مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثالث والعشرون - المجلد التاسع - علوم الحياة - آب 2024 م
doi.org/10.52866/esj.2023.09.23.30

Induction and differentiation of *Momordica charantia* L. callus *in vitro*

Nebras Mahdi Salih
The General Directorate for Education of Diyala-Al-Naqaa secondary
Nebraska.sms@gmail.com

Abstract:

The current study was conducted with the aim of creating callus and the possibility of its differentiation into branches when parts of the stems and Apical tips of *Momordica charantia* plants *in vitro*. Tested parts of the stems and Apical tips separated from sterilized three-week age seedlings to induce callus and from vegetative branches supported by varying concentrations of the growth regulators MS medium alone (0.0,0.5,2.0,2.5) mg.L^{-1} Kin. The interaction was tested between Kin at concentration (2.0,2.5) mg.L^{-1} +(0.2) mg.L^{-1} IAA in replanting and sustaining callus. The results showed that the highest rate of callus formation and weight occurred in the Apical tips with a value of 70% and 4.9g in the medium supplemented with 2.5 mg.L^{-1} Kin. Followed by the stems that recoded the best development rat callus weight reaching 60% and 4.4g at center supplemented with 2.0 mg.L^{-1} Kin respectively. As for the effect of interfering with the nutrient medium containing 2.0 mg.L^{-1}Kin + 0.2 mg.L^{-1} IAA, the highest rate and fresh wight of stem callus was recorded at 5.74g compared to the Apical tips callus, which gave an average fresh weight of 4.85g with the supplemented medium with 2.5mg.L^{-1} Kin+ 0.2mg.L^{-1} IAA. The growing shoot formed a vegetative shoot of 4.5 shoots, vegetative part and average. The number of leaves is 20 leaves , the vegetative portion in medium supplemented with 2.5 mg.L^{-1} Kin, followed by the stems forming the vegetative branches at a rate of 3.5 the branch the vegetative portion and average number of leaves 16 leaves the vegetative portion in medium supplemented with 2.0 mg.L^{-1} Kin respectively.

Keywords: *Momordica charantia* , callus induction , plant growth regulators.

استحثاث وتمايز كالس نبات القرع المر خارج الجسم الحي

م.م نبراس مهدي صالح
مديرية تربية ديالى - ثانوية النقاء للمتفوقين
Nebraska.sms@gmail.com

مستخلص

اجريت الدراسة الحالية بهدف استحداث الكالس وامكانية تمايزه الى افرع عند زراعة اجزاء من السيقان والقمة النامية لنبات القرع المر خارج الجسم الحي. حيث اختبرت اجزاء من السيقان والقمة النامية المفصولة من بادرات معقمة بعمر ثلاث اسابيع لاستحداث الكالس وتكون الافرع الخضرية المزود بتراكيز متباينة من منظمات النمو MS وسط لوحده (0.0,0,5,1.0,2.0,2.5) ملغم.لتر$^{-1}$ Kin . واختبر تأثير تداخل بين Kin بتركيز (0.2,2.5) ملغم.لتر$^{-1}$ +(0.2)ملغم.لتر$^{-1}$ IAA في اعادة زراعة الكالس وادامته. اظهرت النتائج ان اعلى نسبة استحداث ووزن كالس حدثت للقمة النامية قيمة بلغت 70٪ و 4.9غم بالوسط المدعم بتركيز 2.5 ملغم.لتر$^{-1}$ Kin تلتها السيقان التي سجلت افضل نسبة استحداث ووزن كالس بلغ 60٪ و4.4 غم عند الوسط المزود بـ 2.0 ملغم.لتر$^{-1}$Kin. اما عن تأثير تداخل الوسط الغذائي المحتوي على 2.0 ملغم.لتر$^{-1}$ Kin+0.2 ملغم.لتر$^{-1}$ IAA فقد سجل اعلى وزن طري بلغ5.74 غم مقارنة بكالس القمة النامية التي اعطت معدل وزن 4.85 غم بالوسط المدعم بـ 2.5 ملغم.لتر$^{-1}$ Kin+0.2 ملغم.لتر$^{-1}$IAA. وكونت القمة النامية افرع خضرية بلغت 4.5 فرع/ جزء نباتي ومعدل عدد اوراق 20 ورقة/ جزء نباتي بالوسط المدعم بتركيز 2.5 ملغم.لتر$^{-1}$ Kin تلتها السيقان وكونت افرع خضرية بمعدل 3.5 افرع/ جزء نباتي ومعدل عدد اوراق 16 ورقة/ جزء نباتي عند الوسط المزود بـ 2.0 ملغم.لتر$^{-1}$ Kin على التوالي.

