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USING TISSUE CULTURE TECHNIQUE IN MICROPROPAGATION OF SWEET POTATO (*ipomoea batatas)*

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ABSTRACT

The current study was conducted at tissue culture laboratory of Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the period from 2010 – 2012. A protocol is described for rapid and large-scale propagation of the sweet potato (*Ipomoea batatas)* by *in vitro* culture of shoot tips or nodal segments as explants. The best sterilization method for the explants was observed by using Hg Cl_2 at 0.1 % for 14minutes and70%ethanol for 30 seconds. Nodal segments were found to be more efficient than shoot tips for sweet potato shoots regeneration on MS medium (Murashig and skoog, 1962) supplemented with 2ip at 4 mg/l + 0.5 mg/l GA_3. Adding activated charcoal (AC) to the culture medium at 2 mg/l was the most effective treatment to avoid of browning phenomenon occurrence (0.00%). Among the five different cytokinins Thidiazuron (TDZ), kinetin (kin), 6-benzyl aminopurine (BAP), - N6-(2-isopentenyl) adenine 2ip and phloroglycenol (PG) and four concentrations of each, TDZ at 1.00 mg/l gave the best results for shoots multiplication. Concerning the rooting stage, Indole-3-acetic acid (IAA) at 2.0 mg/l clearly in henced roots development. Mixture of peat moss :vermiculite : perlite (1:1:1 v/v/v) recorded the highest results for survived plantlets in terms of survival percentage,

INTRODUCTION

Sweet potato*(Ipomoea batatas)* is a perennial, herbaceous, dicotyledonous species of the morning glory family Convolvulaceace that is grown as an annual crop in many countries. It is one of the most versatile, and yet under-exploited, crop species in the world, its current annual production is estimated at 133 million tons, mainly growing in 100 developing countries and being placed among the five most important food crops in over 50 of these. Despite its major importance sweet potato has not received significant attention from plant biotechnologists (Midmore et al., 2003).

Sweet potato is considered a highest recalcitrant species in terms of regeneration and transformation responses to *in vitro* treatments (Aloufa,2002). Sweet potato is a good source of starch, mineral salts and vitamins. Its tuberous roots are an important root vegetable and the young leaves and shoots are sometimes eaten as greens. Approximately 50 genera and more than 1,000 species of Convolvulaceae. batatas is the only crop plant of major importance. Some others are used locally, but many are actually poisonous. Sweet potato is traditionally propagated vegetatively by vine cuttings or shoot tip cuttings, but this technique favours the propagation of viral infected material which is less productive than healthy ones. The consequent low productivity discourage the growers to continue the cultivation of such crop. So, the aim of the study was to produce high number of a healthy transplant by the technique of micropropagation.

MATERIALS AND METHODS

Plant material.

Sweet potato plant had been obtained from Agricultural Research Center, Egypt. Young healthy shoots of sweet potato were collected from an adult plant growing in the farm of the agriculture Fuclty, Mansoura University. After removing the leaves ,the shoots were nodal segments with 1 auxiliary buds(about 0.5 -1cm long). The excised explants were thoroughly washed under running tap water containing a few amount of household detergent for one hour (outside the culture cabinet) .Thereafter, they are transferred to inside the culture cabinet since, they were surface sterilized by $HgCl_2$ at 0.1 % for 14minutes+70% ethanol for 30 seconds.

Culture media:

Sterilized glass jars, contained 25 ml of MS culture medium (Murashige and skoog, 1962) supplemented with L-tyrosine (100 mg /l), Myo-inositol (100 mg / l), Adenine-hemi-sulphat (60 mg / l), Sucrose (30 g / l), GA3 (0.5 mg /l) and 7 g Agar /l (w/v).

The pH of the all media was adjusted to 5.8 and all growth regulators were added to the media prior to autoclaved at 121 C for 20 minutes except the GA_3 who was added to autoclaved media through 0.45 µm sterile filter.

