reported to infect potatoes, (PLRV, Luteovirus) and (PVX, Potexvirus) are of the most dangerous potato viruses transmitted through seed which cause problem especially in seed potato production, produce small tubers that not preferable in market and reduce the yield by 33-50% but, even greater loss 80-90% may be expected when PLRV occurs in simultaneous infection with PVX or PVY (Khurana, 2000).

The tubers infected with PLRV have rare symptoms but some cultivars such as Green Mountain and Russet Burbank have internal net necrosis which can be seen when cutting the tubers (Mowary, 1994) similarly, infection with PVX reduce more than 10-15% of potato yield without showing any physical symptoms (Gomec, 1977; Beemster and Rozendaal, 1972) therefore, the detection of virus based on symptomatology had disadventage with tubers of masked symptoms (Robert *et al*., 2000).

Obtaining higher yield efficiency of potato is very important and can be achieved by producing potatoes seeds from healthy minitubers (Kawakami *et al*., 2003). Thermotherapy is of the most viable methods for obtaining healthy materials from virus-infected propagative crops also, meristem tip culture was used to enhance thermotherapy in virus eradication (Mori and Hosokawa, 1977). Thermotherapy of the mother plants prior to meristem tip culture has an advantage; it increases the percentage of surviving virus-free explants developed from initiating cultures with large meristem tip. However, PVX is one of the serious viruses as *tobacco mosaic virus* (TMV), *odontoglossum ringspot virus* (ORSV) and *carnation mottle virus* (CarMV) that can be invading the meristematic region (Bhojwan and Dantu, 2013) which considered as an important obstacle in order to eradicate virus by using large meristem tip.

Based on the above facts and due to the serious effect of potato viruses, the present study has been conducted to perform the following objectives:

1- Study the effect of different temperature degrees and meristem culture technique on eradication of PLRV and PVX from the most commonly potato cultivars in Egypt (Spunta and Lady Rosette).

2- Production of potato minitubers from direct transplanting of *in vitro* virus-free plantlets in greenhouse. Also, investigate the effect of different soil mixtures on minitubers production for both cultivars.

MATERIAL AND METHODS

This study includes two parts; the first part was performed at Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), to determine the effect of different temperature degrees and meristem culture technique on eradication of PLRV and PVX from the most common potato cultivars in Egypt (Spunta and Lady Rosette). The second part was based on the results of the first one, production of minitubers in greenhouse from virus-free plantlets that originated from infected tubers. Also, investigate the effect of different soil mixtures on minitubers production for both cultivars. The investigations were carried at Agronomy Department, Faculty of Agriculture, Cairo University.

I-The first part:

Plant material and virus detection:

Two potato cultivars grown in Egypt (Spunta and Lady Rosette) were obtained from EL-Nubaria Region, near Beheira Governorate and detected for the presence of the viruses (PLRV,

PVY and PVX) serologically, directly from tubers by using double antibody sandwich enzyme linked immuno-sorbent assay (Clark and Adams, 1977). Two eyes (One near the rose end and other near the heel end from each tuber) were drilled to a depth of approximately 10 mm by using Cork-borer (10 mm diameter), then extracted and deposited directly into one well of ELISA plate (Gugerli, 1980). ELISA kits were supplied by LOEWE Biochemica, GmbH, DSMZ, Germany.

Virus eradication:

Thermotherapy treatment:

The equipment used in this experiment is VWR/Sheldon 2020 incubator consists of chamber of (18 cu.ft.) capacity with digital temperature control (-10ºC to 45ºC temperature range). Thermotherapy was applied to un-sprouted infected tubers from each cultivar at 37ºC ± 1 and incubated for 21 days. The rate of success in removing viruses was determined at the end of each treatment using DAS-ELISA.

Sprout initiation and surface sterilization:

Infected tubers from more successful thermal treatment were chosen and placed in 4ºC for 3 weeks at room temperature for 2 weeks to break the dormancy. Under aseptic conditions, surface-sterilized was conducted by immersing the formed sprouts in 20% commercial sodium hypochlorite solution and one drop of tween 20 (polyoxyethylene sorbiton monolaurate) for 20 min, sprouts were then rinsed three successive times for 3 min with sterile distilled water.