الكلمات المفتاحية: القرع المر، استحثاث الكالس، منظمات النمو النباتية.

Introduction

Momordica Charantia (Karela), is a creeping annual herbaceous plant. It is known in English as Bitter, melon, and is known by several common names in different languages, such as balsam pear and fuca in China, Ampalaya in the Philippines, Indian Karela, Tamil and Bengali Bramas [1]. It is a sessile or semi-sessile climbing plant that belongs to the Cucurbtiaceae family and is widely distributed in China, Malaysia, Turkey, East Africa, and the Caribbean [2]. All parts of the plant, including the fruits, would be characterized by their oval shape, the many protrusions that protrude from their outer surface, and their bitter taste [3]. It is grown in warm seasons, so the leaves, fruits, and seeds of the plant are used for medicinal purposes in treating diabetics, as it works to lower blood sugar levels in South America and Asia [4]. Bitter gourd contains three different groups of beneficial chemical components that act as effective agents in hypoglycemia. These compounds include a mixture of saponins and type of steroid known as *Charantia*, insulin-like peptide, and alkaloid compounds [5] [6]. It is widely used in the world to treat various diseases. Diseases by individuals residing in those areas to treat bronchitis, anemia, gonorrhea, cancer and to treatment gonorrhea[7]. In addition, the plant contains a group of biologically active phytochemicals, including alkaloids, steroids, and flavonoids, which make the plant antifungal, antibacterial, antiviral, and parasitic [8]. Callus is an irregular tissue of undifferentiated parenchymal cells that arise from areas of cut or Wounding of plant parts grown in nutrient medium to obtain different appearances of callus depending on the type of plant part and the components of the nutrient medium used [9].

Micropropagation is the use of tissue culture to propagate vegetatively and multiply them by growing small parts of plants such as buds, individual nodes, fruits, leaf parts, roots, ect. These parts grow in sterile and controlled media in terms of nutritional and environmental conditions because of the production they provide. Large numbers of plants that are free of pathogens and viruses and are genetically similar to the parent plant at any given time [10]. Among these plants is the bitter gourd plant *Momordica Charantia*. Sultana and Barimaih [11] indicated the possi-

bility of micro vegetative propagation of the bitter branch plant by cultivating tissue nodes and roots derived from seedings growing in sterile conditions and on MS medium supplemented with different concentrations of auxin. And cytokinines. Malike et al [12] were able to study the effect of growth regulators on the production of callus from plant cuttings extracted from bitter gourd seeding, and then the differentiation of the resulting callus. This study aimed to identify the response of the *Momordica charantia* to tissue culture system and the extent of this plants ability to from complete plants from callus.

***Momordica charantia* (Karela) as Bitter melon**

Materials and methods

1- Seed source and sterile seedling production

The experiment was carried out in the plant tissue culture laboratory of Department of life Sciences / College of Education for Pure Science, University of Diyala during the period from 2022 to 2023. The seeds of the bitter gourd plant, *Momordica charantia*, were obtained from local markets imported from India. The seeds were sterilized the day before planting them with water. The next day, they were washed with liquid soap for five minutes to remove any materials stuck to them. They were placed under running water for 15 minutes, after which they were changed the seeds with a solution. Ethyl alcohol at a concentration of %70 for one minute. Then the seed were sterilized by rinsing with commercial grade containing %6 sodium hypochlorite NaOCL which is available in local markets in a ratio of one volume of sterile distilled water for 15 minutes. Then the seeds were washed with sterile distilled three times at a rate of five minutes once to remove the rough coating [13]. The sterilized seeds were planted by placing them on the surface of 20 ml of solid [14]. Me-

dium free of growth regulators in 250 ml glass bottles at a rate of one seed in each bottle. The samples were preserved. Cultivated in a growth room under a light intensity of 1000 lux, a light succession of 16 hours of light and darkness, and a temperature of 25+2m.

2- Callus induction

Sterile seedlings grown in solid MS medium were grown under sterile condition. old 21 days as a source of plant parts. The stems and the Apical tips were separated after their edges were removed using a sterile scalpel. The plant parts were planted in 100ml glass bottles on solid MS medium supplemented with Kin at a concentration of (0.0,0.5,1.0, 2.0, 2.5) $mg.l^{-1}$ .The wet weight of the callus was calculated by weighing the sample in which the plant part was grown after planting and after 50 days, as the difference between the two readings, the weight of the callus.

