A Rapid Micropropagation Protocol for Sweet Potato (*Ipomoea batatas* L.) Via Tissue Culture Technique

Laylan H. Fadaladeen[1], Rafail S. Toma[2], Ahmed A. Saheen[3] and Huda B. Ahmed[4]

[1]Department of Forestry, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region- Iraq.
[2]Department of Horticulture, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region- Iraq.
[3,4]Technicians at College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region- Iraq.
[2]Corresponding author: rshtoma@uod.ac

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Abstract

A rapid and reliable micropropagation protocol was developed for two sweet potato varieties, Mangawy and Mabrokat Al-Shimal, by testing various growth regulators and different carbon sources. Healthy cultures were taken from nodal segment explants taken from potted plants. Adding BA was more effective than Kinetin when used at the same concentration. The highest number of leaves per explant (5.22 leaves/ explant) and the longest shoots (2.88 cm) were achieved when 2 mg L^{-1} BA was added to the culture medium. For Mabrokat Al-Shimal, the highest mean length of shoots (3.77 cm) was obtained when 1 $mg.l^{-1}$ kinetin was used, and the highest leaves number (10.33 leaves/ explant) was obtained when 0.5 $mg.l^{-1}$ kinetin was used. Sucrose was the best carbon source for the multiplication of this Mangawy variety, followed by fructose then glucose. On the other hand, fructose and sucrose showed the best results for the Mabrokat Al-Shimal variety. A 100% rooting was achieved for all tested treatments. IBA was better than NAA Mangawy root formation by giving the best rooting parameters. The highest number of roots per explant (22.33 roots/ explant) was achieved when 1.5 $mg.l^{-1}$ IBA was used. While the highest mean length of roots (12.87 cm) was achieved when added 0.5 $mg.l^{-1}$ NAA. A hundred percent success was achieved at the acclimatization stage for both tested varieties. Both varieties were excellent and tasty, especially from Mabrokat Al-Shimal at open-field cultivation.

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Introduction

Sweet potato (*Ipomoea batatas*) belongs to the Convolvulaceae family. It is one of the most important vegetable crops after potatoes, especially in developing countries (Deng *et al*. 2012). It is considered to be the main food source for many countries in Latin America, South-East Asia, and African people (Dolinski and Olek, 2013) because of its richness with healthy proteins, vitamins, antioxidants, and minerals (Islam 2006 and USDA 2007, and Tumwegamire *et al*. 2011). The global market size of sweet potatoes was 33500 million USD in 2020 (Research Reports, 2021). Sweet potato can be used in cooking as well as in different industrial foods production as a raw material such as in the production of flakes, starch, sweets, and flour in addition to the production of biomass for biofuel (Ferrari *et al*., 2013; Wang *et al*., 2013; Zhang *et al*., 2013). Sweet potato is usually propagated by stem-cuttings, seedlings, and storage roots but these methods face many problems. For example, propagation by stem cuttings and seedlings is associated with bacterial and viral diseases also, propagation by storage roots leads to yield loss (Saiful Islam *et al*. 2002, Ozturk *et al*., 2012, Ndagijimana *et al*., 2014). To overcome these problems, the plant tissue culture

technique could be used whereby a large number of free disease, healthy and uniform plants could be produced. Furthermore, the most suitable explants used for sweet potato micropropagation are apical, axillary, and adventurous buds (Gosukonda *et al.* 1995, González *et al.* 1999, Mukherjee 2002). The research aimed to find a program to propagate two cultivars of sweet potato *in vitro*, test the response of the two cultivars to several kinds and levels of growth regulators and different sources of carbon and test the success of cultivating plants resulting from tissue culture for cultivation in the open field in Duhok Governorate