Culture condition:

In vitro Culture, all experimental treatments were represented by 10 cultured jars (16 light/ 8 dark) photoperiod produced by white fluorescent light lamp with light intensity (2500 lux).

Explants type :

To study the effect of the two explants type (shoot tips and nodal segments)on the production of multiple shoots, the surface sterilized shoot tips and nodal segments were cultured on Murashig and Skoog medium (MS) supplemented with 2ip at 0.00, 1and 2 mg /l combined with GA_3 at 0.5 mg /l . After 30 days of culture, data were recorded for shoots proliferation. A factorial experiment in a complete randomized design was used with 4 replicates for each treatment**.**

Multiplicat of shoot cultures:

An experiment was carried out to determine the optimum type and concentration of cytokinin in interaction with GA3 at 0.5 mg /l . The cytokinin type used were

1-Thidiazuron (TDZ) at (0.025, 0.05, 0.1 and 1.0 mg /l) .
2-Kinetin (Kin) at (1, 2, 4 and 8 mg /l).
3-6-benzyl aminopurine BAB at (1, 2, 4 and 8 mg /l).
4- N6-(2-isopentenyl) adenine 2iP at (1, 2, 4 and 8 mg /l).
5- phloroglycenol PG at (25,50,100 and 200 mg /l).

Multiplication rate characteristics were recorded after 6 weeks from culturing date as number of shoots, leaves and roots and length of shoots and percentage of callus.

Induction of rooting and acclimatization:

For root induction, the regenerated shoots (2.5 – 3 cm long) obtained from the previous proliferation stage were individually cultured on

full or half strength of MS basal medium supplemented with different auxin types, i.e., a naphthalene acetic acid (NAA), indol-3 butyric acid (IBA) and indol-3-acetic acid (IAA) at different concentrations (0.0, 0.5, 1.0 and 2.0 mg/l) in addition to the other additives previously mentioned under culture media item. The *in vitro* rooted plants were taken out from the medium, washed under tap water to remove all traces of media and then individual plants were transferred to plastic pots containing peat moss :vermiculite : perlite (1:1:1 v/v/v). A factorial experiment in a randomized complete block design was used with 3 replicates for each treatment.

RESULTS AND DISCUSSION

Data in Table (1) showed that adding activated charcoal (AC) at 2 mg/l on the culture media significantly inhibited occurs of browning percentage of (0.00 %) when compeared of the other antioxidant treatments, followed by the same antioxidant concentration of 1.00 mg/l, as it was (11.1%) .Using AC at 2 mg/l significantly give the highest value in all characters. Data on this Table indicated that the highest Percentage of callus information with medium supplemented with (150 mg/l Vitamin c , Citric acid 100 mg/l, 150 mg/l Vitamin c + Citric acid 100 mg/l and control) on other hand, the lowest information of callus appeared with AC at (1 and 2 g /l) in addition to A.C improve growth compared to all treatment .

These obtained results are in agreement with the finding of Pan and Van Staden, (1998) who concluded that the AC is often used in plant tissue culture to improve cell growth and development. The addition of Ac to both liquid and semi-solid media is a recognized practice and its influence on growth and development may be attributed mainly to the adsorption of inhibitory substances in the culture medium and this agreement with (Fridborg et al., 1978; Horner et al., 1977; Theander and Nelson,1988; Weatherhead et al., 1978, 1979), drastic decrease in the phenolic oxidation or brown exudate accumulation (Carlberg et al.,1983; Liu, 1993; Teixeria et al., 1994), alteration of medium pH to an optimum level for morphogenesis (Owen et al., 1991) and establishment of a darkened environment in medium and hence simulate soil conditions (Dumas and Monteuuis, 1995). Even though the effect of AC on plant growth regulator (PGR) uptake is still unclear, some workers believe that AC may gradually release certain adsorbed products, such as nutrients and PGRs in addition to the release of substances naturally present in AC which promote plant growth(Johansson and Eriksson, 1977; Johansson et al., 1990). The growth inhibitory chemicals, like 5-hydroxymethyl- furfural produced during autoclaving from sucrose by dehydration, will be removed by AC (Pan and van Staden, 1998).The most crucial impact of adding AC to the culture media is a drastic dip in concentration of PGRs and other organic supplements . This is due to the adsorption of these chemicals by AC.