3- Perpetuating callus

After the formation of callus from parts of the stems and the apical tip, the seedlings of the *Momordica charantia* plant were cut into pieces weighing 1g and replanted on test media for perpetuation, represented by solid MS medium supplemented with a concentration of (2.0,2.5) $mg.l^{-1}$ Kin mixed with IAA at a concentration of 0.2 $mg.l^{-1}$ in glass bottles of size 100. Estimate fresh weight of callus grown on the permaculture media eight weeks after replanting using a weighing scale sensitive.

4- Vegetative branches are formed from calluses Stems and Apical tips

Transferring the newly created callus from a piece of stem and Apical tip, weighing approximately 1g and at 20 days old, marked the surface of solid MS medium supplemented with (0.5, 1.0, 2.0, 2.5) $mg.l^{-1}$ Kin

5- Statistical analysis

A completely randomized design (CRD) was used to implement the study experiments, and the data were analyzed using the program (SAA,1996) and the means were compared according to the Duncan multinomial test under a probability level of %5 [15] Each treatment included 10 replicates. Each replicate contained a part one vegetarian.

Results and Discussion

Production of sterile seedling

The results of the surface sterilization method for the seeds of *Momordica Charantia* plants used showed its efficiency. In terms of obtaining healthy seedlings with complete rooting, plum-

age, and the nature of growth, after several days of planting,(Figure 1، A.) which were characterized by elongated stems and leaves in shape , The suitability of the surface sterilization method for the seeds of the *Momordica Charantia* used in the study showed its characteristics by obtaining healthy signs and good vitality, the use of various sterile materials depends on the time of exposing the seeds as well as their important concentrations and the production of sterile and healthy seedings that are not ripened from sterilization processes [16].

Figure (1): Seedling of a 21-day old *M. Charantia*

Callus induction.

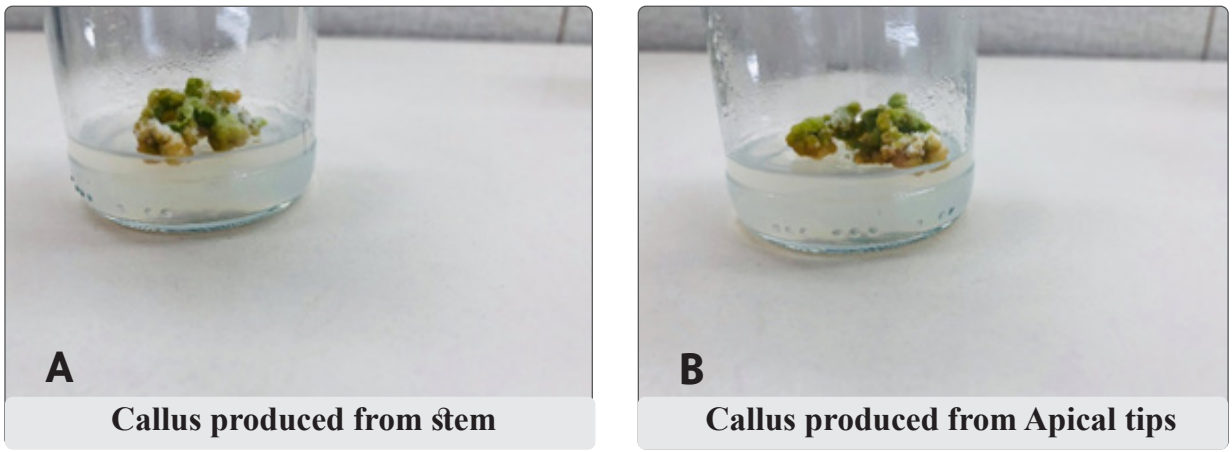
The study showed that the best rate of stem callus generation was %60 with the MS medium supplied with a concentration of 2.0mg.L^{-1} Kin, which in turn gave the largest amount of callus formation, 4.4g, and with a significant difference, the concentration gave 2.5mg.L^{-1} Kin production rate and callus weight reached %30 and1.11 g while the two concentration were 0.5and 1.0mg.L^{-1} of Kin gave the lowest rate of production and a callus weight of %20 , 0.8 g and 1.5g, respectively.

superiority of the apical tips in giving a growth rate and callus weight of %70 and 4.9g when grown in MS medium supplemented with 2.5 mg.L^{-1} Kin, and the treatment concentration was achieved at 2.0mg.L^{-1} Kin. The rate of growth and callus weight of callus reached %50 and1.12 g. The lowest rate of creation and weight of callus was at the two concentrations of 0.5 and 1.0 mg.L^{-1} Kin reached %10, %30 and 1.5g, 1.8g. As for the comparisons treatment, there was no response in the formation of callus tissue in the two parts cultivated vegetarions (Figure 2، A,B).