Meristem excision and culture:

Meristems from sterilized sprouts were excised under laminar flow cabinet by using a zoom binocular microscope. The meristematic dome plus one leaf primordial was separated with scalpel and needle to 0.1-0.2 mm nearly, then cultured in glass tubes (meristem/tube) containing MS medium (Murashige and Skoog, 1962), pH 5.7, supplemented with 30 gL^{-1} sucrose and 9 gL^{-1} agar without any addition of growth regulators. The cultures were incubated at 22°C under a 16h photoperiod with 2000 Lux light intensity for 6-8 weeks. Plantlets derived from meristem were sub-cultured in fresh media and incubated for one month as mentioned before and the rate of success in removing viruses and percentage of surviving plantlets were recorded.

Cultures and micro-propagation:

Shooting stage:

The virus-free plantlets of each cultivar were divided into 6-8 cuttings, then micro-propagated by single node cutting for two subculture times. Each three nodal cuttings were placed in one jar containing modified MS medium, pH 5.7, supplemented with 30 gL^{-1} sucrose, 9 gL^{-1} agar and 2 mgL^{-1} BAP (Benzylaminopurine) and then incubated under the same conditions.

Rooting stage:

For the purpose of root initiation, the solid MS medium was replaced with semi-solid medium supplemented with 30 gL^{-1} sucrose, 5 gL^{-1} agar and 0.5 mgL^{-1} IBA (Indolebutryicacid). The plantlets were cultured by single nodal cutting under the same incubation conditions for 3 weeks. Leaves of plantlets were collected and indexed for viruses' detection using the DAS-ELISA.

II-The second part:

Acclimatization stage and greenhouse study:

Cultured jars from each cultivar were transferred to greenhouse to produce minitubers. The plantlets were removed from jars and washed with tap water, then disinfected by immersion in Benlate Solution (1.0 gL^{-1}) as a fungicide for 5 min.

One plantlet was transferred to plastic pot (8cm D x 7cm H) in each treatment [T_1: vermiculite + sand (4:1 v/v), T_2: peat moss + sand (4:1 v/v), or T_3: peat moss + vermiculite + sand (2:2:1 v/v/v)]. Pots were covered with transparent polyethylene pages for two weeks and gradually they removed before transfer to another plastic pot (15cm D x 13cm H). After one month, the plantlets were transferred in plastic pots (20cm D x 17cm H) until they were harvested, this stage takes about two months and the soluble NPK fertilizers were used.

Measured traits and Statistical analysis:

The studied traits included number of minitubers, minituber weight (g) and minituber diameter (mm). The collected data were statistically analyzed by "MSTATC" computer software package (Freed *et al*., 1989). The obtained data was subjected to the analysis of variance (ANOVA) according to Snedecor and Cochran (1981). Experimental design was a randomized complete block with six replications and two factors: A potato cultivar (Spunta and Lady Rosette) and B ratio of bed components [vermiculite + sand (4:1 v/v), peat moss + sand (4:1 v/v), and Peat moss + vermiculite + sand (2:2:1 v/v/v)]. Means were compared using least significance difference (LSD) test at 5% level of probability.

RESULTS

I. The first part:

1- DAS-ELISA:

Virus detection was relied on the extracted sap from potato tubers by using DAS-ELISA technique. Results of DAS-ELISA test found to be free of PVY or mixed infections while, PLRV infection was detected in 30 potato tubers from Spunta cultivar and 18 infections with PVX from Lady Rosette cultivar.

2- Eradication of potato viruses:

2-1 Effect of thermotherapy on eradication of PLRV and PVX from infected tubers:

Infected potato tubers were subjected for virus eradication by thermotherapy treatments at 36°C, 37°C and 38°C for 3 weeks. Data presented in table (1) showed that the thermal treatment can be considered as a good method to eradicate PLRV infection only. The percentage of PLRV-free tubers were (50%, 70% and 80%) whereas, the percentage of PVX-free tubers were (0%, 16.6% and 33.3%) in case of exposure to 36°C, 37°C and 38°C/3 weeks, respectively. No effect on the survival rate of tubers was detected as a result of the change in the degree of temperature table (1). On the other hand, sprouts (3 cm long) were formed after 35 days nearly of all infected tubers which had been exposed to thermotherapy treatment (38°C) as indicator to successful treatment.

