Plant Cell Biotechnology and Molecular Biology 21(49&50):1-9; 2020 ISSN: 0972-2025

DIRECT SHOOT REGENERATION BY *In vitro* CULTURE OF THE GERBERA (*Gerbera jamesonii* Bolus) CAPITULUM EXPLANTS

MAJID ABDULHAMEED IBRAHIM* AND MAHA MAHDI YASSIN
Department of Horticulture and Landscape Design, Faculty of Agriculture, University of Basrah, Basrah, Iraq [MAI, MMY].
[*For Correspondence: E-mail: majid.abdulhameedl@uobasrah.edu.iq]

Article Information

Editor(s):
(1) Dr. Moaed Al Meselmani, The University of Sheffield, England.
Reviewers:
(1) Bhausaheb Pawar, Humboldt University of Berlin, Germany.
(2) M. Prashanti, Osmania University, India.

Received: 03 August 2020
Accepted: 09 October 2020
Published: 31 October 2020

Original Research Article

ABSTRACT

This study was conducted in the Plant Tissue Culture Laboratory, College of Agriculture, University of Basrah during the 2018/2019 growing season. The aim of the study was to use the capitulum segments as explants in micro propagation of Orange and Malibu cultivars of gerbera plants. The results showed that the capitulum segments of the gerbera plant, Orange and Malibu cultivars that culturing by *in vitro* as explants led to the direct proliferation of adventitious shoots. The results also indicate the significant superiority of the Orange cultivar in its response rate to direct shoot regeneration, which reached 86.72% after 12 weeks of culturing. While the Malibu cultivar was significantly superior in the number and length of the shoots that were formed from direct organogenesis, which reached 10.48 shoots explant^{-1} and 1.78 cm respectively. The concentration of 1.0 mg L^{-1} BA was significantly superior to the other concentrations in the response of explants to direct shoot proliferation and the number and length of shoots recorded 99.22% and 15.90 shoots explant^{-1} and 1.78 cm, respectively. While the explants that cultured on the MS medium that supplied with 5.0 mg L^{-1} BA recorded the lowest response to direct shoot proliferation and the number and length of shoots, which reached 27.50%, and 2.00 shoots explant^{-1} and 0.35 cm, respectively.

Keywords: Adventitious shoot; benzyl adenine; organogenesis; proliferation; rooting.

INTRODUCTION

Gerbera belongs to the Asteraceae family, and it is a perennial herbaceous plant, the common name of the plant is Transvaal Daisy, Barberton Daisy or African daisy [1]. The first to call the gerbera plant was the German naturalist Traugott Gerber and its species were classified by Harry Bolus. Royal Botanic Gardens in Kew, UK was scientifically named under the name *Gerbera jamesonii* [2]. It is ranked fourth as cut flowers in the world after Rose, Chrysanthemum and Tulip [3]. Its flowers are distinguished by their long life after cutting, their resistance to transport damage

