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OPTIMIZATION OF GROWTH CONDITIONS DURING SWEETPOTATO MICRO-PROPAGATION

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ABSTRACT

To establish an efficient technique for sweetpotato (Ipomea batatas L) cv. Abees, an orange fleshed Egyptian cultivar, nodal segments excised from virus free plant material grown in an in vitro culture. Single node cuttings were cultured onto MS (Murashige and Skoog, 1962) established medium supplemented with 0.5 mg/l of IAA (3-Indoleacetic acid), NAA (1-Naphtalene acetic acid) or GA_3 (Gibberellic acid) for one week. After that, nodal segment explants were transferred to MS medium supplemented with 20 or 60 g/l sucrose combined with or without 0.1 mg/l BA (6-Benzyladenine)

The obtained shoots were rooted in hormonal-free MS-medium supplemented with 20 g/l sucrose. The optimum multiplication rate was obtained with segments formerly cultured on 0.5 mg/l GA_3 and thereafter in 60 g/l sucrose. Each nodal segment gave rise to an average of one shoot with 8 nodes.

The survival rate after acclimatization reached 95% and their growth was normal and healthy. Culturing of single-bud explant could produce 2 to 8 million plants following 9 subcultures. This was calculated on the basis of 20% losses of the obtained shoots in each subculture.

RESUME

Pour établir une technique efficiente pour la micropropagation de la patate douce (Ipomea batatas) L., un cultivar égyptien a peau orange, des cultures de nœuds provenant d'un matériel végétal exempt de virus cultive in vitro ont été utilise. Des boutures avec un seul nœud ont été cultive sur un milieu MS (Murashige and Skoog) contenant 0.5 mg/l de IAA (3-Indoleacetic acid), NAA (1-Naphtalene Acetic acid) ou GA_3 (Gibberellic acid) durant une semaine. Les explants ont été transfère a un milieu MS supplémente par 20 ou 60 g/l de sucrose combine avec ou sans 0.1 mg/l BA (Benzyladenine).

Pour leur enracinement, les pousses obtenues ont été places dans un

milieu MS exempt d'hormones supplémenté avec 20 g/l de sucrose. Le taux de multiplication le plus élevé a été observe avec les segments cultive dans du 0.5 mg/l GA_3 et par la suite dans du 60 g/l sucrose. Chaque segment a donne en moyenne 1 pousses, chaque une avec 8 nœuds.

Le taux de survie après l' acclimatation a été de 95%. Approximativement, on a pu produire 2 a 8 million de plantes, a partir de la culture d'explant a un seul bourgeon durant 9 subcultures, même si on a 20% de perte de pousses dans chaque subculture.

INTRODUCTION

Sweetpotato is considered the seventh most important food crop in the world and is ranked fourth in developing countries (FAO, 1997). It is cultivated in more than 100 countries (Horton DE, 1987) as a valuable source of human food, animal feed and industrial raw material (Jarret and Florkowski, 1990). However, pests, diseases and environmental factors prevent the crop from reaching its maximum agricultural potential. Virus diseases have been attributed as the main cause of low yield productivity (Wambugu, 1991) and the major cause of cultivar decline (Carey et al., 1999; Gibson et al., 1998). In sweetpotato, several studies indicated that sweetpotato clorotic stunt virus (spcsv) and sweetpotato feathery mottle virus (spfmv) drastically reduced sweetpotato yields; losses may often reach 65 to 90 % (Karyeija et al., 1998).

Development of new method and transfer technology for producing pathogen-

free clonal seed can overcome this constraint and help to unlock the significant yield potential of this crop. Production of pathogen-free material is the first step of controlling the viral diseases in vegetatively propagated crops. It allows a significant increase in field yield of fresh storage root. Zhang (1995) showed an average yield increase of around 40% in plots using virus-free roots. Plant tissue culture is uniquely suited for obtaining and maintaining mass propagation of specific pathogen-free plants. The provision of a steady supply of indexed planting materials through *in vitro* culture appears economically and technologically feasible. However, the chemical composition of the culture medium is perhaps the most studied aspect of sweetpotato tissue culture; it plays an important role in success of micropropagation. A sub-optimal culture medium may cause physiological disorders or death of tissue (Nas and Read, 2000 and Preece, 1995). Numerous studies aiming to optimize the culture medium constituents have been conducted (George, 1993). The aim of the work was to assess the effect of IAA, NAA, GA_3 on the response of nodal explants during the establishment stage (one week) as well as the effect of BA and sucrose on the incidence growth rate during the multiplication stage (6 weeks) for sweetpotato Abees cultivar.