Table 1. Effect of thermotherapy treatment for three weeks on the eradication of PLRV and PVX from two potato cultivars.

Cultivar	Treatment	Total No. of infected tubers	After treatments*			
			Infected tubers	Healthy tubers	Infected tubers %	Healthy tubers %
Spunta cv. infected with PLRV	36°C	10	5	5	50	50
	37°C	10	3	7	30	70
	38°C	10	2	8	20	80
Lady Rosette cv. infected with PVX	36°C	6	6	0	100	0
	37°C	6	5	1	83.3	16.6
	38°C	6	4	2	66.6	33.3

***Data are based on DAS-ELISA detection**

2-2 Effect of thermotherapy treatment (38°C) combined with meristem tip culture in eradication of PLRV and PVX from infected tubers:

Thirty meristems from each cultivar (0.1-0.2 mm) were excised and cultured on MS medium free of growth regulators. After 6 weeks, meristems was germinated to the size of nearly 10mm with formed new leaves (*Fig.1a*). Data presented in Table (2) showed that thermotherapy followed by meristem tip culture (38°C/21 days) had the highest percentages obtained for Spunta cultivar 96.6% and 93.1% of shoot survival and PLRV-free plantlets respectively. Whereas, the shoot survival percentage of Lady Rosette plantlets was 83.3% with 76% being PVX-free.

All virus-free plantlets were transferred to MS medium containing 2 mgL^{-1} BAP and micro-propagated by single node (*Fig.1b,c*) for 6 weeks (2 sub-culture) followed by rooting medium containing 0.5mgL^{-1} IBA under incubation conditions (22°C, 16h photoperiod for 3 weeks) which showed good results and the development of the Spunta plantlets was rapid with long intensive roots during three weeks (*Fig.1d*) than Lady Rosette plantlets (*Fig.1e*).

Table 2. Effect of combination of thermotherapy and meristem tip culture on shoot survival and virus eradication.

Cultivar	Treatment	Total No. of meristems	Total No. of survival shoots	In*	H*	Survival %	Virus Elimination(%)
Spunta cv. /PLRV	38°C/21 days +Meristem tip	30	29	2	27	96.6	93.1
Lady R. cv. /PVX		30	25	6	19	83.3	76

