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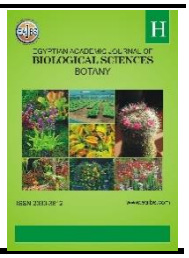

Initiation of *Ipomoea batatas* Through Tissue Culture Technique

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ABSTRACT

Ipomoea batatas is an ornamental foliage plant and important for landscaping and interior due to its attractive foliage, easiness to grow and tolerance to low relative humidity and low light conditions. It is a member of the *Convulvulaceae* family. The goal of this research is to establish a method for initiation of I. batatas plants using single nodal cuttings taken from soft cuttings maintained on full strength MS medium, 30g/l sucrose, 3g/l gelrite, and various doses of plant growth regulators such as NAA, BA, and their combinations. In general, the present study found that when MS medium was fortified with NAA and BA at 1.00 and 0.00 mg/l, respectively, best results for the initiation stage were obtained.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous plant in the Convolvulaceae family. The delicious tuberous root is long and tapering, with a smooth skin that varies in colour from yellow to orange, red, brown, and purple. It is usually a smallholder crop that is resistant to poor growing climates and is commonly cultivated on marginal soils with minimal inputs (Namanda *et al*., 2011).

However, sweet potato is conventionally propagated vegetatively by various methods, especially by vine cuttings or storage roots. Meanwhile, The high cost of vegetative propagation limits majorities production in several situations. (Cantliffe *et al*., 1987).

Therefore, the technique of tissue culture techniques has been suggested an avenue to economically produced crops normally propagated vegetatively such as sweet potato. For instance, the rapid in vitro propagation of disease-free plant material would be of great potential for plant breeders and might help for hybridization and commercial reproduction of sweet potato hybrids (Chee *et al.,* 1990). Therefore, large-scale micropropagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally-propagated crop market (Brown and Thorpe, 1995). Nevertheless, in tissue culture, the use of plant growth regulators plays a pivotal role in influencing different plant processes comprising mostly of growth, differentiation and development e.g., culture establishment, shoot initiation, multiplication and rhizogenesis (Hobbie, 1998). Therefore,

the aim of the present study is to develop a reliable and efficient protocol for the initiation stage of Ipomoea batatas plantlets.

MATERIALS AND METHODS

Experiments on the effect of different concentrations of certain growth regulators and their combinations on the initiation of *Ipomoea batatas* plantlets using nodal segments as explants were carried out between 2018 and 2019 in the Plant Tissue Culture Laboratory of The Faculty of Agriculture Saba Basha, Alexandria University.

Plant Materials:

The explant tissues were collected from healthy mother plants cultivated in the greenhouse of Alexandria University's faculty of agriculture saba-basha. The collected material, were brought to the laboratory for the sterilization process, old leaves were removed and washed after that to be ready for sterilization and tissue culture manipulation, then shoot explants from cuttings were washed thoroughly in the water, using liquid soap for 30 min., then the excised explants were placed under running tap water for 90 minutes then immersed in 70% ethanol for 15 seconds to reduce the harmful effect of ethanol, the explants were rinsed twice with double distilled water after pretreatment with ethanol. Nodal segments of only (1cm) long nodal segment which contained a single node were then surface sterilized with mercuric chloride ($HgCl_2$) at 0.1% (v/v) with a few drops of wetting agent "Tween-20" (surfactant agent) for fifteen minutes and became ready for culturing.

In vitro experimental stage Initiation stage, during this stage explants were established on gelrite (3g/l) solidified Murashige and Skoog medium (1962). The pH of the tested media was adjusted to 5.7 before adding gelrite, and then disinfected autoclaving at 121°C for 20 minutes, before explants were cultured in the given MS medium containing different concentrations of cytokinin (BA at three concentrations: 0.00 (nil), 0.50, and 1.00 mg/l, in combinations with auxin (NAA at four concentrations: 0.00 (nil), 1.00, 2.00, and 4.00 mg/l.

At initiation after 6 weeks in culture, the following characteristics were recorded per propagule: mean number of shoots, mean shoot length (cm), mean number of leaflets, mean number of, mean number of roots.

Experimental design and statistical analysis, all of the tests conducted during this investigation were designed as factorial trials with a completely randomized arrangement (Gomez and Gomez, 1984). The recorded data were statistically evaluated using the analysis of variance technique (ANOVA), and means were compared using Duncan's multiple range tests (Steel *et al*., 1997), with significance set at $p \leq 0.05$.

RESULTS AND DISCUSSION

The collected and analyzed results will be presented and discussed as follows:

Initiation Stage:

Figure (1) and Table (1) showed that different levels of both applied growth regulators and their interactions had a significant influence on the initiation stage features of Ipomoea batatas, except for number of shoots where the interaction only was not significant.

