Regeneration of *Cucumis melo* L. plants from cell suspension derived from hypocotyls callus

تمايز نباتات البطيخ Muskmelon, *Cucumis melo* L. من المعلقات الخلوية المشتقة من كالس السيقان تحت الفلقية

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Abstract

This study aimed to investigate the regeneration capability of cell suspension derived from hypocotyl callus of the vegetable plant, muskmelon, *Cucumis melo* L. Culture of the densities (7.5, 9.8, 13.0, 11.2, 7.8, 4.2)× 10^2 cell/ml produced callus primordia which formed typical callus culture successfully. This green – yellowish color and semi-compact callus regenerated shoots on agar solidified MS medium supplemented with 2.0mgL^{-1}BA. These regenerates rooted readily in MS0 medium. They were adapted and routinely transferred to soil. The conclusion of this work that muskmelon plants have a good response to *in vitro* culture with no need to specific requirements.

المستخلص

هدفت الدراسة الحالية إلى التعرف على قابلية المعلقات الخلوية المشتقة من كالس السيقان تحت الفلقية لاحد محاصيل الخضراوات البطيخ *Cucumis melo L.* . أدت زراعة الكثافات (7.5 ، 9.8 ، 13.0 ، 11.2 ، 7.8 ، 4.2)×10^2 خلية /مل من المعلقات الخلوية إلى تكوين اعداد من منشأت الكالس. وتمكنت هذه المنشأت بنجاح تكوين مزارع نموذجية من الكالس ذو الون الاخضر – المائل للاصفرار شبه الهش في بنيته. واظهر قابليتة على التمايز إلى مجموعة من الافرع الخضرية في وسط التمايز MS الصلب المدعم باضافة 8 غم لتر$^{-1}$ من الاكار ومجموعة الفيتامينات ومدعماً باضافة 2.0 ملغم لتر$^{-1}$ من البنزايل ادنين BA. وجذرت هذه الافرع المتكونة بسهولة حين نقلها إلى وسط التجذير المتكون من املاح وسط موراشيج وسكوج MS0 الخالية من منظمات النمو ونجاح اقلمتها ونقلها إلى التربة . ان الاستنتاج الذي توصلت اليه هذه الدراسة يكمن في سهولة استجابة نباتات البطيخ للزراعة خارج الجسم الحي ولا يتطلب نجاحها متطلبات معينة .

Introduction

Cultures of hypocotyle calli were easily established in MS medium provided with indoleacetic acid and kinetin, and differentiation of the obtained callus when kinetin – containing medium increased up to 6.0mgL^{-1} [1]. Moreover, watermelon plants were steadily regenerated from cotyledonary explants [2]. Many studies investigated the ways to obtain somatic embryos from callus culture of muskmelon mainly, watermelon and squash using various concentration of 2, 4-dichlorophenoxy acetic acid and kinetin [3]. Again, Callus was produced from cotyledonary Leaves in MS medium supplemented with the addition of naphthalene acetic acid and kinetin and differentiated when transferred to medium containing kinetin only [4]. A related study mentioned that addition 0.025 mg L^{-1} of Copper Sulphate ($CuSO_4$) with the presence of growth regulator stimulate callus production of cotyledonary leaves followed by buds formation and muskmelon plant regeneration [5]. This study was interested in whether cell suspension initiated from

Key words: Regeneration , *Cucumis melo* L. , Suspension derived , hypocotyls callus

muskmelon hypcotyl callus retained its potentiality to regenerate plants rather than explants calli which seems to be unlikely.

Material and Methods

1- Axenic shoot cultures

Seeds of *Cucumis melo* L., local variety, were surface sterilized in suitably diluted commercial Bleach i.e 3% sodium hypochlorid (" FAS " Solution, Babylon company for soap industry, Baghdad, Iraq) followed by three washes in autoclaved sterile water [6]. Plants were established by *in vitro* seeds germination aseptically in Murashige and Skoog (MS) based culture medium [7] with 0.8% w:v agar containing 3% w: v sucrose and vitamins but Lacking growth regulator in 250ml Erlenmeyer flasks, each containing 30 ml aliquots at ratio of 3 seeds/ flask . They kept in culture room in dark firstly until starting germination, and then transferred to 16h light. Seedlings were grown in flasks for 6-7 days before been used as source of explants.