Table (1) Effect of Kin in MS medium on callus generation from stems and apical tips of *Momordica charantia* plants on solid MS medium.

Growth regulators Kin mg.L^{-1}	Apical tips		Stems	
	Callus weight g	Response rate %	Callus weight g	Response rate %
0.0	c 0	c 0	c 0	c 0
0.5	b 1.5	b 10	c 0.8	b20
1.0	b 1.8	cd 30	b 1.5	b20
2.0	b1.12	b 50	a 4.4	a60
2.5	a 4.9	a 70	b1.11	b30

Means with similar in each column are not significantly different by Duncan's multiple range test %5



Callus produced from stem **Callus produced from Apical tips**

Figure (2): Callus production on different parts of *M. Charantia* plants.
A- Callus produced from stem pieces at a concentration of 2.0mg.L^{-1} Kin.
B- Callus produced from Apical tips pieces with 2.5mg.L^{-1} Kin

Perpetuating callus

The results of Table (2) showed that the effect of the interaction between the concentration of auxin and cytokinines led to a clear increase in the average wet weight and an increase in the size of the induced callus mass of the plant part, after replanting it on a multiplying medium consisting of 2.0mg. L^{-1} Kin+ 0.2mg.L^{-1} IAA had the highest value of 5.74g, with a significant difference recorded in medium enriched with 2.5mg. L^{-1} Kin + 0.2mg. L^{-1} IAA the callus weight reached 3.22g, which was distinguished by its yellow color and firm texture. In same context the results showed that the treatment with 2.5mg.L^{-1} Kin+ 0.2mg. L^{-1} IAA was superior in giving the highest callus weight. For the apical tip, it reached 4.85g which was distinguished by its green yellow color, and with a sig-

nificant difference, give the medium supplemented with 2.0mg.L^{-1} Kin+ 0.2mg.L^{-1} IAA and the callus weight reached 2.75 which was distinguished by its light yellow color respectively (Figure4, A,B,C,D).

Table (2) The effect of interacting with Kin with IAA in replaced from callus produced from *Momordica charantia* seedling parts in solid MS medium.

Growth regulators mg.L^{-1}	Apical tips Callus weight g	Stem Callus weight g
Kin+ IAA		
2.0+0.2	b2.75	a5.74
2.5+0.2	a4.85	b3.22

Means with similar in each column are not significantly different by Duncan's multiple range test %5

