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Research Paper

Micropropagation of four potato cultivars *in vitro*

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ABSTRACT

In vitro micropropagation is an alternative to conventional (vegetative) propagation of potatoes whereas aseptically meristem cultures were used which gave pathogen free plants. However, three concentrations of disinfectant bleach (Clorox) 15, 20, 25% with two exposure time 15 and 20 min were used for disinfecting the isolated potato sprouts from four potato genotypes named Lady Rosetta, Jaerla, Cara and Hermis. It was found that, as simplest disinfection protocol, concentration 20% Clorox was the suitable one at 20 min of exposure time giving high percentages of survived individuals with low percentage of dead and contaminated individuals. The sterilized sprouts were cut to isolate apical meristems which were then cultured on shoot induction medium containing solidified MS medium with vitamins and free of exogenous plant growth regulators and incubated in a growth chamber at optimized culture conditions in room culture. The initiated shootlets from the aseptic meristem cultures were cut to nodal cuttings which were culture on the previous MS medium for mass propagation of potato plantlets *in vitro*. The results cleared that MS medium with vitamins and solidified by agar without exogenous plant growth regulators can be used for mass propagation of free-pathogen true to type of potato genotype *in vitro* under the optimized culture conditions.

Key words: Plant tissue culture, true to type, free-pathogen plants, meristem culture.

INTRODUCTION

In vitro micropropagation is an alternative to conventional propagation of potatoes (Chandra and Birhman, 1994) that generally occurred vegetatively by tubers and segments of tubers and rarely by seeds. *In vitro* propagation methods using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones since de-differentiation and the subsequent organogenesis/ embryogenesis with the accompanying genetic changes have been reported (Wang and Hu, 1982). Meristem culture *in vitro* provides a reproducible and economically viable method for producing pathogen free plants than the conventional propagation which lead to virus transmission to new generation. As meristem tips are free from viruses, elimination and generation of virus free plants are possible through *in vitro* meristem culture (Jha and Ghosh, 2005). However, there were many reported media formula which were used for *in vitro* propagation of potato (Badoni and Chauhan, 2010; Molla et al., 2011; Motallebi-Azar et al., 2011; Koleva et al., 2012; Qureshi et al., 2014; Khadiga et al., 2015).

The aim of the work is the presentation of easy protocol for *in vitro* induction of potato plantlets stocks free of pathogens which will be used for selection under abiotic

Figure 1. Isolated apical meristem at left and developed shootlets at right initiated on shoot induction medium.

stress.

MATERIALS AND METHODS

This work was conducted in Plant Biotechnology Department, National Research Centre, 12622, Dokki, Giza, Cairo, Egypt and incorporation with Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University, Nasr City, Cairo, Egypt.

Plant material and treatments

Meristems were taken from uniform growing sprouts (~ 1 cm long) established from four potato cultivars (tubers) named Lady Rosetta (T_1), Jaerla (T_2) , Cara (T_3) and Hermis (T_4) and were brought from Egyptian Ministry of Agriculture. The sprouts were cut from sterilized sprouting potato tubers which firstly washed under running tap water for about one hrs with (2-3 liquid soap drops) for about 30 min. The washed tubers were sterilized under aseptic condition in laminar air flow hood by soaking them in 15, 20 and 25% Clorox for 15 and 20 min then rinsed with sterile distilled water five times. However, many reports will be discussed in this respect (Zobayed et al., 2001; Yasmin et al., 2003; Khadiga et al., 2009; Badoni and Chauhan, 2010; Koleva et al., 2012). The sterilized sprouts were cut to isolate apical meristems which were then cultured on shoot induction medium (Figure 1) containing 4.4 g/l of MS medium with vitamins and free of plant growth regulators (Murashige and Skoog, 1962, catalog no, 0222. Duchefa com, Harrlem the Netherland) and 30 g/l sugar with 8 g/l agar for solidification were added to the medium. The medium pH was 5.8 before autoclaving at 121°C and 1-5 kg/cm for 25 min. The Cultures were incubated in a growth chamber at 25±2°C under a photoperiod of 16/8 h (light/dark cycles) giving irradiance of 45 µmol $m^{-2}S^{-1}$ using Philips white fluorescent lamps.

Experimental design and statistical analysis

Analyses averages and standard errors of the recorded data were performed using the online Statistics Calculator (http://www.numberempire.com/statistics calculator.php). Completely randomized experimental design was performed.