and the high prices [4]. Gerbera plants are propagated by division or suckers, and these methods produce a relatively low multiplication rate. As for the method of seed propagation, it is not desirable because the plants obtained from culturing seed are not similar to the mother plant, because the plant is heterozygous [5]. Therefore, some scientists and researchers resorted to the micropropagation of gerbera plants with the aim of rapid multiplication and large numbers of cultivars that have high specifications to obtain true to type plants and free of pathogens and insect infestations through the use of plant organ segment as explants by *In vitro* technique [6]. Also, plant growth regulators have an important role in determining the success of gerbera propagation. Since they are added at low concentrations and absorbed by plant tissues, then they move to their worksites and are linked to a receptor, to stimulate the cell's activity [7-9]. Auxins and cytokinins are the most used in tissue cultures and are among the most important ingredients in culture media, and their effect is directly on the growth of the cultured explant, and plant tissue culture can be considered unsuccessful without these regulators [9-11]. Cardoso and Silva [12] used some explants to propagate the gerbera plant tissue as the shoot tips were the most common explant in micropropagation in addition to inducing the direct adventitious shoots from the floral capitulum. Parvin et al [13] were used also different explants of the gerbera plant (flower buds, leaf segments, leaf petiole and capitulum) and cultured them on MS media, where different combinations of BA at 1, 2, 3, 4, 5 mg L^{-1} and 0.0, 0.01 mg L^{-1} NAA was added to them. The combination of the concentration of 3 mg L^{-1} BA and 0.01 mg L^{-1} NAA recorded the highest response to shoot regeneration, and the flower buds and capitulum were the best explants for propagating the gerbera plant, respectively. Kumari et al. [11] were reported micropropagation of gerbera by culturing the unmaturated capitulum at a diameter of 0.5- 1.0 cm and maturated capitulum at a diameter of 1.5- 2.0 cm. They have obtained shoot proliferation when culturing the explants on MS medium supplemented with 10 mg L^{-1} BA and 1.0 mg L^{-1} IAA as the number of shoots from multiplication reached 10.51 shoots after 47.51 days of culturing. This study aims to micropropagation two cultivars of gerbera (Orange and Malibu) plant by using capitulum segments as explants to obtain large numbers of these two cultivars of this plant that have good properties.

MATERIALS AND METHODS

This study was conducted in the Plant Tissue Culture Laboratory, College of Agriculture, University of Basrah during the 2018/2019 growing season. The medium was prepared from ready-made MS salts [14] produced by Cassion Labs, USA, that added to the medium at a concentration of 4.440 mg L^{-1}. Then, organic compounds consisting of 30 g L^{-1} sucrose, 1.0 mg L^{-1} vitamin for each of nicotinic acid, glycine, Thiamin-HCl and pyridoxine-HCl, 100 mg L^{-1} myo-inositol, 80 mg L^{-1} adenine sulfate, 200 mg L^{-1} sodium hydrogen orthophosphate and 1.0 g L^{-1} polyvinyl pyrrolidone were added. Growth regulators, auxins and cytokinins, were added to the medium at the required concentrations according to the objective of the experiment. Then the pH was adjusted to 5.7-5.8 with titration solutions NaOH and HCl at 0.1 N to each of them. Then Agar was added at 7 g L^{-1} after the medium became homogeneous and clear. The MS medium was poured into the culture tubes of 2.5 x 18 cm in an amount of 20 ml per tube. Then the nozzles of tubes were closed with medical cotton and aluminium foil. The other experiments used glass jars of 4.5 x 15 cm 3 and put 25 ml of MS medium in them. Culture vessels and their contents were sterilized by Autoclave under the pressure of 1.04 kg cm^{-2} and temperature of 121°C for 20 minutes. After the sterilization was completed, the culture tubes were removed from the Autoclave. The culture vessels were cooled at room temperature and kept in the growth room until use in the culture.

In this study, capitulum segments were used as explants that were taken from gerbra (*Gerbera jamesonii* Bolus) plant Orange and Malibu cultivars, obtained from a nursery in Baghdad, Iraq. The capitulum of 1.0 cm in diameter was then cut with a blade into four pieces (Plate 1, A). Then these explants were washed with liquid soap and water, and then washed with distilled water three times. The explants of each cultivar were separately placed in a beaker containing an

antioxidant solution of 150 mg L^{-1} citric acid and 100 mg L^{-1} ascorbic acid for 30 minutes. After that, surface sterilization was performed for them after she was removed from the antioxidant solution. Then the explants of each cultivar were placed in 70% ethyl alcohol for 1-2 minutes and washed with sterile distilled water three times to remove the sterilization solution residue. Then, it was dipped in the Elsa fungicide solution at 500 mg L^{-1} for 15 minutes. Then it was washed with distilled water three times and then placed in a 0.1% mercury chloride solution with 2-3 drops of Tween 20 for 8 minutes. Then they were washed with sterile distilled water three times. The sterilized explants were cultured in glass tubes and jars that contain the previously prepared MS medium. The cultures were then incubated in the growth chamber at a temperature of 25 ± 2ºC and 1000 lux light density for 16 light hours per day. Data were recorded after 12 weeks of culturing.