Table(1): Effect of antioxidants treatments to avoid of browning phenomenon occurrence

Treatment	Browning %	Shoot number	Leaves number	Shoot Length (cm)	Roots number	Root length (cm)	%0F Callus occurrence
Control 0.00	100 %	0.00	0.00	0.00	0.00	0.00	100
Activated charcoal (AC) 1.0 mg/l	11.1 %	0.67	1.70	3.00	1.57	0.82	0.00
Activated charcoal (AC) 2.0 mg/l	0.00 %	1.00	2.77	3.43	2.10	0.83	0.00
Vitamin C 150 mg/l	100 %	0.00	0.00	0.00	0.00	0.00	100
Citric acid 100 mg/l	100 %	0.00	0.00	0.00	0.00	0.00	100
Vitamin C+ Citric acid 150 +100 mg/l	100 %	0.00	0.00	0.00	0.00	0.00	100
L.S.D at 5%	14.29	0.43	0.51	0.30	0.23	0.21	*

Multiplication stage :

Effect of explant type and 2ip at different concentration on the development of sweet potato explants:

Concerning the effect of explants type, the obtained results in Table (2) indicated that culturing nodal segments of sweet potato on MS basal medium at full strength supplemented with 2ip at different concentration (0.00,2.00and 4.00 mg/l)+ 0.5 GA3 recorded the highest values of all studied characters (i.e., shoots number per explants, shoot length and leaves number /shoot)as compared to shoot tips.

Concerning the effect of 2ip concentration, it was observed that increasing 2ip concentration from 0.00 mg/l up to 4.00 mg/l increased the values of shoots number for both explants types. However, the greatest values of shoots number per explant (1 shoot)were obtained with nodal segments and 2ip at 4.00 mg/l. Also, culturing nodal segments on MS medium supplemented with 2ip at 4.00 mg/l recorded the highest values of leaves number per shoot (5.23 leaves) and shoot length (4.63cm) while, control treatments with nodal segments and shoot tip gave the lowest values for these character (3.80leaves) (3.07)cm shoot length for shoot tip. Hence, it was proven that using nodal segments is more efficient than shoot tips for sweet potato shoots regeneration .This result may be due to the apical dominance in the shoot tip explants which decreases number of the grown auxiliary or adventitious buds. Gong *et.al* (2005*)* reported that the best shoot regeneration was achieved from stem explants cultured on basal MS medium supplemented.

In this respect, Fatma *et.al.* (2005) showed that nodal explant was better for shoot formation (100%), shoot length, number of microtubers (2.16) and microtuber weight (164.50 mg/tuber) while shoot tip explant gave significantly more number of leaves (6.61) and higher root formation (81.37%).Maximum shoot development (100%) was observed from nodal

explants . Also, Alam, et al. (2010) found that the developed shoots were further micropropagated by node cutting.

Table (2): Effect of explants type and 2ip at different concentration on the development of sweet potato:

Explants Type	2ip conc. mg/l	Shoot number	Leaves number	Shoot length (cm)	Roots number	Root length (cm)	% of callus occurrence
Nodal sgment	0.00	0.60	1.33	1.27	1.10	0.37	55.6
	(2.00) 2ip+0.5 GA3	1.00	2.77	3.42	2.10	0.97	100
	(4) 2IP+ 0.5 GA3	1.00	5.23	4.63	1.33	1.07	100
Shoot tip	0.00	0.60	1.33	1.00	1.67	0.43	55.6
	(2.00) 2ip+0.5 GA3	1.00	2.67	2.73	2.00	1.07	100
	(4) 2IP+ 0.5 GA3	1.00	3.80	3.07	2.00	1.07	100
L.S.D At 5%	0.56		0.44	0.77	0.69	0.25	47.84

