In vitro osmotic potential for PVY elimination and tuberization of potato by tissue culture technique

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ABSTRACT

Potential osmotic of MS medium is a new method for *Potato virus Y* (PVY) elimination using tissue culture technique. Osmotherapy refers to culture meristem – tip and stem nodes at MS medium saturated with sucrose. Stem nodes of potato were successfully stored for up to 12 month at osmo-preservation medium supplemented with sucrose 50, 60 and 70 gl $^{-1}$ at 21^{0}C. The survival percentage was recorded 85.1, 81.2 and 50.8 for 50, 60 and70 gl $^{-1}$ respectively for 12 months at 21^{0}C. All survival plantlets revealed viability and resumed growth on fresh medium within three weeks. PVY was eliminated from infected potato plants using osmotic potential (Osmotherapy) applied 50 , 60 and 70 gl $^{-1}$ sucrose at 21^{0}C with 73 , 89 and 92 % respectively . In addition to production of microubers *in vitro* under osmotic potential 8.8 (50 gl^{-1}); 8.2 (60 gl^{-1}) and 6.2 (70 gl $^{-1}$) number per 5 plantlets at 17^{0}C . All PVY results were confirmed with DAS – ELISA.

Key words: Osmotic potential; Osmotherapy; Osmopreservation ; potato ; PVY ; DAS- ELISA and Tissue culture .

INTRODUCTION

Plant germplasm is being conserved mainly in the greenhouse or field gene bank which requires much labor money and land. In addition this germplasm is vulnerable to pathogen attack, pests and environmental disasters. Tissue culture systems are aseptic and easily kept free from fungi, bacteria, viruses, parasites and insects (Van Den Houw etal 1995). An important use of tissue culture is micropropagation of meristems and shoot nodes so as to obtained virus free plants applying heat therapy, chemotherapy and osmotherapy as well as subsequent storage of such plants as virus free stocks by plant breeders (Sherin Afifi , 2009 ; EmanYounis , 2008 and El-dougdoug *et al* 2013)

The expense, both in labor and financial terms of maintaining large field collection is a further

factor contributing the use of *in vitro* collection . Also in an ideal tissue culture storage system , genetic erosion is reduced to zero (Dodds , 1991) .

Several approaches can be used to achieve germplasm conservation via incubation at reduced temperature, low light intensity and modification of culture medium by (increasing sugar concentration, decreasing the supply of inorganic nutrients or adding growth retardants to culture medium). Minimal growth storage is a very simple technique that allows storage of plants *in vitro* for period ranging from 6 months to 5 years depending on species. These stored plants can be recultured rapidly through micropropagation when desired (Perez- Tornero, *et al*., 1999).

This work aims to apply osmotic potential for preservation of shoot nodes as well as production of potato plantlet and microtuber virus – free .

MATERIALAND METHOD

Twenty potato tubers cv. Diamond were obtained from naturally infected plants showing severe mosaic, necrosis and dull leaf surface. (Virology greenhouse Fac. Agric.Ain Shams Univ.) El-Dougdoug , (2013) . Healthy potato plantlets as well as PVY inefectd ones were used for preservation explants, production of plantlets , microtubers and PVY elimination under osmotic potential by tissue culture technique. DAS – ELISA assay was applied for detection PVY infected plants and plantlets as described by Clark and Adams (1977) using specific PVY polyclonal antibody (ELISA kit , Sanofi , Sante , Animals , Paris , France) . Potato tubers were washed under running tap water. Breaking dormancy was done by soaking the dormant tubers for one hour in 100 mg/l GA3 and stored in dark for 10-15 days at 25± 1ºC until sprouting.Sprouts of potato tubers were carefully removed then washed in running tap water for several times. Under aseptic conditions, sprouts were soaked in 20% chlorox (5,25% sodium hypochlorite) containing few drops of tween 20 (0.1%) for 20 min.then rinsed 4 times for 5 min. with sterile distilled water. Isolated parts were cultured under aseptic condition onto MS medium supplemented with (0.1 mg/L) thiamine-Hcl, (0.5 mg/L) Nicotinic acid, (0.5 mg/L) Pyridoxin-Hcl, (0.5 mg/L) Glycine and 7 gm/L Bacto-Difco agar. After 21 days, the explants were aseptically cut into single nodal piecesand transferred to liquid MS medium supplemented with (1.0 mg/L) IAA and (1.5 mg/L) Kinetin, 30 gm/L Sucrose, the plantlets were multiplied invitro as nodal cuttings in jars up to 5 subcultures intervals 21 days under 25 °C and 2000 lux for 16 h light days (Edriss *et al.*,1996).

