In Vitro Calli Developing and Characterizing from Sweet Potato (*Ipomoea batatas*) to Maximize Secondary Metabolites Production

E. M. Salah [1,*], M. A. Eissa [1,2], and F, A. El-Feky [1]

[1] *Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo 11651, Egypt*
[2] *Zhejiang BioAsia Institute of Life Sciences, Zhejiang 314200, China*

[*] Corresponding author E-mail: esmaeel.bio@azhar.edu.eg (E. M. Salah)

ABSTRACT

Sweet potato (*Ipomoea batatas*) is considered as an important vegetable according to its extreme nutritive value. It's also a medicinal plant with high source of vitamins, minerals, anti-inflammatory, anticancer and antidiabetic activity. Biotechnology approaches are used to maximize the added value of sweet potato which is needed to fill the gap of world hunger crisis. plant tissue culture technology is a perpetual tool that provides deep understanding of morphogenesis, biochemistry, and molecular physiology in organized and disorganized plant culture. The objective of this study is to find out the effective recommended media composition for callus initiation and induction from leaf explant of sweet potato. Calli are achieved by growing explants on eight treatments which were prepared from MS nutrient medium with different concentrations of plant growth regulators (PGR). Previous treatments were evaluated using cell viability, cell growth and callus type as parameters after nine weeks. Results showed that (2,4-D 2 mg/L to BA 2 mg/L) or (NAA 2 mg/L to BA 2 mg/L) were the best auxin and cytokinin concentrations. Also, results showed that callus initiated from (2,4-D and BA) cultures was more friable than (NAA and BA) cultures. Finally, disorganized cultures (calli) were essential step for continuous cell suspension to produce secondary metabolites.

Keywords: Sweet potato; biotechnology; tissue culture; plant growth regulators; callus.

INTRODUCTION

Sweet potato (*Ipomoea batatas*), one of the most important plants used in human nutrition all over the world, sweet potato can grow in several types of soil under different environmental condition as a result of gene expression responding to surrounding conditions. it is a hexaploidy plant (2n = 6x = 90) (Escobar-Puentes et al., 2022).

Sweet potato was reported to produce different beneficial bioactive secondary metabolites with pharmacological effects on human health, it has a positive effect for hypoglycemic, wound healing, antiulcer, anti-inflammatory, antimutagenicity, cardiovascular, hepatoprotective, immunomodulatory, anti-proliferative, antifungal, and antimicrobial effect (Panda & Sonkamble, 2012).

Leaves of sweet potato contain many antioxidant compounds, and the percentage of phenolics in the leaves higher than petioles (Jang & Koh, 2019), and the results for dried sweet potato leaves showed 3.4 mg/g flavonoids and 36.5 mg/g anthocyanins by 70% ethanol, and 43.8 mg/g of total Phenolic by 50% (v/v) acetone that was the best solvent for extracting polyphenols and antioxidants from sweet potato leaves (Fu et al., 2016). In addition to its phenolic compounds content, sweet potato also contains caffeoylquinic acid derivatives in its leaves (Zhang et al., 2019). Concentrations of antioxidant compounds varies depending on the color of sweet potato flesh (Ooi et al., 2021). Various research databases such as PubMed, scopus, and science direct contain more than 269 scientific articles up to June 2023 that study the effect of sweet potato extracts on type two of diabetes (Arisanti et al., 2023). More biological experiments were conducted on rats by using sweet potato leaves extract treated orally, which showed good results in reducing the level of glucose in the blood, increasing insulin, and improving pancreatic cells (Almoraie, 2019). Where the results of various experiments indicated that sweet potato leaves extract can be used in the production of functional foods to improve the metabolic symptoms caused by diabetes mellitus (Zengin et al., 2017). Sweet potato also contain many important nutrients that have an antitumor and antiproliferative effect, and where those nutrients are distributed in various plant organs, and tested to find out the mechanism of each of them in the anticancer effect (Silva-Correa et al., 2022).