The effect of interaction between five types of cytokinins at different concentration (i.e., TDZ at 0.025, 0.05, 0.1, and 1.0)+0.5GA3, (Kin, BAP,and2iP at 1.0, 2.0, 4.0 and 8.0 mg/l) +0.5GA3mg/l and (PG at 25, 50, 100 and 200 mg/l)on development of nodal segment of sweet potato was shown in Table (3) .

As for shoot number per explants, results clearly showed that the highest shoots number per explant (1.90shoots) was obtained with TDZ at 1.0 mg/l +GA3 at 0.5 mg/l . Concerning leaves number per shoot ,root number and root length data revealed that GA3 improved these two characters, but it was more effective with 2iP at 8.0 mg/l where the highest values 5.13 leaves per shoot compared control (1.22) , and (3.22) of roots number and (3.33) of root length. On other hand the lowest treatment is BAP at 8.0 mg/l +GA3 at 0.5 mg/l on this two characters . The explants cultured on medium containing 2iP at 1.0 mg/l +0.5 GA3 observed the highest value of shoot length (5.19) while, control is the lowest . Table (7) refers to that there was no significant difference on explants cultured on medium containing 2ip at(8 ,4 and2 mg/l) about leaves number and shoot length .

As regard to shoot length clearly and multiplication it was found that application of GA3 to MS containing cytokinin clearly improved these characters. This finding was supported by Esqulbel (1991) *on ipomoea batatas* since the effect of GA3 was to promote multiple shoot growth and elongation of cv.

The promotive effect of a GA3 on shoot multiplication was recorded by Rey and mroginski (1985) described a 50 % increase in the number of regenerated meristems from *Ipomoea batatas.* According to Ashok kumar et al. (2007), TDZ was the best for adventitious shoot induction with an optimal of cvs and medium supplemented with TDZ induced highest percentage of responding explants and maximum number of adventitious shoots followed by BA and 2IP.

Table (3): Effect of different cytokinin types and concentrations on development of sweet potato nodal segments:

Cytokinin type	Concentration	Shoot number	Leaves number	Shoot length	Roots number	Root length	% of callus occurrence
Control	0.00	0.77	1.22	0.75	1.11	0.78	55.6
TDZ +0.5GA3	0.025	0.90.	2.43	2.17	0.77	0.50	77.8
	0.05	2.00	4.33	3.10	1.89	1.39	88.9
	0.10	1.00	3.33	2.87	0.78	0.33	100
	1.00	`1.10	2.80	2.47	1.88	1.30	100
Kin + 0.5 GA3	1.00	1.00	2.33	2.37	0.89	0.66	0.00
	2.00	1.00	2.67	3.77	0.44	0.16	11.1
	4.00	1.10	3.53	3.27	0.11	0.17	0.00
	8.00	1.00	2.90	2.89	0.66	0.35	0.00
BA + 0.5 GA3	1.00	1.00	2.90	2.89	0.33	0.16	11.1
	2.00	1.10	2.57	3.66	0.66	0.31	11.1
	4.00	1.00	2.57	2.61	0.89	0.55	33.3
	8.00	1.10	3.13	2.55	0.0	0.0	55.6
2ip + 0.5 GA3	1.00	1.43	4.90	5.19	1.29	0.66	100
	2.00	1.20	2.93	3.45	1.88	1.35	100
	4.00	1.10	4.80	4.83	2.55	1.04	100
	8.00	1.00	5.13	4.39	3.22	3.33	88.9
PG + 0.5 GA3	25.00	1.00	2.11	2.99	2.11	2.11	0.00
	50.00	1.03	3.22	2.92	1.55	1.55	0.00
	100	1.00	4.44	3.70	1.89	1.89	0.00
	200	1.00	2.55	2.40	0.78	0.79	0.00
L.S.D at 5%		0.22	1.42	1.oo	1.45	0.93	36.18

A: Effect of media strength on root length .