Materials and Methods

Two Egyptian varieties of storage roots sweet potato (Mangawy or "Nashawy" and Mabrokat Al-Shimal) were planted in pots containing sand and peat moss to be used as a source of explants for tissue culture. Nodal segments of the two varieties were taken separately and washed by running tap water for 40 minutes with the addition of several drops of dishwashing liquid detergent. Next, for disinfestations, the explants were shifted to the laminar air-flow cabinet to continue sterilizing with 70% ethyl alcohol for one minute. They were washed with sterilized distilled water. After that, they were treated with bleach at 2.5 % for fifteen minutes. Then, they were washed three times with sterilized distilled water, each for 3 minutes. After sterilization, the explant ends were cut and cultured on an initiation culture medium composed of plant growth regulators–a free MS medium. After four weeks from the culture initiation stage, the establishment parameters were recorded. The micro shoots were then ready to be cut and transferred to the proliferation medium. At the shoot multiplication stage, two cytokinins, BA and Kinetin at different concentrations including 0. 0.5, 1, 1.5, and 2 mg L^{-1} were tested in addition to three types of sugars including sucrose and fructose and glucose at 15, 30, 45, and 60 $g.l^{-1}$. Each experimental unit consisted of 10 culture vessels each containing three explants. After four weeks of culture on multiplication medium, the shoot multiplication parameters, including the number of shoots per explant, mean length of shoots, and the number of leaves, were recorded. At the later root formation stage, the micro shoots were transferred to the rooting medium. Two kinds of auxins (IBA and NAA) were tested at 0, 0.5, 1, and 1.5 mg L^{-1}. After four weeks, the rooting percentage, number of roots per explain, and the mean length of roots were recorded. Then, the plantlets were taken from the medium, washed from the medium, treated with Beltanol fungicide for 10 minutes, and then transplanted in small pots containing peat moss only under greenhouse conditions. During the first week of acclimatization, the plantlets were sprayed three times with ¼ MS salt strength, then at the second week two times and at the third week for one time. After one month of acclimatization, the seedlings of both varieties were planted in the field at the beginning of March, and the harvesting was done at the beginning of September. The whole experiments were arranged according to CRD, and the comparisons between means were done by the SAS program (SAS, 2010).

Results and Discussion

Overall, both varieties of sweet potato showed a successful performance during different developmental stages of propagation. Table (1) illustrates the effect of two cytokinins (BA and Kinetin) in different levels (0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1}) on the shoot multiplication stage of Mangawy sweet potato explants after four weeks in culture on Murashige and Skoog medium. In general, BA shows a better influence on the multiplication stage of Mangawy sweet potato than Kinetin. All explants gave the same number of shoots/ explant by producing only one shoot. The longest shoots and the highest number of leaves were achieved when 2 $mg.l^{-1}$ BA was added to the culture medium by giving 2.88 cm and 5.22 leaves, respectively. However, the shortest shoots were achieved when 1

and 2 mg.l^{-1} kinetin were used by giving only 0.88 cm, while the lowest number of leaves per explant was achieved when 1 mg.l^{-1} kinetin was used, showing only 1.55 leaves per explant. Cytokinins have a significant effect in the multiplication stage due to their role in breaking the apical and lateral buds dominance (Mohammed and Al-Younis, 1991) also they have essential functions in enzymes, RNA, and protein biosynthesis in the cell of the plant, which also has a role in bud growth promotion (Al-Rifae'e and Al-Shobaki, 2002). BA is stronger than Kinetin and more effective in the multiplication stage of Mangawy sweet potato because of its double bonds on the benzyl ringside and its molecular structure (Mohammed, 1985). Moreover, overcoming BA for cell division and apical dominance is more effective than Kinetin (Bashi, 2006; Fadladeen and Toma, 2020).

Table 1. Effects of Benzyl Adenine and Kinetin on multiplication stage of Mangawy sweet potato explants grown on MS medium after four weeks in culture

Cytokinins (mg.l^{-1})		Number of shoots/ explant	Mean length of shoots (cm)	Number of leaves/ explant
BA	0	1	1.61 bc	3.77 c
	0.5	1	1.11 c	4.55 b
	1.0	1	1.83 b	4.33 b
	1.5	1	2.11 b	4.44 b
	2.0	1	2.88 a	5.22 a
Kinetin	0.5	1	1.05 c	3.11 c
	1.0	1	0.88 cd	1.55 e
	1.5	1	1.22 c	2.44 d
	2.0	1	0.88 cd	1.66 e

Table (2) shows the response of Mabrokat Al-Shimal sweet potato multiplication to different concentrations of BA and Kinetin. Generally, for multiplication of Mabrokat Al-Shimal, Kinetin was better than BA by giving the best multiplication parameters (Figure 1: B and C). Accordingly, all cytokinins treatments gave the same number of shoots which was one shoot per explant. The longest shoots were achieved when 1 mg.l^{-1} kinetin was used which gave 3.77 cm. Furthermore, the highest number of leaves was recorded when 0.5 mg.l^{-1} kinetin was used which gave 10.33 leaves per explant. On the other hand, the least mean length of shoots was obtained when 1.5 and 2 mg.l^{-1} BA were used by giving 1.88 cm. However, the least number of leaves was obtained when 1.5 mg.l^{-1} BA was used which gave 3.44 leaves per explant. The differences between both varieties performance might be due to their different internal makeup and endogenous hormonal content.