Plant tissue culture techniques are a method for propagation of plants under controlled conditions. Also, used in the production of virus-free plants in the development of any plant organ separately. propagation of plants that were difficult to

propagate by seeds, through artificial seeds production (Kumar & Mina, 2019; Smith, 2012) . In addition to extracting medicinal and aromatic substances from various plants, it is also possible through plant tissue culture techniques to obtain the disorganized culture, somatic embryogenesis, cell suspension culture, and plant cell lines (Smith, 2012).

Plant growth regulators such as auxin and cytokinin are used in different concentrations with nutrient medium to obtain the best results in terms of callus initiation and induction from sweet potato (Abubakar et al., 2018; ALI et al., 2017; NAWIRI et al., 2017), then get different cell suspension cultures (Mohanraj & Subha, 2017). Auxin and cytokinin are Plant Growth Regulators (PGR) that interact with each other in a complex process, which controls the growth and differentiation in different plant growth stages. Gene expression will be affected depending on the different ratios of auxin and cytokinin. It was found that auxins and cytokinins play a role in cell division process (Coenen & Lomax, 1997).

Callus is a disorganized undifferentiated cells which forms when cells divide irregularly. Callus culture term refers to invitro callus developed from growing of plant tissues and cells (explants) on nutrient medium containing different concentrations of PGR under completely sterile conditions. The process of callus initiation goes through several stages, starting with transformation of organized explants into undifferentiated and unspecialized cells. Callus cultures can be preserved by transferring them to a new nutrient environment containing the same components after a specific period (Kumar & Mina, 2019).

Previous studies indicated the possibility of forming callus from sweet potato plant using different concentrations of PGR. Callus can be initiated and inducted from leaves by using 2 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) (Bett et al., 2015; Wang et al., 2007), 5 mg/L 2,4-D (Bett et al., 2015), 4.0 mg/L 2,4-D was the best concentration for callus induction. Then the callus was transferred to a nutrient medium containing 0.1 mg/L BAP, but no somatic embryos were produced, although it produced single or multiple shoots (Addae-Frimpomaah et al., 2014). Callus culture is used to increase the production of therapeutic active compounds from plant cells (Benjamin et al., 2019).

The maximum of inducted somatic embryos from sweet potato can be obtained from explants cultured on linsmaier and skoog (LS) medium included vitamins and with 0.5 mg/L dichlorophenoxyacetic acid and 0.2 mg/L zeatin riboside. Highest number of inducted shoot was showed after transfer of somatic embryonic callus to embryo maturation (EM) medium contained 2 mg/L abscisic acid (Mbinda et al., 2016). In an experiment to evaluate 36 treatments of naphthalene acetic acid (NAA) and 6-benzylaminopurine (BAP) as a PGR on shoot regeneration, the best treatment was on MS medium with 0.01 mg/L NAA + 1 mg/L BAP. and the plant growth was highest rate when transferred to soil (Sivparsad & Gubba, 2012).

Therefore, this study aimed to investigate the effect of different plant growth regulators (PGR) on callus initiation and induction, determine callus viability and type, and measurement of callus cells growth to maximize secondary metabolites production from sweet potato for future studies.

MATERIALS AND METHODS

The experiments and analysis were carried out at Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Plant material

Egyptian sweet potato plant leaves were collected after five weeks from cultured plants at the growth chamber of Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Preparation and disinfection of Leaves

Two weeks old leaves were placed in a piece of gauze and washed with running tap water for 30 minutes, then disinfected by using a 20% Clorox contained 5% sodium hypochlorite with two drops of commercial detergent for 10 minutes, followed by sterilization using 70% ethyl alcohol for 30 seconds, and washing with sterile distilled water three times, then the surface of the leaves was dissected longitudinally before transferring them to different media.

Effect of Plant Growth Regulators (PGR) on callus initiation and induction

Sweet potato leaves were sterilized and cultured on MS nutrient medium (Murashige & Skoog, 1962), and plant growth hormones 2,4-dichlorophenoxy acetic acid (2,4-D), α-naphthalene acetic acid (NAA) and 6-benzyl adenine (BA) were used to prepare different eight treatments. 4.43 g/L MS + 30 g/L Sucrose

+ 7 g/L Agar + different concentration of plant growth regulators (PGR) were prepared.