2- Preparation cell suspension from hypocotyls callus

Hypocotyle callus which produced on agar- solidified MS medium containing benzyl adenine 5.0 mg L^{-1} [8], was utilized for initiation of cell suspension. One gram of this friable, green callus was placed into 100 ml Erlenmeyer flasks each containing 25 ml aliquot of liquid MS medium supplemented with the concentration (0.5, 1.0, 2.0, 4.0, 6.0, 8.0) mg L^{-1} of BA. Cultures were placed in shaking incubator, (New Brunswick, Edision, USA) in dark, 25 ± 2 C° and velocity was 150 rpm, [9]. Cultures were removed after 24h of incubation and clarified through plastic, sterile sieve 45 µm (PGMG, U.K.) which allow single cells to pass through. The clarified cultures were placed again on shaking incubator at the same conditions [10].

3- Assessment viability of colonized cells.

The viability of cell suspension was performed by adding 0.1ml of Evan blue Millipore filtered stain solution (Chemical Ltd Poole U. K.) to 1.0 ml aliquot of cell suspension and examined under light microscope [11].

4- Assessment cell density for culture.

Density of the cell suspension was determined by taking 0.1 ml aliquot of the culture after 24, 48, 72, 96, 120 and 144 h, of incubation. Each sample was put on haemocytometer slide (0.1 ml Depth, Haemocytometer Labsco, Germany). Number of cells was counted and a density was determined [12].

5- Culture of cell suspension by embedding in media semi- solidified with agar.

Cells are suspended at the double the required plating density in Liquid medium. Six densities (1.0×10^3, 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7) ^{cell}/ml. were selected. One ml aliquot of each density was mixed with 1.0 ml of 0.4% (w : v) warm (40 C°) agarose [13]. The resulting culture was dispersed into drops of 50 ml in size each in the bottom of 3.0 cm diam. plastic Petri dishes (Sterilin, U. K.), then allow cooling. For each 3.0 ml liquid MS medium provided with conc. of BA including (0.5, 1.0, 2.0, 4.0, 6.0, 8.0) mg L^{-1}. Dishes were covered with lids and sealed by Nescofilm. Cultures were incubated in culture room at 25 ± 2C° under low illumination 700 – 800 Lux (day light fluorescent tubes) with a suitable photoperiod of 16 h. Examinations of cultures are carried out periodically and every four days liquid medium was replaced with a same volume of

freshly prepared medium until the development of calls primordial. They were picked up and transferred to propagation medium.

6- Regeneration of plants

Many pieces of 1.0 gm/each of cell suspension derived callus were placed in 15 cm diam. Plastic Petri-dishes (Sterile, U. K.) Containing 15 ml aliquot of agar-solidified MS medium supplied by (0.5, 1.0, 2.0, 4.0, 6.0, 8.0) mgL^{-1} BA. They were kept in culture room at the same condition mentioned previously.

Results

1- Cell suspension derived callus formation.

In relation to cell suspension Figure (1.a) division they exhibit the same normal-type in dividing first division Figure (1.b) through the first week of culture, underwent to second division without any discontinuities forming various number of cell colonies figure (1.c,d) that developed to macro colonies Figure (1.e) and led to the formation of callus primordial Figure (1.f). Generally, primordia were developed from all cultured densities in agar-solidified MS medium provided by various concentration of BA. Numbers of primordia was varied in the different cultures. Interestingly, the densities of 1.0×10^5 and 1.0×10^6 produced numerous number of calli tissues in medium containing 0.5, 1.0 and 2.0 mg L^{-1} BA Table (1) and 4.0 , 6.0 and 8.0 mg L^{-1} BA Table (2). The produced calli, in all cases, was of friable type and green to yellowish-agreen in color Figure (1.g).

Table (1): Formation of callus primordia from hypocotyl cell suspension of Muskmelon, *Cucumis medo* L. in MS medium with BA embedded in agar drops .

Density ($x10^2$ cell/ml)	0.5		1.0		2.0	
	CC	CP	CC	CP	CC	CP
4.2	220	112	150	88	163	140
7.8	250	240	166	112	215	198
11.2	310	262	230	210	320	213
13.0	371	335	245	216	357	312
9.8	256	175	207	198	402	381
7.5	185	130	145	132	610	412

CC: cell colony, CP: callus primordia.

Values represent total number in drops.

Table (2): Formation of callus primordia from hypocotyl cell suspension of Muskmelon *Cucumis medo* L. in MS medium with BA embeded in agar drops.

Density ($x10^2$ cell/ml)	4.0		6.0		8.0	
	CC	CP	CC	CP	CC	CP
4.2	211	162	134	96	122	76
7.8	263	175	211	103	135	88
11.2	250	165	221	111	140	96
13.0	320	185	232	162	107	75
9.8	225	155	175	112	103	86
7.5	187	113	110	96	98	70

CC: cell colony; CP: callus primordial.