Table 2. Effects of BA and Kinetin on multiplication stage of Mabrokat Al-Shimal sweet potato explants grown on MS medium after four weeks in culture

Cytokinins ($mg.l^{-1}$)		Number of shoots/ explant	Mean length of shoots (cm)	Number of leaves/ explant
BA	0	1	3.55 a	8.77 b
	0.5	1	3.50 a	6.88 c
	1.0	1	2.27 c	5.00 d
	1.5	1	1.88 d	3.44 e
	2.0	1	1.88 d	3.66 e
Kinetin	0.5	1	3.61 a	10.33 a
	1.0	1	3.77 a	10.22 a
	1.5	1	3.16 b	7.77 b
	2.0	1	2.83 c	7.44 b

Table (3) shows the effect of different sugars (sucrose, fructose, and glucose) on the shoot-multiplication stage of Mangawy sweet potato explants after four weeks in culture on MS medium. In general, sucrose was the best carbon source for multiplying this variety of sweet potato followed by fructose then glucose. Since sucrose at 60 g L^{-1} gave the highest number of shoots per explant (1.22 shoots/ explant). However, the same sugar at 30 g L^{-1} gave the highest number of leaves per explant (6.66 leaves/ explant). While fructose at 15 g L^{-1} produced the longest shoots (2. 94 cm). Furthermore, glucose at 60 $g.l^{-1}$ showed the least parameters (0.33 shoots/ explant, 0.33 cm and 1 leaf/ explant).

Table 3. Effects of carbon sources on multiplication stage of Mangawy sweet potato explants growing on MS medium after four weeks in culture

Carbon source ($g.l^{-1}$)		Number of shoots/ explant	Mean length of shoots (cm)	Number of leaves/ explant
Sucrose	15	1.00 ab	1.66 d	4.00 c
	30	1.11 a	2.41 b	6.66 a
	45	1.14 a	2.60 b	6.14 a
	60	1.22 a	2.13 bc	5.11 b
Fructose	15	1.00 ab	2.94 a	6.55 a
	30	1.16 a	2.41 b	6.50 a
	45	1.00 ab	1.66 d	5.00 b
	60	0.77 b	1.21 d	3.77 c
Glucose	15	0.88 b	1.33 d	4.00 c
	30	1.00 ab	1.66 d	4.44 c
	45	1.00 ab	1.22 d	3.77 c
	60	0.33 c	0.33 e	1.00 d

Table (4) illustrates the effect of different sugars (sucrose, fructose, and glucose) on the shoot multiplication stage of Mabrokat Al-Shimal sweet potato explants after four weeks in culture on MS medium. In general, fructose and sucrose showed the best results since adding 60 g L^{-1} fructose produced the highest number of shoots per explant (1.83 shoots/ explant). While using 30 g $L^{-1,}$ sucrose gave the highest mean length of shoots (4.27 cm). However, fructose at 30 g L^{-1} gave the highest number of leaves per explant (20.22 leaves/ explant). On the other hand, adding 15 g L^{-1} fructose, 15, 30, and 45 g L^{-1} glucose gave the least number of shoots per explant (1 shoot/ explant). While adding 15 g $L^{-1,}$ sucrose gave the shortest shoots (2.19 cm). Moreover, adding 60 g L^{-1} fructose gave the lowest number of leaves per explant (6.16 leaves/ explant). Carbon sources or carbohydrates are essential in plant tissue culture medium where the culture condition is not appropriate for photosynthesis due to high relative humidity, low intensity of light, and deficiency in gas exchange, so they are essential to preserving the supply of carbon and osmotic potential (Kozai *et al*., 1997). Among carbohydrates, sucrose is the most effective on growth because it is a disaccharide that has a vital role in molecular transporter. It goes easily through the plasma membrane due to its high solubility. On the other hand, sucrose is the cheapest carbohydrate, so the cost will be low compared to other carbohydrates (Baskaran and Jayabalan, 2005; Javid and Ikram, 2008).