Eight different treatments of PGR are (2,4-D 1 mg/L to BA 1 mg/L), (2,4-D 1 mg/L to BA 2 mg/L), (2,4-D 2 mg/L to BA 1 mg/L), (2,4-D 2 mg/L to BA 2 mg/L), (NAA 1 mg/L to BA 1 mg/L), (NAA 1 mg/L to BA 2 mg/L), (NAA 2 mg/L to BA 1 mg/L), (NAA 2 mg/L to BA 2 mg/L) and control medium without PGR (Figure 1). The pH was 5.8 and the medium was autoclaved at 121 °C for 20 minutes, then poured 25 ml in each sterile jar.

Viability and type of callus

Calli were examined to determine their viability and type in terms of being friable or nonfriable. A light microscope with 100x magnification and trypan blue stain (TBS) were used to measure the viability of the cells. Samples were taken from the different calli and placed on microscopic slides, crushed, and TBS drop was added, then covered and examined. Viable cells appear without any stains, while dead cells appear blue (Strober, 1997).

Measurement of callus cells growth

After 9 weeks of culturing the leaves on different treatments. Moisture balance (OHAUS MB27) was used (at the Faculty of Pharmacy for Girls, Al-Azhar University, Nasr City, Cairo, Egypt) to determine the callus fresh weight (FW), moisture percentage (MP), and dry weight percentage (DWP), through which the dry weight (DW) of callus can be calculated as follows:

$$DW = DWP \times FW$$

RESULTS AND DISCUSSION

Effect of Plant Growth Regulators (PGR) on callus initiation and induction

In this study, the callus was obtained from two weeks old leaves (Figure 1 a) grown from the cultivation of Egyptian sweet potato (Figure 1 b). Leaves were applied to MS medium with different eight concentrations from auxins and cytokinins and the control (PGR free) medium. Samples were incubated at 25°C in complete dark. The onset of callus initiation (Figure 1 c, d) after four weeks with (2,4-D 1 mg/L to BA 1 mg/L), (2,4-D 2 mg/L to BA 2 mg/L), (NAA 1 mg/L to BA 1 mg/L) and (NAA 2 mg/L to BA 2 mg/L), and callus initiation after 5 weeks with (2,4-D 1 mg/L to BA 2 mg/L), (2,4-D 2 mg/L to BA 1 mg/L), (NAA 1 mg/L to BA 2 mg/L) and (NAA 2 mg/L to BA 1 mg/L). these results are due to the various effect of different concentrations of PGR on the growth or cells morphogenesis according to (Smith, 2012).

Callus induction (Figure 1 e, f) and growth was appeared clearly after nine weeks from planting the leaves on medium under 16/8 h light/dark period in all treatments (Table 1), where the callus grade showed highest levels with (2,4-D 2 mg/L to BA 2 mg/L), (NAA 2 mg/L to BA 2 mg/L), (NAA 1 mg/L to BA 1 mg/L), (2,4-D 1 mg/L to BA 1 mg/L), (2,4-D 2 mg/L to BA 1 mg/L), (2,4-D 1 mg/L to BA 2 mg/L), (NAA 2 mg/L to BA 1 mg/L), then (NAA 1 mg/L to BA 2 mg/L) respectively (Table 1). Also, results showed that the best PGR concentrations for callus initiation and induction from leaves of sweet potato are (2,4-D 2 mg/L to BA 2 mg/L) (Figure 1 c, e) in 2,4-D and BA treatments like (Al-Hussaini et al., 2015) and (NAA 2 mg/L to BA 2 mg/L) (Figure 1 d, f) in NAA and BA treatments.

Viability and type of callus

The morphological shape differences appeared as a result of different treatments. The combination between (2,4-D and BA) showed that creamy color and friable texture while, callus from (NAA and BA) showed that brownish yellow color and compact texture (Table 1). The light microscope and trypan blue stain were used to examine the cells (Strober, 1997), it became clear due the cells had high viability, as the stain inside the cells had disappeared. However, some molecules with different shapes appeared between cells in samples examined from (NAA and BA) treatments, which may indicate the compact texture of callus from these treatments (Figure 2). The callus inducted from 2,4-D and BA is nodular and while callus inducted from NAA and BA is compact has been documented (JayaSree et al., 2001). and (Cavalcante Alves et al., 1994) reported that callus inducted from 2,4-D is friable.