Valves represent total number in 20 drops

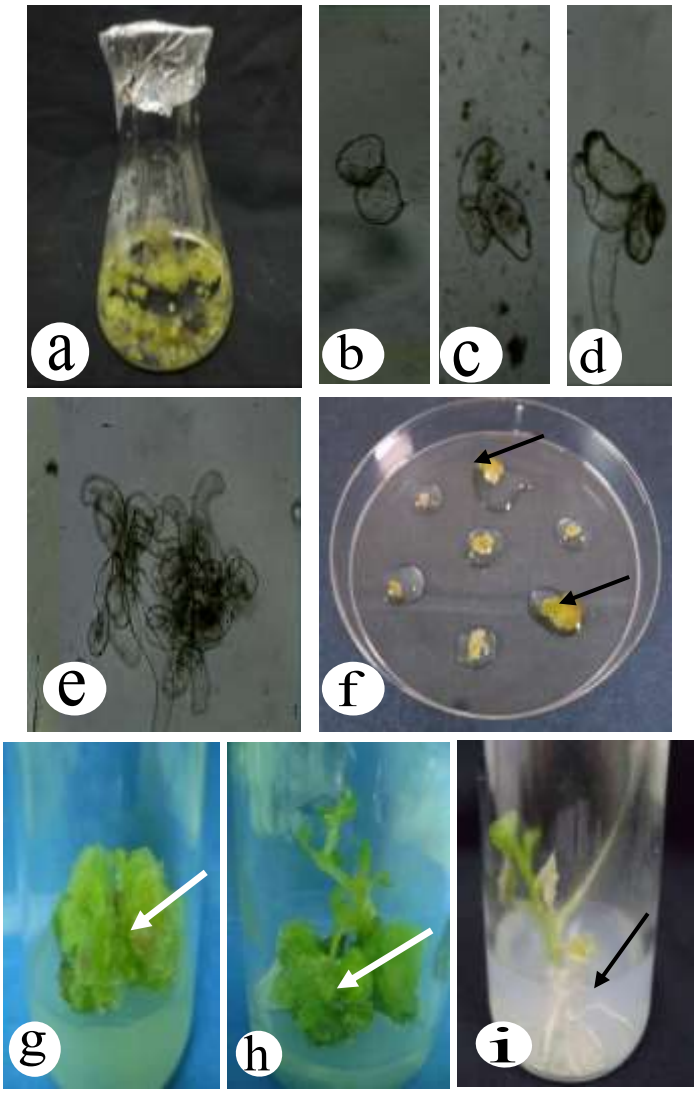

Fig. (1): Plant regeneration of muskmelon, Cucumis melo plants from hypocotyl cell-suspension derived calli.

a- Culture of cell suspension three days old.
b- Division of cells in (a) and development of colonies.
c,d- Formation of micro colonies.
e- Development of macro colonies.
f- Formation of callus primordia from colonies in (e).
g- Callus culture produced from primordia in (f).
h- Shoots regeneration on MS + BA medium.
i- Rooting of shoots (h) in agar-solidifed MS0 medium.

2- Picking – up callus primordia

Small pieces of produced callus were picked -up from agar drops, transferred to 25 ml aliquote of MS medium containing 0.5 mg L^{-1} BA, in 100 ml glass jars. These pieces of callus were sub cultured periodically forming typical callus culture that utilized in shoot regeneration experiments.

3- Regeneration of muskmelon plants

Many callus pieces were cultured on differentiation medium produced tiny green color clusters of structures after 35 days of culture figure (1. i). They were removed aseptically and each transferred to 100 volume glass jars containing 20 ml of the same differentiation medium when they became 5-10cm in length they removed for rooting.

4- Rooting regenerated muskmelon plants

Individual shoot was excised and plunged in 20ml aliquot of agar solidified MS0 medium Table (3). Rooted plants Figure (1. i) were removed from medium, washed with water thoroughly and transplanted to soil. The transplanted plantlets survive for one week then died.

Table (3): Regeneration and rooting of Muskmelon, *Cucumis melo* L. plants.

Experiment No.	No. of Calli	No. of Shoots	No. of Rooted shoots	No. of Transferred Plants
Exp. 1	10	9	8	6
Exp. 2	10	8	8	6

Discussion

The increasing of cells number in suspension cultures of *Cucumis melo L.* may due to the suitability of phytohormones that enhance cellular division subsequently [14]. This is in addition to the numerous single cells produced from hypocotyls callus together with other requirements available in culture medium [15]. Other workers reported that formation of callus from plant cells or protoplasts essentially depend on the hormone effect interaction to cerlain level of balance favorable to cells to start division [16].