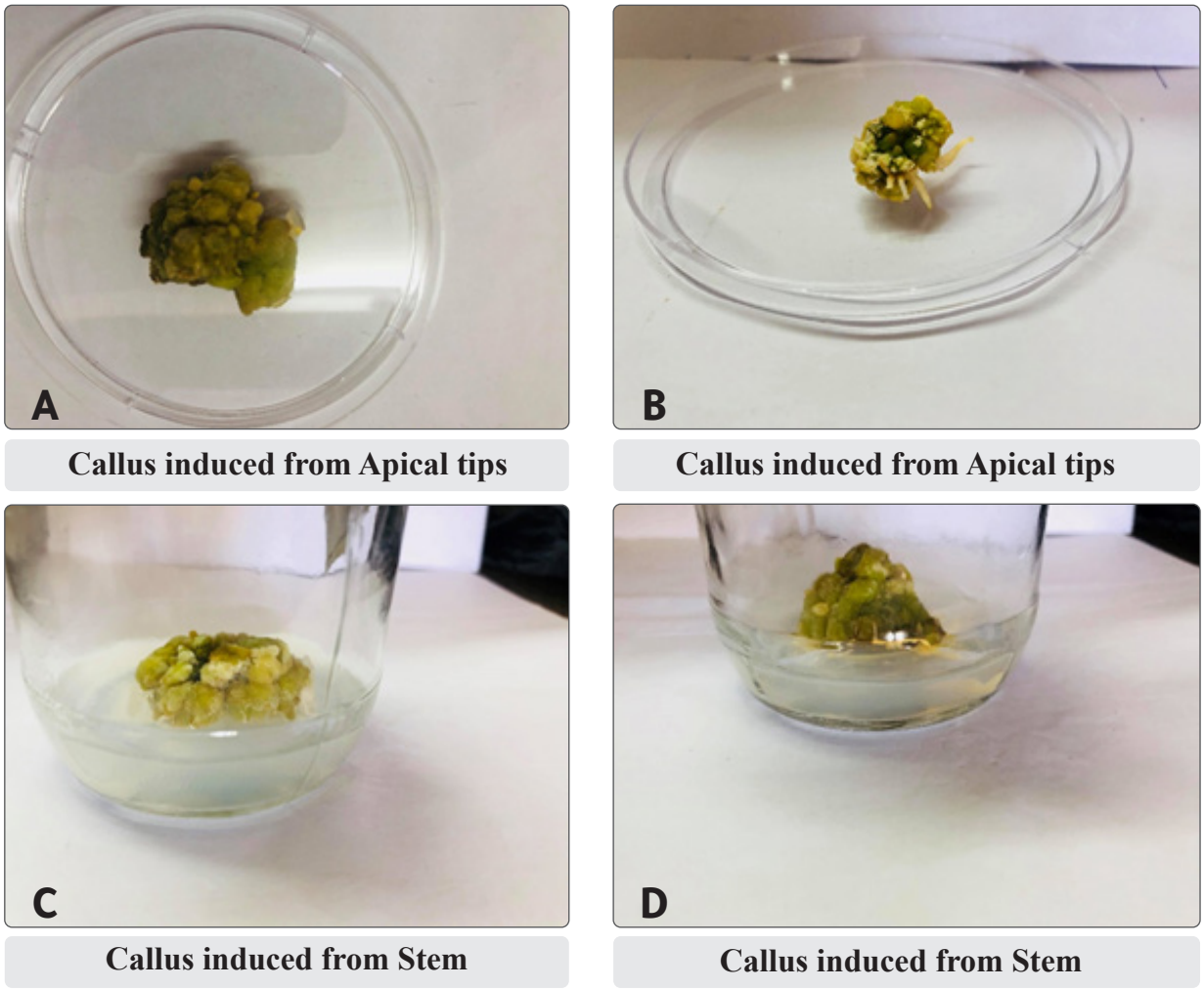


Figure (3): The effect of interfering with IAA with Kin in replanting callus from parts of *M. Charantia* plants on solid MS medium. A- Callus induced from Apical tips parts with 2.5 mg.L^{-1} Kin+0.2mg.L^{-1} IAA. B- Callus induced from Apical tips parts with 2.0 mg.L^{-1} Kin+0.2mg.L^{-1} IAA. C-Induced callus from parts of stems with 2.5mg.L^{-1} Kin+ 0.2mg.L^{-1} IAA. D- Callus induced from stem parts with 2.0mg.L^{-1} Kin+0.2mg.L^{-1} IAA

Vegetative branches are formed from calluses Stems and Apical tips

The results of the Table (3) indicate that planting parts of the stems of the *Momordica charantia* plant showed the ability to form vegetative branches at a rate of 3.5 branches/ plan part bearing the largest number of leaves, amounting to 16 leaves/ plant part at the MS medium supplemented with 2.0mg.L^{-1} Kin. Which differed significantly from the solid MS medium supplemented with a concentration of 2.5mg.L^{-1} Kin, with a number of branches amounting to 2.8 branches/ plant part and carrying to 9 leaves/ plant part. With a significant difference, the two concentrations of 0.5 and 1.0mg.L^{-1} Kin were vegetative branches. It reached 1.0 and 1.6 shoots/ plant part and 3 leaves/ plant part, respectively. The result show that the differentiation of apical tips callus formed vegetative branches amounting to 4.5 branches/ plant parts, and the largest number of leaves was recorded, amounting to 20 leaves/ plant parts, for the medium supplied with MS with concentration of 2.5mg.L^{-1} Kin, while the medium supplemented with 2.0 mg.L^{-1} Kin gave vegetative shoots amounting to 3.0 shoots/ plant parts and 15 leaves/ plant part, and with a significant difference, the two concentrations of 0.5 and 1.0mg.L^{-1} Kin gave vegetative shoots amounting to 1.5 and 1.8 shoots/ plant part and 5,4 leaves/ plant part respectively.(Figure 3, A,B,C,D).