The micropropagatd plantlets (10 plantlets / Jar) were transferred to MS medium containing 30, 50, 60, and 70mg/L sucrose concentrations individually. The cultures were incubated under 21 °C and 2000 lux for 16 h light . Each 10 cultures jars were preserved up to 3, 6, 9 and 12 months. After each preservation period, the plantlets were transferred and subculture to fresh MS medium. The percentage of plantlet survival and PVY - free plantlets were calculated.

potato nodal cuttings from each treatment were transferred on MS liquid medium- free hormone. The cultures were incubated at 21 °C / 16h daylight. After 3 wks, the residual of liquid medium was drawn and replaced by 50 ml/ jar of the tuberogenic liquid MS medium supplement with 0.4 gl^{-1}ca-panthothenate, 0.1 cumarin and 4 gl^{-1} casein under osmotic potential 30, 50, 60 and 70 gl^{-1} sucrose individually. The cultures were incubated at 17 °C / 8h light days. Ten culture jars (10 plantlets / Jar) of each osmotic potential (50, 60 and 70 gl^{-1} sucrose) were incubated at 17±1^{0}C for 10 weeks under 8/16hr as photoperiod/Dark. After incubation period microtubers were harvested, then no. of tubers and weight were determined for each 50, 60 and 70 gl^{-1} sucrose concentration.

RESULTS

Forty out of fifty PVY infected plants with 80% infection were detected in tested potato plants cv. Diamond using polyclonal antibodies by DAS ELISA. These tested plants were used to produce PVY – free plantlets and microtuber.

The stem nodes , with size 2-3 cm of PVY infected and healthy ones were developed to plantlets on MS medium without hormones for establishment stage under convenient conditions . Ten PVY – infected stem nodes were transferred to MS medium containing 30, 50, 60, 70gl^{-1} sucrose individually and incubated at 21 ^{0}C for 3,6,9,12 months under convenient conditions for explants osmopreservation . After 3 and 6 months storage periods , the effect of sugar concentrations were recorded decreasing in percentage of explant survival preservation with 50 , 60 gl^{-1} and 70 gl^{-1} sucrose at 21^{0}C (Table1) . On the other hand after 9 and 12 months preservation, it was found variation in explant survival among sucrose concentration at 21^{0}C, Table 1) .

The study aimed to determine the effect of three different sucrose concentrations capable for PVY elimination and produce microtubers from infected potato explants (subculture 4) cultured on multi placation MS medium. This medium was incorporated individually 50 , 60 and 70 gl^{-1} incubated at 30 days under 15 , 17 and 21 ^{0}C for 16 / 8 hrs light / dark .

The survival percentage was recorded 85.1, 81.2 and 50.8 for 50, 60 and70 gl^{-1} respectively for 12 months at 21^{0}C. In the osmotic potential 50 gl^{-1} gave low PVY – free explants compared 60 gl^{-1} sucrose at 21 ^{0}C. On the contrary 70 gl-1 produced the highest PVY –free percentage and a deleterious effect on the regeneration and growth of the propagated shoots. The potato microtubers were produced from plantlets on MS medium incorporation with different conc. of sucrose (50, 60 and 70 gl^{-1} .It was found that 50 , 60 gl^{-1} sucrose gave increasing in number , weight and size of microtubers at 17^{0}C. On the other hand, 70 gl^{-1} sucrose led to decreasing in number and weight microtubers compared with 50 and 60 gl^{-1} sucrose (Table 2) .

Table (1): The survival percentage and preservation period of stem node potato cv diamond during *in vitro* storage at different sucrose concentrations

Sucrose conc. gl^{-1}	Incubation period (month) at 21				average
	3	6	9	12	
30*	100	100	100	95.2	94.00
50	100	100	95.5	85.1	57.05
60	95.5	100	90	81.2	89.93
70	95.5	75.5	75.5	50.8	74.28

***** Control.

Table (2): Microtubers producing from PVY- free potato plantlets growing under osmotic potential on MS medium .

Sucrose conc. gl^{-1}	Microtuber characters			
	% Survival	% PVY elimination	Microtuber no.	Microtuber wt. (g)
50	100	73	8.8a	0.9a
60	100	89	8.2b	0.7b
70	92.5	92	6.2c	0.6c
LSD at 5%	-	-	0.5	0.2

- Calculating per 5 potato plantlets .