***Data are based on DAS-ELISA detection. In= Infected. H= Healthy**

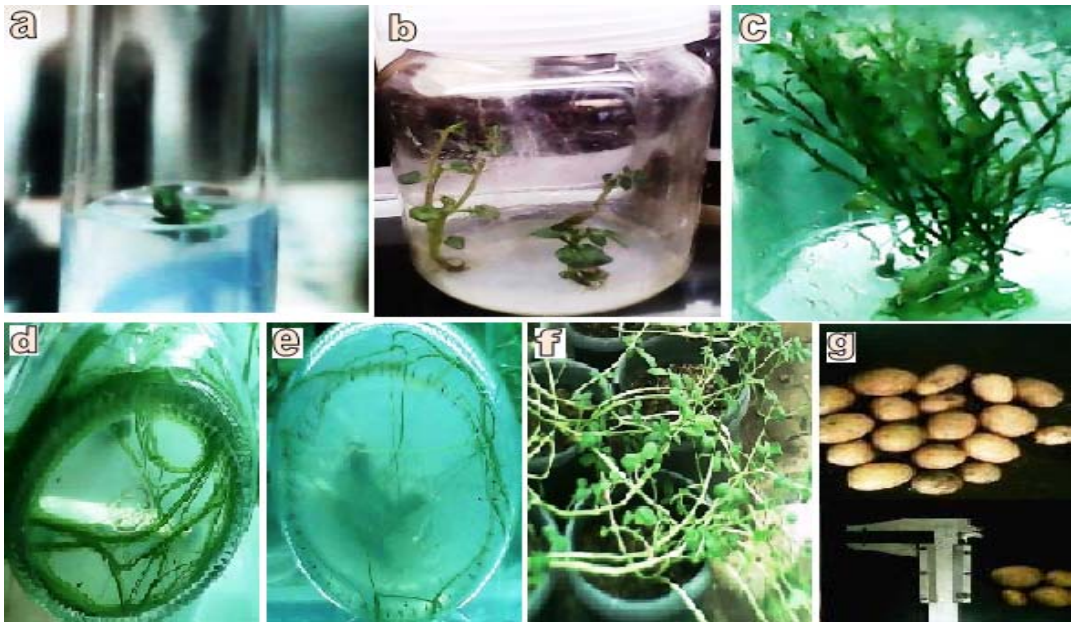

Figure 1. Meristem grown in solid medium **(a)**. Virus-free plantlets after subculture in MS medium **(b)**. *In vitro* shoot proliferation **(c)**. Root development of Spunta and Lady Rosette cultivars respectively **(d,e)**. Potato plants development under greenhouse conditions **(f)**. Minitubers production and measurements **(g)**.

II- The second part: Effect of Soil Mixtures on Minitubers Production:

These processes were established in the greenhouse to produce minitubers, (*Fig.1f.g*). Rooted plantlets (Stock Microplants) of both cultivars (100% virus-free) were transferred from the aseptic culture environment (*in-vitro*) to mixture of vermiculite and sand at the ratio 4:1(T_1), mixture of peat moss and sand at the ratio 4:1(T_2) or mixture of peat moss, vermiculite and sand at the ratio 2:2:1(T_3). The results of harvested minitubers after four months nearly were tabulated as indicated in Table (3).

Analysis of variance and mean comparisons:

Analysis of variance showed that cultivar and ratio of bed components and their interaction had insignificant (P>0.05) effects on minituber number and minituber weight, while ratio of bed components and interaction with cultivars was significantly (P<0.05) affect on minituber diameter (Table 4).

Number of minituber:

Statistical analysis of the data showed that cultivars, ratio of bed components and their interactions did not show any significant change in number of minituber (Table 4). However, among ratio of bed components, maximum number of minituber (2.00) was recorded for Vermiculite + sand (4:1) and minimum (1.75) for Peat moss + vermiculite + sand (2:2:1). Also, the results showed that the maximum number of minituber (2.06) was recorded for Spunta cultivar while, minimum (1.67) were recorded in Lady Rosette cultivar (Table 5).

Minituber weight (g):

Data recorded on minituber weight is presented in (Table 4). Analysis of the data revealed that cultivars, ratio of bed components and their interactions did not show any significant (P>0.05) change in minituber weight (Table 4). Between the cultivars, maximum minituber weight (1.89) and minimum (1.80) was noted for Spunta and Lady Rosette respectively. Among ratio of bed components, maximum minituber weight (1.98) was recorded for vermiculite + sand (4:1) and minimum (1.74) for peat moss + sand (4:1) (Table 5).

Minituber diameter (mm):

Minituber diameter (mm) data was non-significant due to cultivars, while ratio of bed components and interaction with cultivars was significantly (P<0.05) affect on minituber diameter (Table 4).

Studying means of minituber diameter showed that maximum minituber diameter (13.52 mm) was found in Lady Rosette, followed by Spunta that produced 13.48 (Table 6). Among interaction, maximum minituber diameter (13.64 mm) was recorded for Lady Rosette treated with Vermiculite + sand (4:1), followed by Spunta (13.53 mm) applied with Vermiculite + sand (4:1). Pots received Vermiculite + sand (4:1), produced highest mini-tuber diameter, while minimum was recorded for pots having Peat moss + sand (4:1) (Table 6).

Table 3. Effect of different soil mixtures on minitubers production under greenhouse conditions.

Cultivar	Ratio of bed components	Total minitubers		
		Number	Weight (g)	*Diameter (mm)
Spunta	(T_1)	32	64.09	433.90
	(T_2)	29	41.89	388.70
	(T_3)	29	53.97	390.72
Lady Rosette	(T_1)	27	51.45	366.26
	(T_2)	24	34.36	321.82
	(T_3)	26	41.21	349.98

T_1: vermiculite + sand (4:1); T_2: peat moss + sand (4:1); T_3: Peat moss + vermiculite + sand (2:2:1). 15 plants per each teatment. *Diameters measured by the Vernier caliper.

Table 4. Analysis of variance for effect of cultivars and ratio of bed components on number of minitubers, minituber weight (g) and minituber diameter (mm).