Effect of Different Concentrations of BA on Direct Shoot Regeneration

The capitulum segments were cultured by five replicates on MS medium supplied with different concentrations of BA at 1.0, 2.0, 3.0, 4.0, 5.0 mg L^{-1} and constant concentration at 0.1 mg L^{-1} NAA and 0.5 mg L^{-1} GA3. The following data were recorded after 12 weeks of culturing:

1. The Response to direct shoot regeneration (%).
2. The number of shoots per explant.
3. Shoot Length (cm).

Effect of Different Concentrations of Auxin NAA on Rooting

The shoots that producing from direct organogenesis were cultured in five replicates for each treatment in MS medium supplied with different concentrations of auxin NAA at 0.1, 0.3, 0.5, 0.7and 0.9 mg L^{-1} plus the constant concentration of BA at 0.1 mg L^{-1}. The following data were recorded after 12 weeks of culturing:

1. The response to rooting (%).
2. Number of roots per plant.
3. Root length (cm).

Experiment Design and Statistical Analysis

The experiments of this study were designed according to the Randomized Complete Design. The results were analyzed using the statistical program SPSS version 24. The mean of the treatments were compared between them by using the Revised Least Significant Difference Test at a probability level of 1% according by Al-Rawi and Khalaf Allah [15].

RESULTS AND DISCUSSION

The Plate 1, B-J show the direct shoot regeneration stages from the capitulum cultures of the gerbera plant by *in vitro* culture. As the cells of the capitulum tissues began to swell and discolor white (Plate 1, B-D). Then the cells of this tissue differentiated, resulting in the emergence of adventitious vegetative buds, colored green (Plate 1, E-H). After that, the shoots were formed directly from the growth of the adventitious vegetative buds and their elongation 12 weeks after culturing (Plate 1, I-J).

The results from Tables 1, 2 and 3 indicate the significant superiority of the Orange cultivar in its response rate to direct shoot regeneration, which reached 86.72% after 12 weeks of culturing. While the Malibu cultivar was less responsive to direct organogenesis, which was 79.72%. As for the Malibu cultivar, it was significantly superior in the number and length of the shoots that were formed from direct organogenesis, which reached 10.48 shoots explant^{-1} and 1.78 cm respectively (Plate 1, L). While the Orange cultivar recorded the lowest mean number and length of shoots, which reached 6.56 shoots explant^{-1} and 0.86 cm respectively (Plate 1, K).

The same tables also show that the concentration of 1.0 mg L^{-1} BA was significantly superior to the other concentrations in the response of explants to direct organogenesis and the number and length of shoots recorded 99.20% and 15.90 shoots explant^{-1} and 1.78 cm, respectively after 12 weeks of culturing. While the explants when cultured on the MS medium supplied with 5.0 mg L^{-1} BA recorded the lowest response rate to direct shoot regeneration and the number and length of shoots, which reached 27.50%, and 2.00 shoots explant^{-1} and 0.35 cm, respectively.

Table 1. The effect of cultivar, BA concentration and the interaction between them on the response of capitulum explants to direct shoot regeneration after 12 weeks of culturing

Cultivar	BA concentration (mg L^{-1})					Cultivar effect
	1.0	2.0	3.0	4.0	5.0	
Orange	99.20	98.80	98.40	89.20	48.00	86.72
Malibu	99.20	98.40	97.20	96.80	7.00	79.72
BA effect	99.20	98.60	97.80	93.00	27.50	
RLSD p≥0.01	Cultivar		BA concentration		Cultivar × BA conc.	
	**		4.09		5.78	