Table (2) Formation of vegetative shoots from stem callus and apical tips of *Momordica charantia* plants on solid MS medium

Growth regulators Kin mg.L^{-1}	Apical tip		Stems	
	Number of papers	Number of branches part/ plant	Number of papers	Number of branches part/ plant
0.5	c 5	c 1.5	c 3	c 1.0
1.0	c 4	c1.8	c 3	c 1.6
2.0	b 15	b3.0	b16	a 3.5
2.5	a 20	a 4.5	a19	b2.8

Means with similar in each column are not significantly different by Duncan's multiple range test %

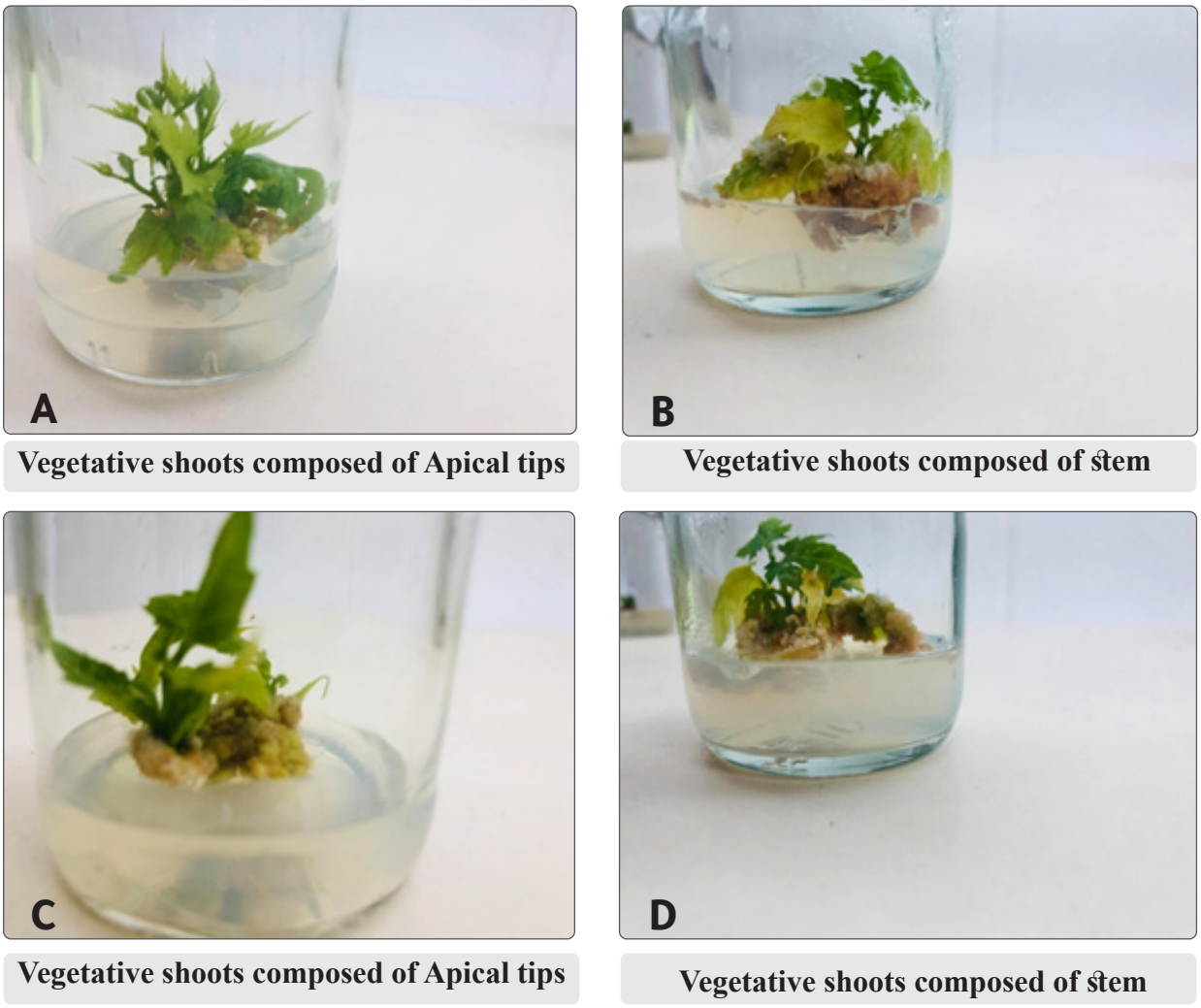


Figure (4): Formation of vegetive shoots from callus of M. Charantia on solid MS medium supplemented with the addition of growth regulator Kin. A-vegetative shoots composed of Apical tips callus with 2.5mg.L-1. B- Vegetative shoots composed of stem callus with2.0mg.L-1. C- Vegetative shoots composed Apical tips with 0.5mg.L-1. Vegetative shoots composed of stem callus with 1.0mg.L^{-1}

The formation of callus from bitter gourd (*Momordica charantia*) in this study is an important and desirable case due to the lack research that has dealt with achieving this matter. Therefor the study aimed to identify the response of the *Momordica charantia* to the tissue culture system and the extent of this plants ability to form complete plants from callus. As it depends on the type of plant, the sterilization material used, and the internal content of plant hormones and the growth regulators added to the nutritional media used.