Data in Table (4) showed that using MS full strength media significantly produced the tallest roots length of (3.88cm) when compared with using half strength media as it was (2.57cm)

B: Effect of auxin type and concentration on roots length .As shown in the same Table adding IAA at 2 mg /l significantly produced the tallest roots length of (12.23cm) when compared with all of the other treatments. On the other hand, using free auxin medium significantly gave the shortest roots length of |(0.62 cm).

C:Effect of the interaction between media strength and auxin type and concentration using IAA at 2 mg /l significantly gave the highest rate of root length (15.9) .On the other side, the control media gave shortest length (0.57) (0.67) for full strength and half strength media respectively .

The auxin type and their concentration have the upper hand in that characteristic than the other treatment (media strength) . Alam et al. (2010) showed that Indole-3-acetic acid (IAA) at a concentration of 2.0 mg /l gave the highest number of root (13.41) and it is the most effective for root development compared to IBA at 2.0 mg/which given (5.95).

Table (4): Effect of auxin type, auxin concentration, medium strength and their interaction on root length (cm) of sweet potato shoots.

Auxin type (B) / Stren.(A) Media	Control	IAA			IBA			NAA			Mean of (A)
		0.5mg/l	1mg/l	2mg/l	0.5mg/l	1mg/l	2mg/l	0.5mg/l	1mg/l	2mg/l	
full strength	0.57	1.53	4.93	15.90	1.07	1.90	2.43	2.53	2.70	5.27	3.88
Half strength	0.67	1.67	3.03	8.57	1.17	3.00	2.30	1.83	1.23	2.27	2.57
Mean of (B)	0.62	1.66	3.98	12.23	1.12	2.45	2.37	2.18	1.97	3.77	
L.S.D at 5%	A: 0.48 B: 0.97 AB: 1.32										

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استخدام تقنية زراعة الأنسجة في الاكثار الدقيق للبطاطا

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أجري هذا البحث بمعمل زراعة الأنسجة بقسم الخضر والزينة- كلية الزراعة- جامعةالمنصورة في الفترة من 2009-2012 بهدف الوصول إلى بروتوكل لإكثار سريع لنبات البطاطا بواسطة تكتيك زراعة الأنسجة باستخدام القمة النامية أو قطع ساقية برعمية صغيرة وكانت أهم النتائج مايلي: استخدام القطع العقدية الساقية كان أكثر كفاءة عن القمة النامية على بيئة موراشيج وسكوج والتي تحتوي على 4مللجرام/لتر من الأيذوبنتينيل ادنين كما ان اضافة الفحم النشط بتركيز 2 جرام / لتركان الاكثر تاثيرا على القضاء على ظاهرة اللون البنى .

كما وجد أن استخدام الثيازورون بتركيز 1 مللجرام/ لتر مع 0.5 مللجرام/لتر حمض الجبريليك كلن الأكثر كفاءة في تضاعف الأفرع بالمقارنة مع الكايتين، البنزيل أمينوبيورين والايذوبنتينيل ادنين والفلوروجليسرون مع حمض الجبريليك.

وأن أفضل تعقيم للجزء النباتي المستخدم كان باستعمال كلوريد الزئبقيق بتركيز 0,1 مللجرام/ لتر لمدة 14 دقيقة مع 70% ايثانول لمدة 30 ثانية. أيضا خلصت النتائج إلى أن استحدام الاندول أسيتك أسيد قد أعطى أفضل تطور للجذور على الأفرع الخضرية وأن خليط البيت+فيرموكوليت+ البرليت (1:1:1بالحجم) كان الوسط الأكثر ملاءمة لعملية الأقلمة قبل النقل إلى البيئة الخارجية.

قام بتحكيم البحث

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