Measurement of callus cells growth

Callus dry weight (DW) was determined as a measure that was used to compare the growth rate of callus in different treatments. A moisture balance was used to measure fresh weight (FW), moisture percentage (MP) and dry weight percentage (DWP) of callus. When measuring FW of callus from different treatments after 9 weeks of planting the leaves on different treatments, the results appeared that (2,4-D 1 mg/L to BA 1 mg/L) = 2.193 g, (2,4-D 1 mg/L to BA 2 mg/L) = 1.416 g , (2,4-D 2 mg/L to BA 1 mg/L) = 1.641 g, (2,4-D 2 mg/L to BA 2 mg/L) = 3.227 g, (NAA 1 mg/L to BA 1

mg/L) = 2.417 g, (NAA 1 mg/L to BA 2 mg/L) = 0.787 g, (NAA 2 mg/L to BA 1 mg/L) = 0.941 g and (NAA 2 mg/L to BA 2 mg/L) = 2.603 g (Table 2).

MP showed 85.73%, 87.04%, 86.93%, 86.19%, 83.17%, 84.02%, 83.95% and 83.30% (Table 2). DWP displayed 14.27%, 12.96%, 13.07%, 13.81%, 16.83%, 15.98%, 16.05% and 16.70% (Table 2). and DW was calculated by the following equation:

DW = DWP × FW

The results after calculated were 0.3129411 g, 0.1835136 g, 0.2144787 g, 0.4456487 g, 0.4067811 g, 0.1257626 g, 0.1510305 g and 0.434701 g of callus respectively (Table 2). From the previous results, it appeared that (2,4-D 2 mg/L to BA 2 mg/L) was the best concentration for callus inducted by using 2,4-D and BA, and (NAA 2 mg/L to BA 2 mg/L) then (NAA 1 mg/L to BA 1 mg/L) were the best concentrations for callus inducted by using NAA and BA. Results above correspond to knowledge that humidity in plant cells between 80% to 90%, according to (Kramer, 1955).

CONCLUSIONS

The results of this study revealed the possibility of obtaining callus from sweet potato leaves using different concentrations of auxin and cytokinin. The results showed that (2,4-D 2 mg/L to BA 2 mg/L) or (NAA 2 mg/L to BA 2 mg/L) concentrations were the best combinations for callus initiation and induction. This study results indicated that callus initiated from 2,4-D and BA was friable, while the callus initiated from NAA and BA was compact. Callus is considered an essential step for cell suspension cultures to produce secondary metabolites. At present, in-depth study of metabolic engineering using callus will open new ways for wider applications. Therefore, confirming the effect of callus types on the amount of secondary metabolites production may be taken into consideration in future studies.

ACKNOWLEDGMENTS

The authors thanks faculty of Pharmacy for Girls, Al-Azhar University, Nasr City, Cairo, Egypt for allowing us to use a Moisture Balance (OHAUS MB27).

REFERENCES

Abubakar, A., Yahaya, S., Shaibu, A., Ibrahim, H., Ibrahim, A., Lawan, Z., Isa, A. 2018: In vitro propagation of sweet potato (Ipomoea batatas (L.) Lam.) cultivars. *Agricultural Science Digest-A Research Journal*, *38*(1), 17-21.

Addae-Frimpomaah, F., Arkorful, E., Tengey, T. 2014: The effect of 2, 4-D on callus induction using leaf lobe of sweet potato as a source of explant. *Int. J. of Agronomy and Agric. Res*, *5*(1), 16-22.

Al-Hussaini, Z., Yousif, S., Al-Ajeely, S. 2015: Effect of different medium on callus induction and regeneration in potato cultivars. *International Journal of Current Microbiology and Applied Sciences*, *4*(5), 856-865.