S.O.V.	df	MS		
		Number of minituber	Minituber weight (g)	Minituber diameter (mm)
Replications	5	0.29444ns	0.32511 ns	0.02382 ns
Cultivars (A)	1	1.36111 ns	0.08507 ns	0.01480 ns
Ratio of bed components (B)	2	0.19444 ns	0.17187 ns	0.065800s
A x B	2	0.02778 ns	0.31910 ns	0.021270s
Error	25	0.6011100	0.1932100	0.0097800

Note: s = Significant at 5% and ns = not significant. SOV= Source of variation. df = degrees of freedom. MS= Mean Squares.

Table 5. Mean Comparison of the effect of cultivar and Ratio of bed components on number of minitubers, minituber weight (g) and minituber diameter (mm).

Main effects	Traits		
Cultivars	Number of minituber	Minituber weight (g)	Minituber diameter (mm)
Spunta	2.06 A	1.89 A	13.48 A
Lady Rosette	1.67 A	1.80 A	13.52 A
LSD at 5%	NS	NS	NS
Ratio of bed components			
Vermiculite + sand (4:1)	2.00 A	1.98 A	13.59 A
Peat moss + sand (4:1)	1.83 A	1.74 A	13.45 B
Peat moss + vermiculite + sand (2:2:1)	1.75 A	1.82 A	13.46 B
LSD at 5%	NS	NS	0.083

Means of the same category followed by different letters are significantly different from one to another at P≤0.05 using LSD test.

Table 6. Minituber diameter (mm) of two potato cultivars as affected by different ratio of bed components.

Cultivars	Ratio of bed components			Mean
	Vermiculite + sand (4:1)	Peat moss + sand (4:1)	Peat moss + vermiculite + sand (2:2:1)	
Spunta	13.528 AB	13.422 B	13.488 B	13.479 A
Lady Rosette	13.642 A	13.482 B	13.437 B	13.520 A
Mean	13.585 A	13.452 B	13.463 B	

Means of the same category followed by different letters are significantly different from one another at P≤ 0.05 using LSD test.

DISCUSSION

The management of potato viruses is fundamental to potato production in Egypt and the entire world, and without proper management, disease incidence reach 100% within a few years, and cause serious problems espicially by infected tubers with viruses which have no visible symptoms. The early detection using DAS-ELISA technique directly from the tubers is more rapid and effective because of the high concentration of PLRV or PVX present in the vascular tissue of the heel end or the rose end in addition, this technique is a current standard method in the most of the world`s potato seed certification schemes. (Gugerli and Gehriger, 1980; Holland and Jones, 2005).

In the present study, thermotherapy treatment has been used singly or in combined with meristem tip technique to free potato tubers of PLRV or PVX. Thermotherapy treatment alone succeded to eradicate PLRV with higher percentage than PVX. Thermotherapy treatment was more successful at 38°C/3weeks as evidenced by the high percentage of eradicating PLRV (80%) and the survival percentage of tubers (100%). This is in agreement with Stace-Smith and Mellor (1968) who found that PVX is not readily eradicated by thermotherapy alone. Similarly, Rozendall (1952) who failed to eradicate PVX and succeeded in eradicate PLRV from infected tubers at 38°C for 3 weeks. However, our results differed from results of Roland (1952) using thermal treatment at 37°C for 25 days and failed to eradicate PLRV in spite of, PLRV the only virus that can be eradicated from potato tubers by thermal treatment (Fernow *et al*., 1962; IPC, 1977).

Effectively eradicated during the present study may be due to alterations in viral particles as a result of thermal treatment, such as breaking of virus RNA or inactivation of enzymes necessary for viral protein synthesis and consequently prevent virus replication (Panattoni, *et al*., 2013).

Meristem tip excision (0.1-0.2mm) from thermo-treated Spunta or Lady Rosette tubers in the present study led to great success in eradication of PLRV or PVX. Our results are in agreement with Stace-Smith and Mellor (1968) and MacDonald (1973) who reported that most stable potato viruses (PVX and PVS) could be eradicated by taken meristem tip from thermal-treated mother plants, but PVS was more difficult to eradicate than PVX. Similarly, Maureen, *et al*. (2014) reported that meristem tips of size (1mm) and thermotherapy at 38°C were efficiently used in the eradication of cassava brown streak virus (CBSV) from cassava plants. Generally, thermotherapy treatment followed by meristem culture has successfully used for eradication of many viruses in potato (Stace-Smith and Mellor, 1970; Pennazio and Redolfi, 1973).