**: High significant

Table 2. The effect of cultivar, BA concentration and the interaction between them on the number of shoots after 12 weeks of culturing

Cultivar	BA concentration (mg L^{-1})					Cultivar effect
	1.0	2.0	3.0	4.0	5.0	
Orange	13.20	9.20	5.60	3.40	1.40	6.56
Malibu	18.60	13.20	10.20	7.80	2.60	10.48
BA effect	15.90	11.20	7.90	5.60	2.00	
RLSD p≥0.01	Cultivar		BA concentration		Cultivar × BA conc.	
	**		0.836		1.259	

**: High significant

Table 3. The effect of cultivar, BA concentration and the interaction between them on the shoot length after 12 weeks of culturing

Cultivar	BA concentration (mg L^{-1})					Cultivar effect
	1.0	2.0	3.0	4.0	5.0	
Orange	1.92	1.06	0.60	0.40	0.34	0.86
Malibu	1.64	2.06	2.26	2.58	0.36	1.78
BA effect	1.78	1.56	1.43	1.49	0.35	
RLSD p≥0.01	Cultivar		BA concentration		Cultivar × BA conc.	
	**		0.177		0.250	

**: High significant

The interaction treatment between the Orange or Malibu cultivar and a concentration of 1.0 mg L^{-1}BA recorded the highest response rate to direct shoot regeneration, which recorded 99.20% in both treatments, which did not differ significantly with most of the interaction treatments. While the interaction treatment between the Malibu cultivar and the concentration of 5.0 mg L^{-1}BA recorded the lowest response rate to direct organogenesis, which was 7.00%, which differed significantly with all interaction treatments (Table 1). The interaction treatment between the cultivar Malibu with a concentration of 1.0 mg L^{-1}BA recorded the highest mean number of shoots that were formed from direct organogenesis, which amounted to 18.60 shoots $explant^{-1}$, which differed significantly with all interaction treatments. While the interaction treatment between Orange cultivar and the concentration of 5.0 mg L^{-1}BA, recorded the lowest mean of the number of shoots, which reached 1.40 shoots $explant^{-1}$ (Table 2). The interaction treatment between the cultivar Malibu with a concentration of 4.0 mg L^{-1}BA recorded the highest mean of the shoot length that were proliferated from direct organogenesis, which amounted to 2.58 cm, which differed significantly with all interaction treatments. While the interaction treatment between Orange cultivar and the concentration of 5.0 mg L^{-1}BA, recorded the lowest mean of the shoot length, which reached 0.34 cm (Table 3).

The results from Table 4 indicate the significant superiority of the Orange cultivar in its response

to shoot rooting, which reached 68.56%. While the Malibu cultivar recorded the lowest response to shoot rooting, at 62.60%. The data from Table 5 and 6 also indicate the significant superiority of the Malibu cultivar in the number and length of the main roots, which reached 3.84 roots plant^{-1} and 3.12 cm, respectively (Plate 1, N). While the Orange cultivar recorded the lowest mean of main root number and length, at 2.72 roots plant^{-1} and 2.81 cm, respectively (Plate 1, M).

The data from Tables 4, 5 and 6 indicate the significant superiority of the shoot that cultured on the MS medium supplied with 0.5 mg L^{-1} NAA in the shoot response to rooting, and the number and length of the main roots, which reached 97.80% and 5.90 roots plant^{-1} and 4.54 cm, respectively. As for the shoots that were cultured on the MS medium supplied with 0.9 mg L^{-1} NAA, recorded the lowest response to rooting of shoots and the number and length of the main roots were 10.00%, 0.90 roots plant^{-1} and 0.72 cm, respectively.