ALI, N.M., Rashed, M., ABDEL-AZEEM, A., EL-DIN, T.N., Metry, E. 2017: regeneration and transformation system in egyptian sweet potato (Ipomoea batatas Lam.) CULTIVARS. *Egyptian Journal of Genetics and Cytology*, *46*(2).

Almoraie, N.M. 2019: The Role of Ipomoea Batatas Leaves Extract on the Treatment of Diabetes Induced by Streptozotocin. *Diabetes*.

Arisanti, C.I.S., Wirasuta, I.M.A.G., Musfiroh, I., Ikram, E.H.K., Muchtaridi, M. 2023: Mechanism of Anti-Diabetic Activity from Sweet Potato (Ipomoea batatas): A Systematic Review. *Foods*, *12*(14), 2810.

Benjamin, E.D., Ishaku, G.A., Peingurta, F.A., Afolabi, A.S. 2019: Callus culture for the production of therapeutic compounds. *American Journal of Plant Biology*, *4*(4), 76-84.

Bett, B., Machuka, J., Gichuki, S., Ateka, E. 2015: Effect of 2, 4-D levels on callus induction of leaf and stem explants of 5 local farmer-preferred sweet potato varieties in Kenya. In.

Cavalcante Alves, J., Sihachakr, D., Allot, M., Tizroutine, S., Mussio, I., Servaes, A., Ducreux, G. 1994: Isozyme modifications and plant regeneration through somatic embryogenesis in sweet potato (Ipomoea batatas (L.) Lam.). *Plant Cell Reports*, *13*(8), 437-441.

Coenen, C., Lomax, T.L. 1997: Auxin—cytokinin interactions in higher plants: old problems and new tools. *Trends in plant science*, *2*(9), 351-356.

Escobar-Puentes, A.A., Palomo, I., Rodríguez, L., Fuentes, E., Villegas-Ochoa, M.A., González-Aguilar, G.A., Wall-Medrano, A. 2022: Sweet potato (Ipomoea batatas L.) phenotypes: From agroindustry to health effects. *Foods*, *11*(7), 1058.

Fu, Z.F., Tu, Z.C., Zhang, L., Wang, H., Wen, Q.H., Huang, T. 2016: Antioxidant activities and polyphenols of sweet potato (Ipomoea batatas L.) leaves extracted with solvents of various polarities. *Food bioscience*, *15*, 11-18.

Jang, Y., Koh, E. 2019: Antioxidant content and activity in leaves and petioles of six sweet potato (Ipomoea batatas L.) and antioxidant properties of blanched leaves. *Food science and biotechnology*, *28*, 337-345.

JayaSree, T., Pavan, U., Ramesh, M., Rao, A., Jagan Mohan Reddy, K., Sadanandam, A. 2001: Somatic embryogenesis from leaf cultures of potato. *Plant cell, tissue and organ culture*, *64*, 13-17.

Kramer, P.J. 1955: Water content and water turnover in plant cells. *Encyklopedia of Plant Physiology (ed. Ruhland, W.)*, *1*, 194-217.

Kumar, P., Mina, U. 2019: *Life Sciences : Fundamentals and Practice II* (Seventh edition ed., Vol. II).

Mbinda, W., Anami, S., Ombori, O., Dixelius, C., Oduor, R. 2016: Efficient plant regeneration of selected Kenyan sweetpotato (Ipomoea batatas (L.) Lam.) cultivars through somatic embryogenesis. *Journal of Tissue Science & Engineering*, *7*, 1000176.

Mohanraj, R., Subha, S. 2017: Optimization of process parameters for bioproduction, isolation and purification of 4-Ipomeanol from cell suspension cultures of Ipomoea batatas (L.) Lam.

Murashige, T., Skoog, F. 1962: A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473-497.

Nawiri, S.O., Oduor, R.O., Jalemba, A.M. 2017: Genetic engineering of sweet potatoes (Ipomoea batatas) using isopentenyl transferase gene for enhanced drought tolerance. *Asian Journal of Agriculture*, *1*(2), 85-99.