The production of callus from cell suspension of cotyledonary explants of muskmelon, *Cucumis melo* using N6 medium provided by the addition of 2,4-dichlorophenoxy acetic acid (2,4-D) and benzyl adenine (BA) was reported [17]. In this study, hypocotyl-derived cells were potential in producing small pieces of callus from all densities cultured in all experiments, using multiple drop arrays (MDA) technique [18]. Although, in this study few shoots were regenerated from cell suspension derived callus, the fact that shoots regeneration potential in this species *C. melo* was exhibited by [19]. It seems that cell suspension – derived callus is much amenable in culture than explants callus. Therefore, the conclusions that cell suspension system probably represent an efficient strategy to overcome barriers facing callus differentiation from this plant.

References

1. Chee, P. P. (1991). Somatic embryogenesis and plant regeneration of squash *Cucurbita pepo L.* Plant Cell Repts. 9: 620-622.
2. Compton, M. E., Gray, D. J. and Elmstrom, G. W. (1993). Identification tetraploid regenerants from cotyledons of watermelon cultured *in vitro*. Euphytica. 87:167-172.
3. Kintozoms, S., Sereti, E., Bluchos, P., Drosopoulos, J. B., Kitsaki, C. K. and Liopa-Tsakalidis, A. (2003). Growth regulator pretreatment improves somatic embryogenesis from leaves of squash *Cucurbita pepo L.* and *Cucurbita melo L.* Plant Cell Repts. 21: 1–8.
4. Everson, J., Citadin, I. and Antonio, J. (2006). Effect of different culture medium in the *in vitro* regeneration of melon (Cucumis *melo L.*).
5. Souza, F. V., Sogo, B. G., Sonza, A. S., Juan, P. and Morento, V. (2006). Morphogenetic response of cotyledons and leaf explants of melon, *Cucumis melo* . Brazilian Archiv. Boil & Techn. 49: 21 – 27.

6. Sultana, R. S., Bari, M. A., Rahman, M. H., Siddique, N. A. and Khatun, N. (2004). *In vitro* rapid regeneration of plantlets from explants of watermelon *Citrullus lanatus* Biotech. 3:131 – 135 .

7. Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 54: 457 - 497.

8. Nasr, M. I., Ibrahim, A. I., Habib, H. M. and Kaplem, T. Y. (2006). Rapid Micropropagation of diploid and tetraploid watermelon (Citrullus *Lanatus)* cultivars. Plant Cell, Tiss. & Org. Cult. 1: 1 - 13.

9. Morris, P. and Fowler, M. W. (1981). A new method for the production of fine plant cell suspension culture. Plant Cell, Tiss. & Org. Cult. 1: 15 – 24.

10. Rõper, W. (1979). Growth and cytology of callus and cell suspension of *Vicia faba*. Z. Pflanzen physiol. 93: 245 – 257.

11. Kanai, R. and Edwards, G. E. (2010). Purification of enzymatically isolated mesophyll protoplasts from C3, C4 and CAM plants using aqueous dextran polyethylene glycol two phase system. Plant Physiol. 52: 84 - 90.

12. Majewska-Sawka, A., Nakashima, H., Mori, K. and Shimaoto, Y. (1990). Protoplasts isolation from mesophyll of *Beta* species . Proc. Japan. Soc. Sugar Beet Technol. 32: 129 - 133.

13. Tang, K. X., Zhan, E. P., HU, O. N., Yao, I. M. and Wo, A. Z. (2000). A simple and efficient procedure to improve plant regeneration from protoplasts isolated from long-term cell-suspension cultures of indica rice. *InVitro* Cell Develop. Biol-Plant. 36: 362 - 365.

14. Aequaah, G. (2004).Understanding Biotechnology, Pearson Education, Inc. U. S. A.

15. Hartman, H. T., Kester, D. F., Davis, F. T. and Genever, R. L. (2002). Plant Propagation, Prinicples And Practice, Hall, New Jersey, U. S. A.

16. Jimenez, V. W. (2001). Regulation of *in vitro* somatic embryogenesis with emphasis on the role of endogenous hormones. Rev. Bar. Fisiol. Veg. 13: 196 - 223.

17. Ordiate, T., Alsnmi, H. Ito, S, and Araki, H. (1992). Genetic differences in somatic embryogenesis from seeds in melo (*Cucumis melo L.*). Plant Cell, Tiss. & Org. Cult. 29: 27 - 30.

18. Dixon, R. A. (1985). Plant Cell Culture. A Practical Approach. IRL Press, Oxford.

19. Debeanjo, I. and Branchand, M. (1991). Somatic embryogenesis and organogenesis from protoplasts derived culture of muskmelon, *Cucumis melo L.* Acta Horticulture, 289: 225 - 226.