The major effect of NAA and BA on the mean number of shoots per propagule revealed that the presence of both growth regulators in MS medium had no significant influence on the provided characteristic.

In terms of the influence of NAA on the mean number of shoots developed per propagule, the results of NAA showed that NAA at 1.0 mg/l produced the longest shoot length. However, the influence of BA had no significant effect on the provided attribute. The interaction of NAA and BA at 0.0 and 1.0 mg/l resulted in the production of the longest shoot length.

Concerns about the number of leaflets and the number of nodes established per propagule, as well as the effect of NAA, had no effect on the provided characteristic.
The major effect of BA, on the other hand, and the interaction between both growth regulators had a significant effect on the original characteristics. In terms of the major effect of BA, the presence of BA at 0.5 mg/l resulted in the highest mean value.
The highest mean values were obtained from the interaction of NAA and BA at 0.0 and 0.5 mg/l, respectively.

In relation to the number of roots generated per propagule, both growth regulators had a significantly significant influence. The main effect of NAA stated that MS-basal medium with 1.0 mg/l produced the highest mean value. In the instance of the BA major effect, increased basal medium free hormone resulted in the highest mean value of roots generated per propagule. The combinations of both given levels of NAA at 1.0 mg/l and BA at 0.0 mg/l, on the other hand, resulted in the production of the greatest mean number of roots.

Concerning the major effect of NAA, the results tabulated in Table (1) revealed that the presence of the given auxin at 1.0 mg/l had a significant effect on the given trait when compared to the control treatment. This discovery may have been achieved by the method of action of auxin (NAA) within cultured tissues, which may boost and control a variety of unique processes such as cell growth and elongation (George and Sherrington, 1984). Wilkins (1989) also stated that auxin-induced a number of responses, including cell division, cell enlargement, protein and nucleic acid synthesis, all of which are concomitants of auxin-induced growth and changes in plant cell wall plasticity, which increase apical dominance because growth and elongation are essential and rapid processes. The presence of auxins in the culture media, on the other hand, enhanced the mean shoot length of *Trigonella foenum*-graecum (Aasim *et al*., 2010).

This observation may be related to the efficacy of cytokinins in stimulating cell division and morphogenesis (shoot initiation/bud development) in tissue culture, as well as the break of apical dominance and the release of lateral buds (Stern *et al*., 2004; George *et al*., 2008). About the major influence of NAA measured levels on the abovementioned characteristics, NAA levels had a substantial effect on the aforementioned characters. The highest mean values for shoot length, number of leaflets, and number of roots generated per propagule were always obtained when the culture medium was added with 1.00 mg/l NAA.

Fig. 1. Initiation *stage Ipomoea batatas* axillary buds are grown *in vitro* on MS medium augmented with NAA at 1.00 mg/l and BA at 0.0 mg/l over 45 days.

Table 1. Effect of different levels of NAA and BA (mg/l) and their combinations on the initiation stage of *Ipomoea batatas* cultured *in vitro* for 6 weeks.

Characters	BA levels mg/l	NAA levels (mg/l) 0.00	0.50	1.00	1.50	Mean NAA	Significance NAA	BA	NAAXBA
(a) Mean number of shoots formed/propagule:									
	0.00	1.00	1.00	1.00	1.00	1.00	ns	ns	ns
	0.50	1.00	1.00	1.00	1.00	1.00			
	1.00	1.00	1.00	1.00	1.00	1.00			
Mean (NAA)		1.00	1.00	1.00	1.00				
L.S.D. (0.05)							0.05	0.04	0.84
(b) Mean shoot length (cm)/propagule:									
	0.00	2.00	2.00	4.00	2.00	2.50	**	ns	**
	0.50	2.00	2.00	2.50	2.40	2.23			
	1.00	2.50	2.56	2.00	2.30	2.34			
Mean (NAA)		2.17	2.19	2.83	2.23				
L.S.D. (0.05)							0.31	0.27	0.53
(c) Mean number of nodes and leaflets formed/propagule:									
	0.00	2.90	2.90	5.00	2.90	3.43	ns	*	**
	0.50	5.10	3.80	3.90	3.40	4.05			
	1.00	3.50	3.90	2.90	3.80	3.53			
Mean (NAA)		3.83	3.53	3.93	3.37				
L.S.D. (0.05)							0.65	0.56	1.12
(d) Mean number of roots formed/propagule:									
	0.00	5.50	0.00	6.30	2.00	3.45	**	**	**
	0.50	0.00	1.00	5.00	1.00	1.75			
	1.00	0.00	0.00	1.00	0.00	0.25			
Mean (NAA)		1.83	0.33	4.10	1.00				
L.S.D. (0.05)							0.21	0.18	0.36

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.
*, **: Significant or highly significant, ns: not significant

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