The data in Table 4 indicate the significant superiority of the interaction treatments between Orange cultivar and the concentration of 0.3, 0.5, or 0.7 mg L^{-1} NAA in the shoot response to rooting, which amounted to 99.60%, which did not differ significantly with the interaction treatment between Malibu cultivar and concentration of 0.5 mg L^{-1} NAA. Whereas, the results in Table 5 indicate the significant superiority of the interaction treatment between the Malibu cultivar and the concentration of 0.5 mg L^{-1} NAA in the number of main roots, which reached 6.60 roots plant^{-1} roots, compared with other interaction treatments. The results from Table 6 also indicate the significant superiority of the two interaction treatments between the Orange or Malibu cultivar and the concentration 0.5 mg L^{-1} NAA in the mean root length, which were 4.56 cm and 4.52 cm, respectively. As for the data in Tables 4, 5 and 6, it shows that the interaction treatment between Orange cultivar and 0.9 mg L^{-1} NAA, recorded the lowest response to rooting of shoots and the number and length of main roots, which were 9.00%, 0.60 roots plant^{-1} and 0.24 cm, respectively.

The Plate 1, O, P indicate the success of the acclimatization of gerbera plant for both cultivars, Orange and Malibu, when cultured in the agricultural medium consisting of peat moss and sand at a ratio of 2 : 1 after 12 weeks of culturing, with a high success rate of 88.87%.

Table 4. The effect of cultivar, NAA concentration and the interaction between them on the response of shoots to rooting after 12 weeks of culturing

Cultivar	NAA concentration (mg L^{-1})					Cultivar effect
	0.1	**0.3**	**0.5**	**0.7**	**0.9**	
Orange	35.00	99.60	99.60	99.60	9.00	68.56
Malibu	62.00	62.00	96.00	82.00	11.00	62.60
NAA effect	48.50	80.80	97.80	90.80	10.00	
RLSD p≥0.01	Cultivar		NAA concentration		Cultivar × NAA conc.	
	**		3.82		5.40	

**: High significant

Table 5. The effect of cultivar, NAA concentration and the interaction between them on the number of main roots after 12 weeks of culturing

Cultivar	NAA concentration (mg L^{-1})					Cultivar effect
	0.1	**0.3**	**0.5**	**0.7**	**0.9**	
Orange	1.80	3.40	5.20	2.60	0.60	2.72
Malibu	3.40	3.60	6.60	4.40	1.20	3.84
NAA effect	2.60	3.50	5.90	3.50	0.90	
RLSD p≥0.01	Cultivar		NAA concentration		Cultivar × NAA conc.	
	**		0.59		0.87	

**: High significant

Table 6. The effect of cultivar, NAA concentration and the interaction between them on the root length after 12 weeks of culturing

Cultivar	NAA concentration (mg L^{-1})					Cultivar effect
	0.1	0.3	0.5	0.7	0.9	
Orange	1.66	4.14	4.56	3.44	0.24	2.81
Malibu	3.10	3.16	4.52	3.62	1.20	3.12
NAA effect	2.38	3.65	4.54	3.53	0.72	
RLSD p≥0.01	Cultivar		NAA concentration		Cultivar × NAA conc.	
	**		0.12		0.17	