Ooi, S., Sukri, S., Zakaria, N., Harith, Z. 2021: Carotenoids, phenolics and antioxidant properties of different sweet potatoes (Ipomoea batatas) varieties. IOP Conference Series: Earth and Environmental Science,

Panda, V., Sonkamble, M. 2012: Phytochemical constituents and pharmacological activities of Ipomoea batatas l.(Lam)–A review. *International Journal of Research in Phytochemistry and Pharmacology*, *2*(1), 25-34.

Silva-Correa, C.R., Hilario-Vargas, J., Villarreal-La Torre, V.E., Calderón-Peña, A.A., González-Siccha, A.D., Aspajo-Villalaz, C.L., Cruzado-Razco, J.L. 2022: Potential Anticancer Activity of Bioactive Compounds from Ipomoea batatas. *Pharmacognosy Journal*, *14*(3).

Sivparsad, B., Gubba, A. 2012: Development of an efficient plant regeneration protocol for sweet potato (Ipomoea batatas L.) cv. Blesbok. *African Journal of Biotechnology*, *11*(84), 14982-14987.

Smith, R.H. 2012: *Plant tissue culture: techniques and experiments*. academic press.

Strober, W. 1997: Trypan blue exclusion test of cell viability. *Current protocols in immunology*, *21*(1), A. 3B. 1-A. 3B. 2.

Wang, Y., Wang, F., Zhai, H., Liu, Q. 2007: Production of a useful mutant by chronic irradiation in sweetpotato. *Scientia Horticulturae*, *111*(2), 173-178.

Zengin, G., Locatelli, M., Stefanucci, A., Macedonio, G., Novellino, E., Mirzaie, S., Orlando, G. 2017: Chemical characterization, antioxidant properties, anti-inflammatory activity, and enzyme inhibition of Ipomoea batatas L. leaf extracts. *International journal of food properties*, *20*(sup2), 1907-1919.

Zhang, C., Liu, D., Wu, L., Zhang, J., Li, X., Wu, W. 2019: Chemical characterization and antioxidant properties of ethanolic extract and its fractions from sweet potato (Ipomoea batatas L.) leaves. *Foods*, *9*(1), 15.

Table 1: Effect of plant growth regulators (PGR) on callus induction, color and texture

Plant growth regulators (mg/L)	Callus grade	Callus color	Callus texture
Control	-	-	-
1.0 2,4-D + 1.0 BA	+++	Creamy	Friable
1.0 2,4-D + 2.0 BA	++	Creamy	Friable
2.0 2,4-D + 1.0 BA	++	Creamy	Friable
2.0 2,4-D + 2.0 BA	++++	Creamy	Friable
1.0 NAA + 1.0 BA	+++	Brownish yellow	Compact
1.0 NAA + 2.0 BA	+	Brownish yellow	Compact
2.0 NAA + 1.0 BA	+	Brownish yellow	Compact
2.0 NAA + 2.0 BA	+++	Brownish yellow	Compact

Results showed that the order of callus levels were (2,4-D 2 mg/L to BA 2 mg/L), (NAA 2 mg/L to BA 2 mg/L), (NAA 1 mg/L to BA 1 mg/L), (2,4-D 1 mg/L to BA 1 mg/L), (2,4-D 2 mg/L to BA 1 mg/L), (2,4-D 1 mg/L to BA 2 mg/L), (NAA 2 mg/L to BA 1 mg/L), then (NAA 1 mg/L to BA 2 mg/L) respectively. The Calli inducted from (2,4-D and BA) were creamy and friable, while the Calli inducted from (NAA and BA) were brownish yellow and compact.