The part is the plant is a determining factor in the development of callus [17]. The variation in the response of plant parts to callus induction may be due to the effect of the added growth regulator and the difference in their internal content of plant hormones and growth regulators added to the nutrient media used [18] [19]. Although the presence of auxins is of great importance in inducing callus, alone or with the presence of cytokinines, it leads to a balance between the externally added regulators and internal hormones to obtain the best cell division. However, adding cytokinines alone to the medium led to the induction of callus from plant parts, and the reason may be attributed to this. In herbaceous plant, the hormonal content of auxin is sufficient to produce a response to callus induction without external addition [20] [21]. Fresh weight is considered one of the most important biological measures for the growth of newly developed callus, as growth regulators played a distinct role in this path through its direct effect on the construction of proteins and nucleic acids by stimulating the important enzymes in their construction. This is what was found in terms of an increases in fresh weight rates after 40 days of growth [22].

The effect of the interaction of auxin with cytokinines was clear in increasing the wet weight of callus on the tested permaculture media. The reason for this is that the creation of callus, its increase in quantity, and the formation of roots may be the result of the physiological balance between auxin and cytokinines, and adding both to the culture medium is necessary for the creation of callus, as it works cytokinines

in the presence of auxin is a key to starting cell division, and the adenine that formed the cytokinines molecule may be the part that led to the optimal balance (23). The reason for the difference in wet weight rates of plant parts is also due to the potential energy of the cells and their number, which supports them to divide at rates to from callus [24]. The ratios of auxins to cytokinines that are added to the nutrient medium determine the growth trends of the cultivated cells, and they can control the morphological composition by changing these ratios (25). Auxins encourage cell expansion, division, and elongation. Which comes from increasing the plasticity of the cell wall, and this role is completed by the pres-

ence of cytokinines, which stimulate cell division, because it contains auricles. Thus, the presence of auxins and cytokinines together in nutrient media is essential for the direction of growth cultured cells for morphological formation and for the formation of callus from plant parts [25].

The formation of vegetative aggerates from callus is an essential step in the integration of the system for producing new plants using tissue culture technology. Therefore, the results demonstrated the ability of apical tips are the best part of the plant in producing the highest number of leaves compared to the stems. This is attributed to the presence of physiological factors related to the tissue content of food, hormones, and the production of auxins for their ability to re-differentiate and from vegetative branches [26]. The ability of stems to differentiate and from vegetative branches is due the occurrence of a state of balance between the internal hormones of the cultivated plant part with the external growth regulators added to them, and the achievement of a state of balance between them, which led to the formation of branches. On the other hand, the process of callus formation of vegetative branches, the level of hormones and vitamins as well as the components of the nutrient medium and the external addition of growth regulators concentration that enhance cell division of plant parts and their dependence on the source [27].

The reason may be that the growth of callus and its incitement to differentiation and organ formation a accomplished by means of various growth additives, and to the presence of stimulation resulting from endogenous growth materials, or the compatibility of the nutrient medium and the external addition of growth regulators, the cells will divide, grow, and the resulting plant tissues will differentiate. Scientists have proven that the number of branches the vegetative effect resulting from the differentiation of the plant piece depends on the genetic composition of plant piece itself and on the concentration of growth regulators [28].

We conclude from this current study that the apical tip is superior to stems in the rate of callus generation when using Kin alon, as the possibility of generating callus and forming vegetative branches from parts of the apical tip and stems. The stems excelled in increasing the rat of wet weight and volume when using the effect of the inter-

action of IAA with Kin. Therefore, it is necessary to subject this plant to for further research in the field of tissue culture because it is one of the important plants from a medical standpoint.

References

1. Jadhv D. Medicinal plants. A Compendium of 500 species, ed Orient Longman Ltd., Madras. 2008; 4: 48-51.

2. Thiruvengadam, M, Rekha K. T, Yang C. H, Tayabalan N. Chung. T.M. High Frequency shoot regeneration from leaf explants through organogenesis in bitter melon (*Momordica charantia* L.) plant Biotechnology Reports. 2010; 4. 321-328.