Table 4. Effects of carbon sources on multiplication stage of Mabrokat Al-Shimal sweet potato explants grown on MS medium after four weeks in culture

Carbon source ($g.l^{-1}$)		Number of shoots/ explant	Mean length of shoots (cm)	Number of leaves/ explant
Sucrose	15	1.11 b	2.19 cd	9.00 d
	30	1.11 b	4.27 a	10.60 c
	45	1.11 b	3.77 b	10.44 c
	60	1.11 b	4.08 a	8.77 d
Fructose	15	1.00 c	3.66 b	10.55 c
	30	1.11 b	3.77 b	20.22 a
	45	1.25 b	3.90 b	12.00 b
	60	1.83 a	2.50 c	6.16 f
Glucose	15	1.00 c	2.66 b	7.83 e
	30	1.00 c	3.33 b	8.88 d
	45	1.00 c	2.88 c	9.11 d
	60	1.00 c	1.88 d	5.66 g

Table (5) represents the response of Mangawy sweet potato explants to the culture medium supplemented with different concentrations of both NAA and IBA after four weeks of culture on MS medium. Overall, the whole treatments gave the same rate of rooting percentage by reaching 100% and IBA was better than NAA by giving the best rooting parameters for Mangawy sweet potato. The highest number of roots per explant was achieved when 1.5 $mg.l^{-1}$ IBA was used by giving 22.33 roots/ explant. While the highest mean length of roots (12.87 cm) was achieved when 0.5 $mg.l^{-1}$

NAA was added. Moreover, the lowest number of roots per explant (6.42 roots/ explant) was obtained when 1.5 mg.l^{-1} NAA was added and the shortest roots (7.88 cm) were achieved when 1.5 mg.l^{-1} was used.

Table 5. Effects of NAA and IBA on root formation stage of Mangawy sweet potato explants grown on MS medium after four weeks in culture

Auxins (mg.l^{-1})		Number of roots/ explant	Mean length of roots (cm)	Rooting percentage
NAA	0.0	7.55 d	12.11 b	100
	0.5	7.75 d	12.87 a	100
	1.0	8.66 c	11.77 b	100
	1.5	6.42 e	12.71 a	100
IBA	0.5	8.88 c	10.16 c	100
	1.0	12.44 b	10.94 bc	100
	1.5	22.33 a	7.88 d	100

Table (6) demonstrates the effect of various concentrations of NAA and IBA on the rooting stage of Mabrokat Al-Shimal sweet potato explants grown on MS medium after four weeks in culture. Overall, the whole treatment gave the same rooting percentage, which reached 100%, and NAA gave the best results compared to IBA. Accordingly, NAA at 0.5 mg L^{-1} produced the highest number of roots per explant (19 roots/ explant). At the same time, IBA at 0.5 mg L^{-1} gave the highest mean length of roots (16.31 cm). Furthermore, IBA at 0.5 mg.l^{-1} produced the least number of roots per explant (5.50 roots/ explant), and NAA at 1.5 mg.l^{-1} gave the shortest roots (7.33 cm).

Table 6. Effects of NAA and IBA on root formation stage of Mabrokat Al-Shimal sweet potato explants grown on MS medium after four weeks in culture

Auxins (mg.l^{-1})		Number of roots/ explant	Mean length of roots (cm)	Rooting percentage
NAA	0.0	10.40 e	7.50 e	100
	0.5	19.00 a	9.75 c	100
	1.0	18.66 b	11.66 b	100
	1.5	11.66 d	7.33 e	100
IBA	0.5	5.50 g	16.31 a	100
	1.0	8.71 f	9.42 c	100
	1.5	12.25 c	8.12 d	100

Although the two varieties of sweet potato were rooted in free growth regulators medium, the auxin had a significant role in increasing the number of roots per explant from 7.55 to 22.31 roots per explant for Mangawy sweet potato as well as from 10.40 to 19.00 roots per explant for Mabrokat Al-Shimal sweet potato. The enhancement role of auxins in root induction and development is due to the physiological effect of the auxin in the adventurous root stimulation and cell division and elongation (Fadladeen and Toma, 2020). Both sweet potato varieties were successfully acclimatized (Figure 1: D) with a hundred percent. After four weeks of growth in the greenhouse, they were transplanted in the permanent field under open-air conditions (Figure 1: F) at the beginning of March.

Figure 1. Propagation stages of sweet potato:

A. Mangawy and Mabrokat Al-Shimal varieties at the initiation stage.
B. B and C. Shoot multiplication stage.
D. Acclimatization Stage
E. Sweet potato transplant produced in the greenhouse ready for transplanting.
F. Open-field grown plants.
G. Sweet potato yield at harvesting time.

Conclusion

General conclusions from the current research are that sweet potato can be propagated by tissue culture technique in the Kurdistan Region of Iraq toward mass production for this most nourishable vegetable crop. Mabrokat Al-Shimal is a

good choice for future propagation and field production of sweet potato under Duhok governorate conditions. Further studies are needed on other factors affecting the growth and development of sweet potatoes in open-field conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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