Table 2: Effect of Plant Growth Regulators (PGR) on callus fresh weight (FW), moisture percentage (MP), dry weight percentage (DWP) and dry weight (DW):

Plant growth regulators (mg/L)	FW (g)	MP (%)	DWP (%)	DW (g)
Control	-	-	-	-
1.0 2,4-D + 1.0 BA	2.193	85.73	14.27	0.3129411
1.0 2,4-D + 2.0 BA	1.416	87.04	12.96	0.1835136
2.0 2,4-D + 1.0 BA	1.641	86.93	13.07	0.2144787
2.0 2,4-D + 2.0 BA	3.227	86.19	13.81	0.4456487
1.0 NAA + 1.0 BA	2.417	83.17	16.83	0.4067811
1.0 NAA + 2.0 BA	0.787	84.02	15.98	0.1257626
2.0 NAA + 1.0 BA	0.941	83.95	16.05	0.1510305
2.0 NAA + 2.0 BA	2.603	83.30	16.70	0.434701

Results showed that callus inducted from (2,4-D 2 mg/L to BA 2 mg/L) was the best concentration by 2,4-D with BA, and (NAA 2 mg/L to BA 2 mg/L) then (NAA 1 mg/L to BA 1 mg/L) were the best concentrations for callus inducted by NAA with BA.

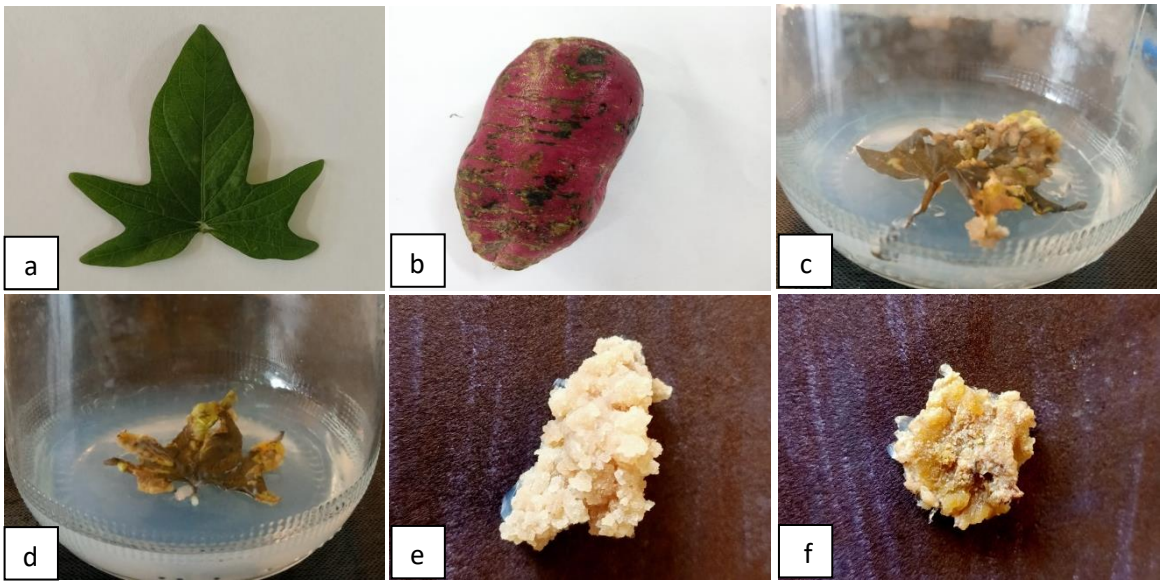

Figure 1: Callus initiation and induction of Egyptian sweet potato from leaves after 9 weeks on MS culture medium with different concentration of plant growth regulators (PGR). (a) Egyptian sweet potato, (b) Leaf of egyptian sweet potato, (c) callus initiation with (2.4-D 2 mg/L to BA 2 mg/L), (d) callus initiation with (NAA 2 mg/L to BA 2 mg/L), (e) Friable callus inducted and grown with (2.4-D 2 mg/L to BA 2 mg/L) and (f) Compact callus inducted and grown with (NAA 2 mg/L to BA 2 mg/L).