RESULTS

Tuber sprouts sterilization

The success of *in vitro* cultures depends on getting sterilized starting plant material. In this respect, all tested sprouts were exposed to 15, 20 and 25% Clorox with two exposure time (15 and 20 min). Data in Table 1 showed that, the low concentration of Clorox (15%) showed 80-90% and 70-80% of contamination at 15 and 20 min of exposure time respectively with all potato types. However, 15% Clorox showed 10-20% and 20-30% of disinfected individuals at 15 and 20 min of exposure time respectively. There are no dead individuals at the 15% of Clorox. On the other hand, concentration of Clorox (20%) at 15 min showed 30-40% and 10-20% of contamination at 15 and 20 min of exposure time respectively with all potato types. However, 20% Clorox showed the high disinfected individuals 50-60% and 60-80% at 15 and 20 min of exposure time respectively. There were 0-10% and 10-20%

Table 1. Effect of various Clorox concentrations and two exposure times on disinfection of potato sprouts isolated from four potato cultivars.

Time	Disinfected survival %				Death %				Contamination %			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
						15% Clorox						
15 min	20	10	20	20	0	0	0	0	80	90	80	80
20 min	30	20	30	20	0	0	0	0	70	80	70	80
						20% Clorox						
15 min	60	50	60	50	0	10	0	10	40	30	40	30
20 min	80	70	80	60	10	10	10	20	10	20	10	20
						25% Clorox						
15 min	30	20	30	10	70	80	70	90	0	0	0	0
20 min	40	10	40	10	60	90	60	90	0	0	0	0

T_1 = Leady Rosetta; T_2 = Jaerla; T_3 = Cara and T_4 = Hermes. Three replicates each had 10 explants per treatment.

Table 2. the effect of MS media on development of micro-propagated nodal cuttings taken from four potato genotypes.

Type	Mean of shootlet length (cm)	Mean no. of leaf/shootlet	Rooting	Mean of fresh mass of plantlet (g)
T_1	9.03 ± 0.044502	9 ± 0.203419	+++	1.9 ± 0.042885
T_2	3.1 ± 0.043681	2 ± 0.143839	++	0.322 ± 0.002366
T_3	12.3 ± 0.021972	4 ± 0.239732	+++	0.93 ± 0.009954
T_4	8.3 ± 0.037139	3 ± 0.143839	++	0.569 ± 0.006628

+++ = Many roots with vigorous growth. ++ = Few roots with weak growth.

of dead individuals at 15 and 20 min of exposure time respectively on 20% Clorox. Whereas, 30-40% and 10-20% of tested explants showed contaminations at 15 and 20 min of exposure time respectively. Moreover, with all potato types at 25% Clorox, there was no contamination at 15 or 20 min of exposure time. Twenty five percent (25%) Clorox showed 10-30% and 10-40% of the disinfected individuals at 15 min and 20 min of exposure time respectively. There were 70-90% and 60-90% of dead individuals at the 15 and 20 min of exposure time respectively at 25% Clorox.

In vitro propagation

Data in Table 2 and Figure 2 showed the response and development of the nodal cuttings of 4 potato genotypes to *in vitro* mass micropropagation on MS media with vitamins without any exogenous plant growth regulators. It was clear that, after 21 days of culture, the growth parameters such as shootlet length, numbers of leaves per shootlet, the root phenotype and growth and the fresh matter of the plantlets showed various responses to the culture medium under the same culture conditions. However, T_1 plants and T_3 were best growing types followed by T_4 and the less type in response was T_2. However, T_3 showed the highest shootlet length giving average of 12.3 cm followed by T_1 which gave average 9.03 cm whereas T_4 gave average of 8.3 cm while T_3 gave the lowest average of 3.1 cm. Data cleared that averages of leaf numbers per shootlet were highest in T_1 followed by T_3, T_4 and T_2 as 9, 4, 3 and 2 leaves per shootlet respectively. On the other hand, T_1 and T_3 gave numerous roots with vigorous growth whereas T_2 and T_4 gave few roots with weak growth. Moreover, the average masses of fresh matter of the initiated plantlets were the highest for T_1 gave 1.9 g followed by T_3 gave 0.93 g then T_4 and finally the less fresh matter showed in T_2 and gave 0.322 g.