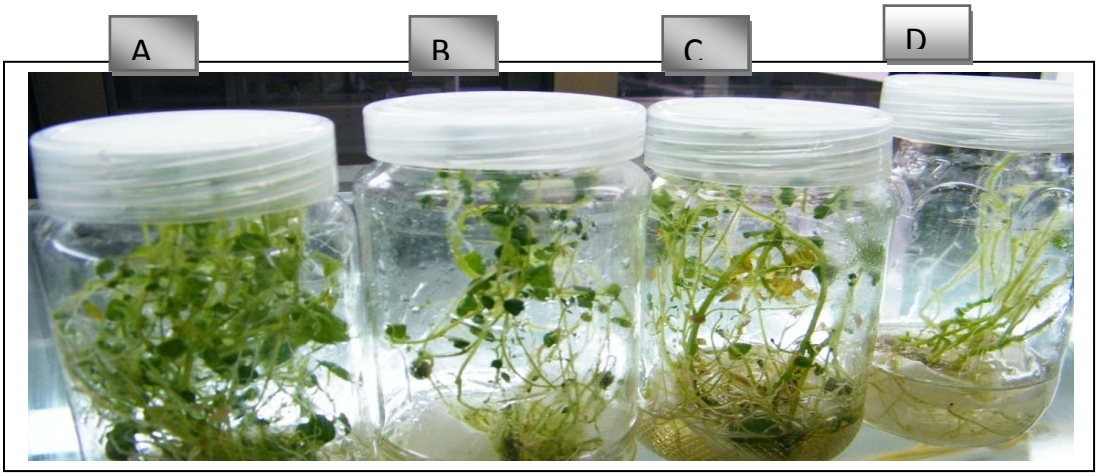

Fig 1: Micropropagatd PVY- free potato plantlets growing under osmotic potential on MS medium at 25^0C. (A) at 30 gl^{-1} (B) at 50 gl^{-1} sucrose , (C) at 60 gl^{-1} sucrose and (D) at 70 gl^{-1} sucrose.

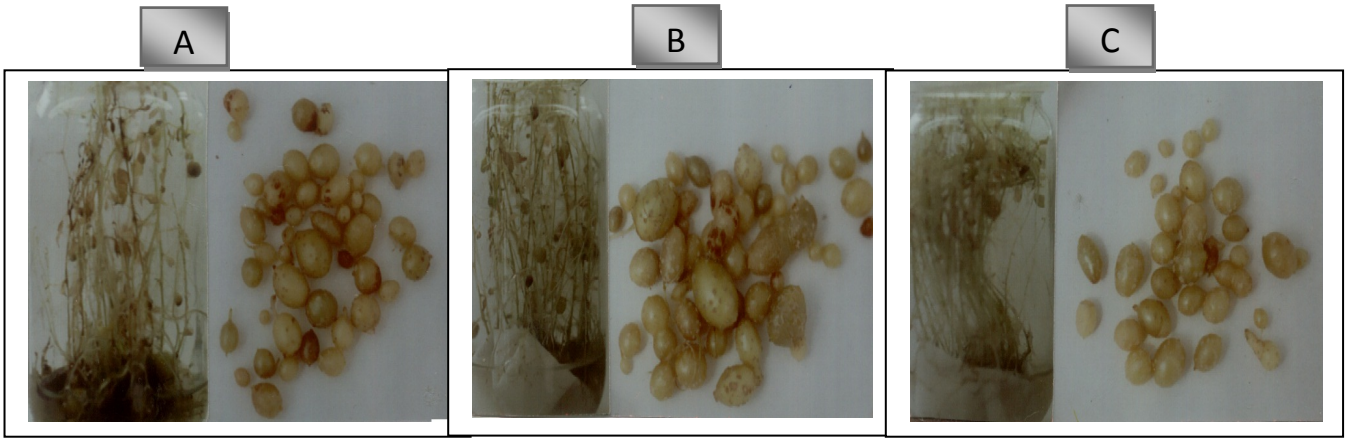

Fig 2: Microtubers produced from PVY- free potato plantlets growing under osmotic potential on MS medium at 17^0C. (A) 50 gl^{-1} sucrose , (B) 60 gl^{-1} sucrose and (C) 70 gl^{-1}sucrose.

DISCUSSION

Infected potato tuber samples cv diamond were obtained from virology greenhouse , Fac . Agric . Ain Shams Univ. It was confirmed that PVY was detected in unclarified extracts of infected tubers using DAS-ELISA, The highly sensitive detection for many viruses (Clark and Adams , 1977 and Sherin , 2009) .