MATERIALS AND METHODS

Plant material

Virus-free sweetpotato plant material Abees cultivar were used as the explant source .Abees cultivar was micropropagated *in vitro* using the nodal cutting as described by Roca *et al*. (1978) and were routinely sub-cultured every 6 weeks on a fresh MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose. The cultures were maintained at 28 ± 2 ºC under 300 foot-candle for 16 h lights per day.

Culture media

Stem segments containing one axillary bud (c. 2.0 cm) were obtained from 6 weeks-old culture. Stem segments were cultured into glass jars 250 ml containing 15 ml of MS medium. The basal MS medium was supplemented with 3% sucrose, 0.5 mg/l of Indole-3-acetic acid (IAA), Naphtalene acetic acid (NAA) or Gibberellic acid (GA_3). The pH was adjusted to 5.7 and solidified with 0.6% Bacto agar. The cultures were kept under 28 ± 2 ºC and 300 foot-candle for 16 h light per day, for one week. At the end of one week the explant appearance was visually evaluated. After this period, stem segments were transferred onto glass jars 500 ml containing 30 ml of propagation MS medium supplemented with 20 or 60 g/l sucrose combined with or without 0.1 mg/l Benzyladenine (BA) and incubated for 5 weeks under the same previous conditions.

The obtained shootlets were rooted in MS-medium free hormones supplemented with 30 g/l sucrose. Each treatment consisted of 10 glass jars, each jar contained three cuttings. After six weeks growth analysis, expressed as the number of leaves, number of shoots, length of shoots, number of roots, length of roots as well as fresh and dry weights for shoots and roots, were recorded. The experiment was repeated two times.

Total Chlorophylls and total Carotenoides determinations

The total chlorophyll and total carotinoides pigments were extracted by using N.N.Dimethyl formamide as previously described by Nornai (1982). Total chlorophylls and total carotinoides were carried out triplicate and values were expressed as means and standard error was statistically analyzed using the Standard Error analysis.

Data of each treatment was statically evaluated using the analysis of variance as outlined by Gomez and Gomez (1984) based on MSTATC program. The differences between means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

In all studied treatments (Table 1, 2 and 3), growth characters of plants cultured on 6% sucrose in a combination with growth regulators or without were higher than those cultured on 2% sucrose. This improvement in growth was noted for all growth parameters measured, i.e. plant height, number of leaves and roots, fresh and dry weight for shoots and roots. This was also acompanied by an increase in total chloeophylls and carotenoides (Table 4).

These results are in agreement with Faria *et al*. (2004) Who reported that the presence of 6% sucrose was the most efficient treatment for increasing plant height and fresh weight of Dendrobium plantlets propagated *in vitro*.

A reduction in sucrose concentration has been shown to effectively inhibit growth of carnation and tomato microplants *in vitro* (Schnapp and Preece, 1986). In fact, the importance of sucrose for both establishment and multiplication stages of micropropagation has been pointed out by several authors (Jusaitis, 1997; Marino *et al*., 1993; Sansberro *et al*., 2000).

In this study it was found that increasing plant height, number of leaves, number of roots, total fresh and dry weight and total chlorophylls and carotenoides of sweetpotato internodes under high sucrose concentration considerably allow to manipulate explants more easier for subculture and transfer during *in vitro* multiplication cycles as well as it greatly facilitated the handling and the efficiency during transfer of sweetpotato plants from *in vitro* culture to *in vivo* soil conditions.

Contrarily, Yong-*et al*., (2000) found that no need to maintain high sucrose concentration in the culture medium under proper culture conditions (increased number of air exchanges, CO_2 supply, positive difference in photoperiod and dark period temperatures (DIF) and high photosynthetic photon flux (PPF), showing greater plantlet growth on sucrose-free medium. There are other reports (Myster and Moe, 1995) that confirmed the distinctive effect of increased number of air exchanges in culture vessels on growth of *in vitro* strawberry and *Brassica Campestris* plantlets (Kozai and Sekimoto, 1988; Kozai 1991 and Fujiwara and Kozai, 1995).

Table (1) indicated that presence of BA in the culture medium reduced plant height and number of leaves as compared with BA-free medium while number of roots was not affected by absence or presence of BA. Also BA caused a reduction in total fresh and dry weight of whole plant (Table 2 and 3) this was accompanied by a negative effect of BA on total chlorophylls and carotenoides concentrations (Table 4).

In this work, the results indicated that BA is inappropriate for sweetpotato micropropagation, yet this contradicted with the results reported by Edison *et al*., (2002), who demonstrated the usefulness of BA for micropropagation of *Dioscorea* species. Similar to previous findings Mroginsky *et al*. (1999), Sansberro *et al*. (2000) found that different concentrations of BA induced rapid development of *I. Paraguariensis* plantlets. These contradictory results could be related to the effect of plant species, genotype, type of explants or culture conditions in different laboratories.