Effectively eradicated in current study, may be due to the effect of thermal treatment which make the union of the protein subunits that protect the nucleic acid of the virus become weaker (Idrees *et al*., 2010) and the fact that concentration of virus in apical meristems of infected plants may be very low or non-existent due to lack of vascular system subsequently led to slow movement of virus particles from cell to cell and with the presence of high concentration of auxin within the small meristem (0.1-0.2mm) prevented the virus replication (Faccioli, 2001).

On the other side, the high percentages of survival rate of the tubers or *in vitro* plantlets at thermal treatment alone or followed by meristem tip excision from thermo-treated tubers respectively, are not a surprise and in agreement with Baker

and Phillips (1962) and Berg and Bustamante (1974), they reported that using high temperature 40ºC or even 43ºC could be inhibited viruses without damaging the host plant. This is completely different from results of Biniam and Tadesse (2008), they reported that plantlets survival rate decreased from 100 to 90% at the end of the first week, then to 55% after 2 weeks, then to 0% at the end of third week when applied heat treatment 37°C only to *in vitro* plantlets to eradicate PVX.

The *in vitro* micropropagation allow supplying plant material continuously in a fast way, identical to the mother plants in genotype and the successfully *in vitro* micropropagation or multiplication are based on the formation of shoots and roots induced on the explant in order to obtain virus-free plantlets (*in vitro*) and minitubers in greenhouse later, which were achieved in the present study by using 2 mgL^{-1} BAP and 0.5 mgL^{-1} IBA with different response towards *in vitro* shoot and root development that may be due to genetic makeup of different potato cultivars (Ghaffoor *et al.*, 2003 and Abdel Aleem *et al*., 2009). Similar observations have been reported by Modarres and Jami (2003) and Taveira *et al*. (2009), they indicated that, using 2 mgL^{-1} BAP was more effective to produce shoot materials while, 0.5 mgL^{-1} IBA was more effective for root development especially for Spunta cultivar (Miassar *et al*., 2011) as evidenced by the intensive roots.

In the present study, the production of potato minitubers was confirmed in all different soil mixtures. The use of semi-solid medium (*in vitro*) was facilitated removing the traces of adhering agar and avoids roots damage. There are many factors that affect the transferred plantlets under greenhouse conditions, among them soil components are of considerable importance (Jami *et al*., 2001). In this study, except for minituber diameter, no significant differences were found between cultivars Spunta and Lady Rosette. Our results in agreement with Jami *et al*. (2001) who noted that, no significant difference was recorded between potato cultivars when the number and weight of minitubers. Considering the results with soil components, it was noted that mixture of vermiculite and sand (4:1) producing maximum diameter (13.53 mm and 13.64 mm) of Spunta and Lady Rosette respectively in all attempts of the study compared with those from peat moss and sand (4:1) or peat moss, vermiculite and sand (2:2:1) that mean, high response of Spunta and Lady Rosette cultivars to soil that containing vermiculite. This is in contrast to the results obtained by Jami *et al*. (2001), who introduced peat moss as the best mixture but in agreement with Obradovic and Sukha (1993), who showed that planting bed with 80% of vermiculite and 20% of sand was found to be best for plantlet growth that resulted in the production of many minitubers in the greenhouse. In addition, Ahloowia (1994) reported that the micro-propagated plantlets produced minitubers range between 9-15 mm in greenhouse and 5-25 mm diameter in the field. So, our results are nearly appropriate with these findings and such variation in the results may be to genetic diversity among potato cultivars and their response to the nature of soil components.

CONCLUSION

The combination between thermotherapy treatment and meristem culture technique have increased the efficiency of virus-free plant production. The relationship between *in vitro* plantlets that can easily produced throughout the year, and soil components may allow increasing minitubers production from direct transplanting of *in vitro* virus-free

plantlets which becomes a source of pre-basic potato seed amongst the potato growers of Egypt, thereby limiting the entry pests or diseases as well as covering the requirements of local consumption, manufacturing and exportation.

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