The growth expression potential during development of living organisms has been affected by a number of environmental stresses, either affected internally (viruses) or externally (medium composition or culture incubation condition results into growth limitations . During this study 4 cultures were maintained for 4 weeks , viz ., (a) plant micropropagation culture of healthy plantlets ; (b) micropropagation culture of PVY infected plantlets ; (c) multiplication of healthy potato culture under sucrose stressed condition and (d) micropropagation culture of PVY infected plantlets under sucrose stressed conditions . Differential bio-matrics in the micropropagated plantlets were observed among these stressed culture . With the decrease in micropropagation efficiency, plant night was also observed to be reduced . (Ikram. Ul – HAQ *et al.*, 2012).

The osmotic potential (sucrose) and PVY infection have been adversely affecting quantitative as well as quantitative characteristics of the multiplying potato plantlets. The growing plantlets are getting certain amendment in their interval constituents in according to the applied osmotic potential (Haq. e*t al*., 2011 and 2012). The developed complex phenomena may be adopted by the plantlets in future.

Early response of plantlets against applied osmotic potential could usually be helpful to enhance their tolerance against the applied osmotic potential .

Their accumulation of certain metabolites like as praline involved in the prevention of stress injury among the tissues. It is an indicator for cell' injury as well as acts as osmoprotactent . Their over – productions can decrease the rate of injury due to sucrose stress, enzyme inhibiting factors and pathogen toxins. They are developing stability for ongoing metabolism within the cells during different stages of the cell' cycles .

The first concern of the present study was production of PVY – free potato plantlets from infected tubers through applied osmotic potential (osmotherapy) The second without subculturing for longer period storage (osmopreservation) . The third concern production the microtubers *in vitro* under controlled conditions. Generally , it could be concluded the stem nodes plantlets potato cv. Diamond were successfully eliminated PVY and preserved for 12 months on MS medium supplemented with 60 or 70 gl^{-1} sucrose at 21^0C incubation period . The stored stem nodes resumed growth and started the regeneration during 21 days after transferring on proliferation MS medium at 21 ± 2^0C. These results suggest that, potato plants may survive in osmopreservation at intermediate temperatures (17 to 21 ^{0}C).

Alternatively growth media may be altered to slow growth. The microtubers produced on MS medium incorporation with 50, 60 and 70 gl^{-1} sucrose at 17. Rooted epically dominate plantlets of banana were maintained for 12 months without subculture when 60 gl^{-1} sucrose

in growth medium (Eman Younis, 2006) .As well as production of *banana bunchy* top virus – free banana plantlets on MS medium incorporation with 70 gl^{-1} sucrose at 21^0C (El-Dougdoug *et al.*, 2013). Potato microtuber production on MS medium supplemented with 80 gl^{-1} sucrose (Sherin Mahfoze, 2009). In general the carbohydrates play a prominent part in the nutrition and structure of the plant. The carbohydrates could have been caused by effects on water potential or metabolism uptake differences (El Habashy 2002). Temperatures range 15 and 21^0C in preservstion chambers are 21 days frequently utilized to reduce the growth rate (Van Den Houw*et.al*, 1995). The correlation between sucrose and protein accumulation could be exploited to improve further studies on minimal growth storage and cryopreservation of oil palm embryonic culture (Tarmizi*e .al* , 1993) .

The increase of the sucrose levels from 50 to 70 gl^{-1} on MS medium results in a lowered survival rate of potato shoot tips cultures at 21^0C and increased continuous PVY elimination . These results suggested that ,the high sucrose levels cause hyper – methylation of DNA , possibly as on adaptive response to conserve cellular resource during osmotic stress . The growth rate of explants decreased when the sucrose concentration revealed 60 % . The moisture content was reduced in all sucrose concentration(Uargomi,1991).The dry materials which accumulated in banana plants during *in vitro* growth appears to be linked to the quantity of sucrose concentration used during this stage between 70 to 80 mg / l (Murchal and Filliot , 1992) . The proposed modes of sucrose results in a slow reduction in moisture content. Histological studies or pre – cultured banana meristem revealed the synthesis or accumulation of sugar – like of sugar – like compound inside the cytoplasm after pre- culture.

This is confirmed by the sugar analysis. At 0.6 M sucrose only 17% of the inoculated bud display growth. At 0.75 M, no buds growth and all become brown . It is assumed that, the osmotic check reaction of living tissues to stress as well as reduce growth by osmotic stress . These results suggested that , high sucrose levels can there for be used to maintain cultures in a dormant conditions of long period , this appears to be an osmotic effect (Tarmizi , et.al , 1993 ; Harding , 1994 ; Panis , et.al ,1996 ; Hassan , 2002 and EmanYounis 2006) .

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