**: High significant

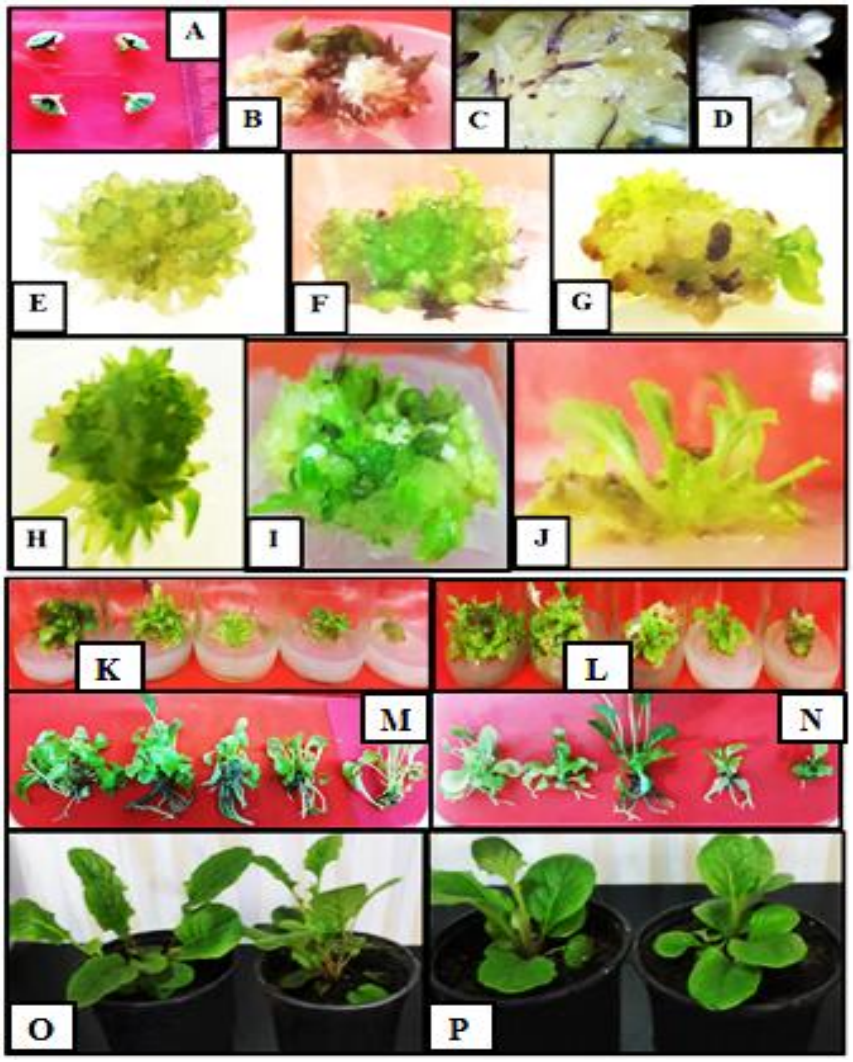

Plate 1. Micropropagation of gerbera (*Gerbera jamesonii* Bolus) plant. Capitulum segments (A); Direct organogenesis stages (B- J); Direct shoot regeneration at 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1} BA (Left to right, respectively) in Orange cultivar (K) and Malibu cultivar (L); Root formation at 0.1, 0.3, 0.5, 0.7 and 0.9 mg L^{-1} NAA (Left to right, respectively) in Orange cultivar (M) and Malibu cultivar (N); Plant acclimatization in Orange cultivar (O) and Malibu cultivar (P)

The significant superiority of the Orange cultivar in the capitulum response to direct adventitious shoots and shoot rooting, and the significant superiority of the Malibu cultivar in the number and length of shoots and roots after 12 weeks of culturing due to the genetic differences between them [6,8,16].

The significant superiority of the MS medium that supplied with 1.0 mg L^{-1}BA + 0.1 mg L^{-1} NAA + 0.5 mg L^{-1} GA_3 in the response of the capitulum to direct shoot regeneration and the number and length of branches is due to the fact that this concentration of benzyl adenine is optimum for stimulating the capitulum tissue to dedifferentiation and form new cells. These new cells begin to be re-differentiated by the components of the MS medium that leads to the initiation of adventitious buds that grow and develop as direct adventitious shoots on the capitulum tissue. The interaction between endogenous hormones in the tissue and the exogenous hormones that are added to the MS medium may have a positive role in the initiation adventitious buds and stimulate the division and elongation of cells that leads to the formation of direct shoots on capitulum segments [6,17-20,21].

The significant superiority of the shoot that culturing on the MS medium that supplied with 0.5 mg L^{-1} NAA + 0.1 mg L^{-1} BA in their response to rooting and the number and length of the roots is due to the stimulation of the tissues in the bases of the shoots to form cells through the interaction between this optimum concentration with the endogenous and exogenous hormones that were added to the MS medium as well as its components. These cells re-differentiate, grow and develop into adventitious roots [11,21-25].