DISCUSSION

Many sophisticated treatments for surfaces sterilization could be used according to the used part and kind of explants. The presented treatments of surface sterilization of potato explants were the simplest treatments of surface sterilization comparing to that used by other researchers. In this respect, Zobayed et al. (2001) used 10% (v/v) sodium hypochlorite solution for sprout sterilization. On the other hand, Yasmin et al. (2003) reported that for surface sterilization, isolated sprouts of potato were first sterilized with 70% (v/v) ethanol for few seconds. The sprouts were then rinsed twice with sterile distilled water then immersing in 0.1% $HgCl_2$ solution for 2 min then washed several times with sterile distilled water. On the

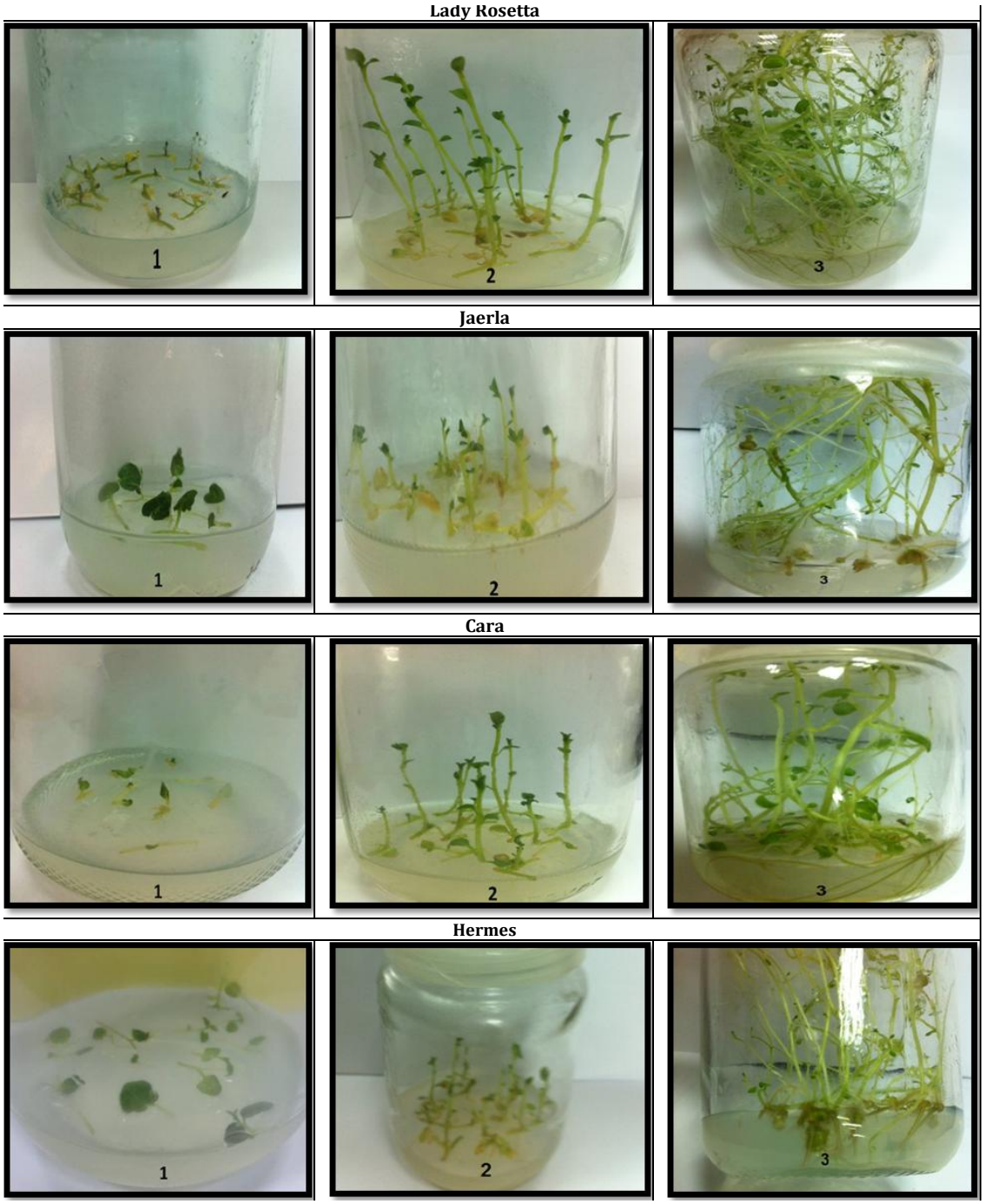

Figure 2. Shootlets initiated and developed from nodal cuttings of four potato genotypes, (1) is nodal segments, (2) developed shootlets 7 days and (3) elongated plantlets 21 days.