In all studied treatments, GA in the presence of sucrose or BA or both induced significant higher values of plant height,

number of leaves, number of roots and total fresh and dry weight for the whole plant comparing with both tested auxins or control (Table 1-3). However, its influence on total chlorophylls and carotenoides were negative (Table 4). Similar results were obtained with Moacir *et al*., (2004) who reported that presence of GA induced excessive elongation during micropropagation of fig.

Concerning IAA and NAA, data could not detect any positive influence of NAA and IAA on all studied growth parameters. The lack of significant response to the IAA treatment has been previously been attributed to its chemical lability (Gamburg, 1988). IAA has been shown to degrade in the presence of light (Dunlap and Robacker., 1998). On the other hand, David *et al*., 1996) mentioned that after only one week of NAA treatment, more than 90% of NAA was conjugate

The present study clearly proved that the presence of sucrose at high concentration and GA are environmental constraints leading to the enhancment of photosynthesis, or precisely, to the growth and development of micropropagated sweetpotato plantlets. Under micro-environmental controled conditions, *in vitro* sweetpotato plantlets were expected to have a high percent of survival when transferred to the greenhouse or to the open field, which is often expected to be stressful due to a high PPF or low relative humidity.

In summary, sweetpotato (*Ipomea batatas* L) cv. Abees plantlets could be micropropagated by single-node cutting under photoautotrophic conditions. The growth and photosynthesis of *in vitro* plantlets increased with the increasing of sucrose concentration in the presence of gibbrillic acid.

Further studies on the optimal conditions for an effective micropropagation system of sweetpotato need to be carried out.

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Table (1): Effect of BA on plant height, number of leaves/plant and number of roots/plant during two plant stages of sweet potato internode explants cultured for one week on MS medium containing 0.5 mg/l IAA, NAA or GA3 as well as recultured for five weeks on 20 or 60 g/l sucrose.

Plant age (weeks)	2				6			
BA (mg/L)	0.0		0.1		0.0		0.1	
Sucrose (g/L)	20	60	20	60	20	60	20	60
Hormone treatment				Plant height				
Control	1.30^{D}	2.0^{CD}	1.15^{D}	1.3^{D}	2.5^{CD}	5.0^{C}	2.35^{D}	1.0^{D}
IAA	1.35^{D}	3.9^{B}	1.09^{D}	1.2^{D}	2.51^{CD}	9.60^{B}	2.0^{D}	1.75^{D}
NAA	1.50^{CD}	2.25^{CD}	2.00^{CD}	1.00^{D}	2.8^{CD}	7.40^{B}	1.30^{D}	0.70^{D}
GA_3	2.10^{CD}	5.80^{A}	1.20^{D}	2.70^{C}	3.35^{CD}	9.1^{B}	2.35^{D}	12.0^{A}
				Number of leaves				
Control	3.5^{CDEF}	5.00^{CDE}	3.00^{DEF}	2.90^{DEF}	5.7^{DEFG}	9.7^{CD}	4.1^{EFG}	1.2^{G}
IAA	3.8^{CDEF}	7.4^{B}	4.8^{EFG}	3.60^{EFG}	6.2^{DEF}	16.0^{AB}	4.8^{EFG}	3.60^{EFG}
NAA	3.1^{DEF}	5.4^{BCD}	2.6^{EF}	2.3^{F}	4.8^{EFG}	12.4^{BC}	2.2^{FG}	1.3^{G}
GA_3	4.1^{CDEF}	12.2^{A}	2.1^{F}	5.7^{BC}	7.3^{DE}	19.3^{A}	4.4^{EFG}	11.5^{C}
				Number of roots				
Control	2.0^{DE}	1.4^{E}	3.6^{BCD}	2.1^{DE}	1.9^{EF}	1.8^{EF}	3.0^{CDEF}	1.4^{F}
IAA	3.5^{BCD}	2.6^{CDE}	3.7^{BCD}	3.4^{BCD}	3.9^{C}	3.5^{CDE}	4.3^{C}	3.7^{CD}
NAA	2.2^{DE}	2.2^{DE}	3.1^{BCD}	2.1^{DE}	2.0^{DEF}	2.2^{DEF}	1.9^{E}	2.1^{DEF}
GA_3	6.1^{A}	4.4^{B}	3.5^{BCD}	4.1^{BC}	6.6^{AB}	8.1^{A}	4.3^{C}	5.9^{B}

Means in each group of hormones treatments, followed by the same letters are not significantly different at p=0.05 of Duncan's multiple range tests.