CONCLUSIONS

The capitulum segments of the gerbera plant, Orange and Malibu cultivars that culturing by *In vitro* as explants led to the direct proliferation of adventitious shoots. The shoots of the Orange cultivar were significantly superior in their response to direct organogenesis and rooting compared to the Malibu cultivar, which was significantly higher in the number and length of shoots and roots. The shoots of gerbera plant that cultured on the MS medium that supplied with 1.0 mg L^{-1} BA led to their significant superiority in their response to direct proliferation, number and length of shoots, and the medium that supplied with 0.5 mg L^{-1} NAA led to the significant superiority of the shoots in their response to rooting and the number and length of the main roots.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh VK, Prasad VM, Suspriya K, Preeti R, Pragati M. Identification of the suitable hardening protocol and hardening medium in micropropagation of *Gerbera* (*Gerbera jamesonii* Bolus) Int. J. Curr. Microbiol. App. Sci. 2017;6(7):2476-2484. Available:https://doi.org/10.20546/ijcmas.2017.607.292
2. University of Helsinki. Gerbera laboratory—History of *Gerbera*; 2012. Available:http://www.helsinki.fi/agriculturalsciences/research/Gerberalab/History.html, 2012 [Last accessed 9 May 2013].
3. Teeri TH, Elomaa P, Kotilainen M, Albert VA. Mining plant diversity: *Gerbera* as a model system for plant developmental and biosynthetic research. Bioassays. 2006; 28(7):756–767. Available:https://doi.org/10.1002/bies.20439
4. Chung MY, Kim MB, Chung YM, Nou IS, Kim CK. *In vitro* shoot regeneration and genetic transformation of the *Gerbera* (*Gerbera hybrida* Hort.) cultivar 'Gold Eye'. J. of Plant Biotechnology. 2016;43: 255–260. Available:https://doi.org/10.5010/JPB.2016.43.2.255
5. Shelahi SA, Majeed DM, Hassan SM. Propagation of *Gerbera* (*Gerbera Jasmonii* Bolus) by using a tissue culture technique. Journal of the Biotechnology Research Center. 2013;7(1): 50-58.