other hand, Khadiga et al. (2009) used surface sterilization of potato tubers by washing under running tap water and laundry bleach for 20 min. Then the tubers were sprayed with 70% alcohol and cleaned with a clean towel before transfer to a laminar flow. Under a laminar flow tubers were cut into pieces and surface sterilized by immersing in 70% alcohol for 1 min, washed three times with sterilized distilled water to remove the trace of alcohol then immersed in 25% (v/v) sodium hypochlorite solution supplemented with 2 drops of liquid soap for 20 min and finally rinsed five times with sterilized distilled water. However, Badoni and Chauhan (2010) used two different chemicals for sprout sterilization. Mercuric chloride (0.1%) and sodium hypochlorite (1%) were used with duration of 2, 5 and 8 min. Moreover, Koleva et al. (2012) reported that the sprouts were surface sterilized by washing under flow

of tab water for 10-15 min. After washing, the sprouts are surface sterilized by dipping in 70% alcohol for 2 min, followed by 0.1 $HgCl_2$ solution for 3-5 min, then were washed several times with sterilized distilled water. However, the data in Table 1 cleared that, as simplest disinfection protocol, concentration 20% Clorox was the suitable one at 20 min of exposure time giving high percentages of survived individuals with low percentage of dead and contaminated individuals.

Potato plant is one crop seriously affected by viruses and microbes infections whereas it is vegetatively propagated, however, to avoid such infections, *in vitro* micropropagation is an alternative to conventional propagation of potatoes (Chandra and Birhman, 1994 and Jha and Ghosh, 2005), where it done using meristem cultures under aseptic conditions which is followed by nodal cultures that conserve the genetic background of used plants *in vitro* (Wang and Hu, 1982). In this respect, the presented work agreed with Badoni and Chauhan (2010) who reported that potato sprouts were cultured on MS medium without hormones to get complete plantlets and that agreed with the presented work. However, Molla et al. (2011) reported that *in vitro* regeneration of potato is easily done from different explants on MS medium supplemented with different auxin and cytokinin for diseases free good quality seeds and pathogen free planting materials. They also reported that among the BAP, TDZ and ZR, ZR showed the very good performance in respect of direct regeneration from potato explants. On the other hand, Motallebi –Azar et al. (2011) used nodes that were cut into pieces of 0.3 – 0.5 cm, containing one axillary bud in each explant and were cultured on MS media containing three concentrations of NH_4NO_3 (800, 1900 and 2400 mgl^{-1}) and three concentrations of hydrolyzed casein (0.0, 100 and 200 $mg.l^{-1}$), 3% sucrose, 0.8% agar and supplemented with two concentrations of BAP (0.0 and 2 mg.l-1) for growth of lateral shoots. However, Koleva et al. (2012) used MS medium, supplemented with different hormonal combinations. For sprouts, MS + 4 mg/l KIN and MS + 2 mg/l BAP were used as an initial explants, and for development of nodal explants, MS + 4mg/l KIN + 1mg/l IAA and MS + 2 mg/l BAP+1 mg/l NAA were used. Moreover, Qureshi et al. (2014) used (MS) medium containing 1.0 mgl^{-1}Ca-pentothenate, 0.25 mgl^{-1} Gibberellic acid (GA3), 100 mgl^{-1} Myoinsitol and 30 gl^{-1} sucrose at pH 5.7 was used in this for culturing nodal cuttings of potato cultivar 'Desiree'. On the other hand, Khadiga et al. (2015) used plantlets which were cut to 1.0-2 cm long segments, each with about two nodes (2 axillary buds), incubated on Murashige and Skoog MS medium17. MS media with 6 and 8% sucrose without hormone or supplemented with thiadizuron (TDZ) and benzylaminopurine (BAP) each alone at two concentration (5.0 and 8.0 mg/l) and the two sucrose concentration. It is cleared that from the previous reports that shoot proliferation in potato *in vitro* is influenced by many factors including medium contents, culture conditions, endogenous and exogenous plant growth regulators and plant genotype.

Conclusion

It was concluded that MS medium with vitamins and solidified by agar without exogenous plant growth regulators can be used for mass propagation due to the type of potato genotype *in vitro* under the optimized culture conditions and conserving money which is consumed for the purchase of the exogenous plant growth regulators.

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