Table (2): Effect of BA on shoot, root and whole plant fresh weights (g/5 plants) of sweet potato internode explants cultured for one week on MS medium containing 0.5 mg/l IAA, NAA or GA_3 as well as recultured for five weeks on 20 or 60 g/l sucrose.

BA (mg/l)	0.0		0.1	
Sucrose (g/l)	20	60	20	60
Hormone treatment				
		Shoot		
Control	2.15^{DE}	3.8^{CD}	0.88^{E}	1.70^{E}
IAA	1.55^{E}	6.51^{AB}	1.07^{E}	0.04^{E}
NAA	1.53^{E}	5.51^{BC}	0.39^{E}	0.11^{E}
GA_3	1.65^{E}	7.5^{A}	0.85^{E}	4.75^{BC}
		Root		
Control	0.65^{D}	2.10^{CD}	0.70^{D}	0.10^{D}
IAA	0.67^{D}	5.95^{B}	0.90^{D}	0.60^{D}
NAA	0.20^{D}	3.45^{C}	0.20^{D}	0.35^{D}
GA_3	1.10^{D}	9.00^{A}	1.25^{D}	5.5^{B}
		Whole plant		
Control	2.8^{D}	5.9^{C}	1.58^{D}	1.8^{D}
IAA	2.22^{D}	12.1^{B}	1.97^{D}	1.00^{DE}
NAA	1.73^{D}	8.96^{B}	0.59^{E}	0.46^{E}
GA_3	2.75^{D}	16.5^{A}	2.1^{D}	10.25^{B}

Means in each group of hormones treatments, followed by the same letters are not significantly different at p=0.05 of Duncan's multiple range tests.

Table (3): Effect of BA on shoot, root and whole plant dry weights (g/5 plants) of sweet potato internode explants cultured for one week on MS medium containing 0.5 mg/l IAA, NAA or GA_3 as well as recultured for five weeks on 20 or 60 g/l sucrose.

BA (mg/l)	0.0		0.1	
Sucrose (g/l)	20	60	20	60
Hormone treatment				
		Shoot		
Control	0.08^{D}	0.36^{C}	0.06^{D}	0.02^{D}
IAA	0.12^{D}	0.69^{B}	0.06^{D}	0.10^{D}
NAA	0.11^{D}	0.51^{BC}	0.04^{D}	0.02^{D}
GA_3	0.10^{D}	0.85^{A}	0.08^{D}	0.55^{B}
		Root		
Control	0.05^{C}	0.24^{C}	0.07^{C}	0.02^{C}
IAA	0.19^{C}	0.55^{BC}	0.07^{C}	0.10^{C}
NAA	0.02^{C}	0.38^{BC}	0.02^{C}	0.02^{C}
GA_3	0.10^{C}	0.95^{AB}	0.05^{C}	1.35^{A}
		Whole plant		
Control	0.13^{C}	0.06^{D}	0.13^{C}	0.04^{D}
IAA	0.31^{C}	1.24^{B}	0.13^{C}	0.20^{C}
NAA	0.13^{C}	0.89^{BC}	0.06^{D}	0.04^{D}
GA_3	0.2^{C}	1.8^{A}	0.13^{C}	1.9^{A}

Means in each group of hormones treatments, followed by the same letters are not significantly different at p=0.05 of Duncan's multiple range tests.

Table (4): Effect of BA on total chlorophyll and total carotenoids (mg/g f.wt.) concentrations in sweet potato leaves of internode explants cultured for one week on MS medium containing 0.5 mg/l IAA, NAA or GA_3 as well as recultured for five weeks on 20 or 60 g/l sucrose.

BA (mg/l)	0.0		0.1	
Sucrose (g/l)	20	60	20	60
Hormone treatment				
		Total cholorophyll		
Control	0.32 ± 0.06	1.03 ± 0.28	0.46 ±0.08	1.21 ± 0.3
IAA	0.18 ± 0.04	0.74 ± 0.2	0.13 ± 0.01	0.23 ± 0.04
NAA	1.23 ± 0.03	0.64 ± 0.13	0.94 ± 0.04	0.63 ± 0.21
GA_3	0.64 ± 0.09	0.76 ± 0.15	0.85 ± 0.14	0.68 ± 0.27
		Total carotinoides		
Control	0.11 ± 0.03	0.41 ± 0.14	0.16 ± 0.04	0.62 ± 0.16
IAA	0.08 ± 0.02	0.30 ± 0.08	0.04 ± 0.01	0.08 ± 0.02
NAA	0.46 ± 0.1	0.25 ± 0.05	0.30 ±0.06	0.18 ± 0.03
GA_3	0.24 ± 0.04	0.28 ± 0.07	0.32 ± 0.08	0.67 ± 0.34

Values are the means ± SE of three samples

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