6. Son NV, Mokashi AN, Hegde RV, Patil VS, Lingaraju S. Response of *Gerbera* (*Gerbera jamesonii* Bolus) varieties to micropropagation. Karnataka Journal of Agricultural Sciences. 2011;24(3):354-357.
Available:https://doi.org/10.1.1.846.1216&rep=rep1&type=pdf
7. Kadu AR. *In vitro* micropropagation of *Gerbera* using auxiliary bud. Asian J. Bio. Sci. 2013;8(1):15-18.
8. Shylaja MR, Sashna P, Chinjusha V, Nazeem PA. An efficient micropropagation protocol for *Gerbera jamesonii* bolus from flower buds. International Journal of Plant, Animal and Environmental Sciences. 2014; 4(3):641-643.
Available:http://www.rroij.com/open-access/an-efficient-micropropagation-protocol-for-gerbera-641-643.pdf
9. Nazari F, Khosh-khui M, Azadi P. A simple and efficient direct shoot organogenesis method using New Zealand study tour project report; 2016.
Available:http://www.jsceint.org/system/files/2016_02-1_STG_Report_Mr.%20HABIBIE%20RAZAK%28Indonesia%29.pdf
10. Akter N, Hoque MI, Sarker RH. *In vitro* propagation in three varieties of *Gerbera* (*Gerbera jamesonii* Bolus.) from flower bud and flower stalk explants. Plant Tissue Culture and Biotechnology. 2012;22(2): 143-152.
Available:https://doi.org/10.3329/ptcb.v22i2.14203
11. Kumari S. Pal AK, Sarkhel S, Singh P, Kumar R. Standardization of *In vitro* Mass multiplication protocol for *Gerbera* cv. partrizia. Int. J. Curr. Microbiol. App. Sci. 2018;7(4):514-519.
Available:https://doi.org/10.20546/ijcmas.2018.704.060
12. Cardoso JC, Silva JAT. *Gerbera* micropropagation. Biotechnology Advance. 2013;31:1344-1357.
Available:https://doi.org/10.1016/j.biotechadv.2013.05.008
13. Parvin S, Ona AF, Ahmad H, Nahiyan AS M, Jamal Uddin AFM. Callus induction of gerbera explants with different growth regulator compositions. Int. J. Bus. Soc. Sci. Res. 2017;6(1):68-73.
Available:https://www.ijbssr.com/currenttissueview/14013243
14. Murashige T, Skooge F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 1962; 15(3):473-497.
Available:https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
15. Al-Rawi KM, Khalaf Allah AM. Design and analysis of agricultural experiments. Dar Al Kutub for Printing and Publishing, College of Agriculture and Forestry, University of Mosul, Ministry of Higher Education and Scientific Research, Iraq. 2000;488.
16. Khatun MM, Ali MH, Desamero NV. Effect of genotype and culture media on callus formation and plant regeneration from mature seed scutella culture in rice. Plant Tissue Culture. 2003;13(2):99-107.
Available:https://doi.org/10.3126/jiaas.v26i0.607
17. Kanwar JK, Kumar S. *In vitro* propagation of *Gerbera*- A Review. Hort. Sci. 2008;35(1): 35-44.
Available:https://www.agriculturejournals.cz/publicFiles/00765.pdf
18. Wojciechowicz MK. Organogenesis and somatic embryogenesis induced in petal cultures of sedum species. ACTA Bio. Cra. Ser. Botancica. 2009; 51(1):83-90.
Available:https://www.researchgate.net/publication/242719750_Organogenesis_and_somatic_embryogenesis_induced_in_petal_cultures_of_Sedum_species
19. Ying Ko C, Al-Abdulkarim AM, Al-Jowid SM, Al-Baiz A. An effective disinfection protocol for plant regeneration from shoot tip cultures of strawberry. African Journal of Biotechnology. 2009; 8(11):2611-2615.
Available:https://www.ajol.info/index.php/ajb/article/view/60783
20. Hasan MN, Nigar S, Rabbi MAK, Mizan SB, Rahman MS. Micropropagation of strawberry, (*Fragaria×ananassa*) Int. J. Sustain. Crop Prod. 2010;5(4):36-41.

21. Ibrahim MA, Al-Taha HA, Seheem AA. Effect of cytokinin type and concentration, and source of explant on shoot multiplication of pineapple plant (*Ananas comosus* 'Queen') *In vitro*. Acta Agriculturae Slovenica; 2013;101(1): 15-20.
Available:https://doi.org/10.2478/acas-2013-0002
22. Ibrahim KM, Abdul–Hameed S. The nurseries. The University of Mosul. Univ. Mosul, Iraq. Press. Ministry of Higher Education, Iraq; 2001.
23. Hartmann HT, Kester DE, Davies FT, Geneve RL. Plant propagation. Principles and practices. 8th ed. New Jersey. 2010;915.
Available:https://www.amazon.com/Hartmann-Kesters-Plant-Propagation-Principles/dp/0135014492
24. Hopkins WG, Huner NPA. Introduction to plant physiology. 3rd. John Wiled and sons, New York; 2004.
Available:https://www.amazon.com/Introduction-Plant-Physiology-William-Hopkins-ebook/dp/B006R6I850
25. Winarto B, Yufdy MP. Establishment of *In vitro* propagation protocol of *Gerbera jamesonii* bolus ex hook f.: Explant and media selection to plantlet acclimatization. Journal of Agricultural Science. 2017;1:32-40.
Available:https://doi.org/10.15159/jas.17.03