3. Taylor, L. ((Tropical plant databases. Available at: http:// www. tree.com /plants. htm. Accessed)). 2004; 08/10/04.

4. Nadarni, K. M. Indian Materia Medica. 1993; Vol (1) pp: 805-806.

5. Agarwal, M. and Kamal, R. In vitro clonal propagation of *Momordica charantia* L. Ind J. Biotech. 2004; Vol 3pp 426-430.

6. IBakare, R.; Mabgabeole, O.A.; Akinwande, A.L. and Okunowo,O.W. Nutritional and Chemical evaluation of Momordica charantia J. Med Plant Research. 2010; Vol 14(21), pp: 2189-2193.

7. Madhu, G.; Suhil, S.; Ajay, G. and Rekha, B. *Momordica charantia* Linn. (Karela): Natures Silent Healer. Int J, pharmaceu Sci Res,2011; Vol 11(1), pp: 32-37.

8. Zafar, R. and Neerj, A. Momordica charantia- a review Hamdard Medicine 1991; 34pp: 49-61.

9. George, E. F., and P.D. Sherrington. Plant propagation by tissue culture.2008; Fourth edition. Ltd. England

10. Gupta, S.D. and Iarakl Y. Plant Tissue Culture Engineering.2006; Vol. 6. The Background. Springer. 469 pages.

11. Sultana, R. S. and Bari Miah, M.A. In vitro propagation of karalla (*Momordica charantia* L.) J. Biol, Sci., 2003; Vol3(12) pp: 1134-1139.

12. Malike, S.; Zia, M.; Rehman, R. and Chaudhary, F. In vitro regeneration from direct and indirect organogenesis of *Momordica charantia*. Pak, j. Biol. Sci., 2007; Vol (10), pp: 4118-4122.

13. Borkowska, B. Micropropagation of sour cherry cultivars Schatten

morelk. fruit Sci. Rep. 1983; 10: 59-66.

14. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant., 1962; 15: 473-497.

15. Al- Sahoki, M.; and Karima, MW. Application in the design and design and anlaysis of experiments Baghdad.1990; University Ministry of Higher Education and Scientific Research Iraq.

16. Nabi, A., Rashid, M., AL-Amin, M. and Rasul, M. G. Organogenesis in teasle gourd (*Momordica dioica* Roxb.) Plant Tissue Cult., 2002; Vol (12) pp:173-180

17. Anzidei, M.; Bennici ; A.; Schiff. S.; Tani, C.; Mori, B. Plant cell Tissueand organ culture, 2000; 61(1):69-79.

18. Abu Zaid, N. Plant hormones and agricultural application. 2003; Second Edition, Arab House for publishing and Distribution, Egypt.

19. Al-Mamari, A.; Muhmamed S.; Bashar, K. B;. and Iyad G. AL. Determination of alkaloids callus in plant Catharanthus roseus L. *in vitro*. Syrian Journal of Agricultural Research. 2018; 5(3):21-36.

20. Delloloio, R. Cytokininus determine Arabidopsis root-meristem size by controlling cell differentiation. Curr. Biol.,2007; 17: 678-682.

21. Hedden. P and Stephen, G. Plant Signaling. Black well publisher Ltd. 2006; pp: 97-101.

22. Muhammad, A.; and Rana, T. Differention from the callus of the Stems of Subcotyledonous white *Lupinus albus.* Iupine plants. Mesopotamia, Jounral. 2018;18(5):99-102.

23. Bryant, J. and D. Chianat. Plant Cell proliferation and its Regulation in Development. 1997; Jhon wiley an Sons. New York.

24. Beloin, N.; Gbeassor, M.; Akpagana, K.; Hudson, J.; de Soussa, K.; Koumaglo, K. and Arnason, JT. Ethonmedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J, Ethnopharmacol.2005. 96 pp 49-55.

25. Moore, T. C. Physiology and Biochemistry of Plant Hormones. 1976; Acadimic Press, New York.

26. Bowes, S. G. A colour Atals of plant propagation and Conserva-

tion. Manson publishing. 1990; Ltd, London. U.K.

27. Hartmann, H.T.; Keter, D.E.; Davies, F.T. and Geneve, R.L. Plant Propagation, Principles and Practices. 7th Ed. 2000; Prentice Hall, Upper Saddle River, N.J.

28. Al- Akidi, H.; Nawaf, and Bashar. Z. K.B. Nodules multiplication, Development and differentiation of plant Atropa belladonna L.in Iraq, Tekrit Univresity Journal of Agricultral Sciences.2018; 18(1):99-112.