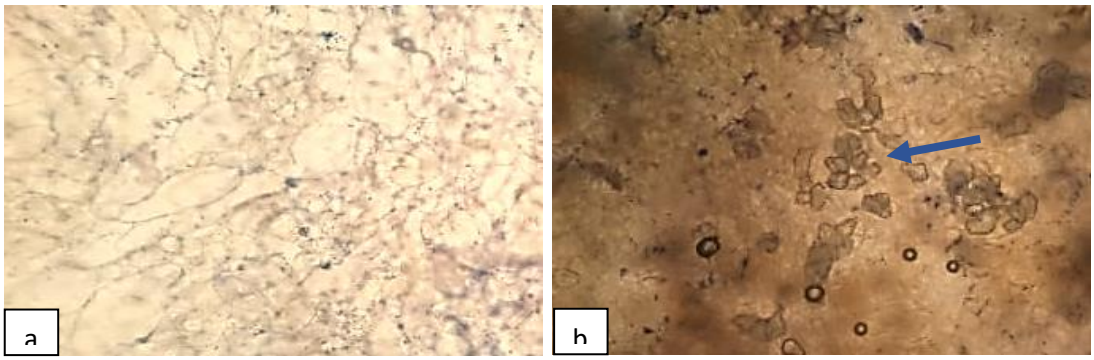

Figure 2: Determine of callus viability and type through trypan blue staining and light microscopic examination at 100x magnification. (a) Examination of initiated callus cells by using (2,4-D 2 mg/L to BA 2 mg/L) (b) Examination of initiated callus cells by using (NAA 2 mg/L to BA 2 mg/L), and some molecules with different shapes appeared between cells.

استحداث وتوصيف الكالس معملياً في نبات البطاطا الحلوة لزيادة إنتاج الميتابولايتس الثانوية

اسماعيل محمد صلاح[1،*]، محمد أحمد عيسى[2،1]، فوزي علي الفقي[1]

[1] *قسم التقنية الحيوية، كلية الزراعة، جامعة الازهر، مدينة نصر، القاهرة، مصر*

[2] *معهد جيجيانج بيو آسيا لعلوم الحياة، جيجيانج، الصين*

[*] البريد الإلكتروني للباحث الرئيسي: esmaeel.bio@azhar.edu.eg

الملخص

تعتبر البطاطا الحلوة واحدة من الخضروات المهمة وفقًا لقيمتها الغذائية المرتفعة. هي أيضًا نبات طبي، ومصدر عالي للفيتامينات والمعادن ومضادات الالتهابات والسرطان ومرض السكري. وتستخدم طرق التقنية الحيوية لتعظيم القيمة المضافة للبطاطا الحلوة اللازمة لسد فجوة أزمة الجوع العالمية. تعتبر تكنولوجيا زراعة الأنسجة النباتية أداة مهمة توفر فهمًا عميقًا للشكل المورفولوجي والتركيب الكيميائي الحيوي ووظائف الأعضاء في مختلف النباتات. الهدف من هذه الدراسة هو معرفة البيئة المغذية المناسبة لتكوين الكالس من أوراق نبات البطاطا الحلوة. تم تصميم تجربة معملية لثماني معاملات تختلف فيما بينها في نوع وكمية منظمات النمو النباتية (الاوكسين والسيتوكينين) المضافة للبيئة المغذية. تم تقييم المعاملات الثمانية السابقة باستخدام فحص حيوية الخلايا ومعدل نموها ونوع الكالس كمقاييس، وذلك بعد تسعة أسابيع. أظهرت النتائج أن المعاملات (2 ملجم 2،4،داي كلورو فينوكسي اسيتيك اسيد / 2 ملجم بنزيل أدنين)، (2 ملجم نفثالين اسيتك اسيد / 2 ملجم بنزيل أدنين) و أعطوا أفضل النتائج في تكوين الكالس ونموه إلا أن المعاملات التي تم فيها أستخدام 2،4،داي كلورو فينوكسي اسيتيك اسيد أعطت كالس أكثر هشاشة عن المعاملات التي تم فيها استخدام نفثالين اسيتك اسيد حيث كان الكالس الناتج متكتل. تعتبر مزارع الكالس خطوة أساسية في انشاء معلقات الخلايا المستمرة لإنتاج الميتابولايتس الثانوية.

الكلمات الاسترشادية: البطاطا الحلوة، التقنية الحيوية، زراعة الأنسجة، منظمات النمو النباتية، الكالس.