Establishment of Callus Cultures of Chrysanthemum (*Dendranthema* × *grandiflorum*) var. 'Zembla yellow'

Babiker, Y. F.,[1] Eltorky, M. G.,[1] Elmokadem, H. E.,[1] El-Naggar, H. M.,[1] and Meheissen, M. A. M.[2]
[1]Dept. of Floriculture, Ornamental Horticulture and Landscape Gardening, Faculty of Agriculture, Alexandria University, Egypt.
[2]Clinical Oncology Department, Alexandria University, Egypt.

ABSTRACT

Dendranthema x grandiflorum is an important species of the genus *Dendranthema*, and for the development of callus cultures. In this study, explants such as leaves and internodes were used to evaluate the effects of different types of explants, plant growth regulating agents in various concentrations on callus production. The results showed that, the leaf explant was significantly enhanced callus production than internode explant according to the percentage of callus formation, fresh and dry weight parameters. MS supplemented with 0.2 mg/l^{-1} BAP and 0.5 mg/l^{-1} 2,4-D yielded the highest efficiency of callus formation after 2 month of culture. The BAP 0.2 mg/l^{-1} was significantly enhanced callus production in the both fresh and dry weight parameters than BAP 0.1 mg/l^{-1}. The use of 0.5 mg/l^{-1} 2,4-D excelled all other treatments for fresh weight although, there is no significant differences from the 1 mg/l^{-1} 2,4-D treatment, the high 2,4-D concentrations 2 mg/l^{-1} also performed better than the lower concentrations for dry weight. Similarly, the least fresh and dry weight were obtained from the control which was devoid of 2,4-D. Using 0.5 mg/l^{-1} 2,4-D leaf explant excelled all other treatments for fresh weight than internode explant, but using 2 mg/l^{-1} 2,4-D leaf explant excelled all other treatments for dry weight than internode explant. Similarly, the control of leaf and internode explant in the both concentrations (0 and 0.5 mg/l^{-1}) yielded the lowest fresh weight, but in dry weight the least was obtained from the control of leaf explant. Using 0.1 mg/l^{-1} BAP with 0.5 mg/l^{-1} 2,4-D and 0.2 mg/l^{-1} BAP with (0.5, 1, and 1.5 mg/l^{-1}) 2,4-D excelled all other treatments for fresh weight, but the best is using 0.1 mg/l^{-1} BAP with 0.5 mg/l^{-1} 2,4-D. The least fresh weight was obtained from the control which devoids of plant growth regulators. Using 0.1 mg/l^{-1} BAP with 2 mg/l^{-1} 2,4-D leaf explant excelled all other treatments with espect to dry weight. The least dry weight was obtained from the control which devoids of plant growth regulators.

Keywords: *Dendranthema x grandiflorum*, explants, plant growth regulators, *in vitro*, callus induction cultures, callus formation

INTRODUCTION

Chrysanthemum (*Dendranthema x grandiflorum* (Ramat.) Kitam, previously *Chrysanthemum morifolium* Ramat.) (Shinoyama *et al*., 2006), is an important genus within the Asteraceae family which is comprised of about 40 species, including some economically valuable species, mostly used as ornamentals and insecticides (Kumar *et al*., 2006). The plant is native to China and Northern Japan (Padmadevi and Jawaharlal, 2011), cultivars are hexaploids, with 2n = 6x = 54 chromosomes (Nazeer and Khoshoo, 1983). It is an extremely attractive and charming short-day plant that serves both as an annual herb and as a perennial herb (Arora, 1990). It is ranked second after roses in terms of its market value (Guan *et al*., 2017). It is one of the most commonly grown ornamental plants in the world. Due to its wide range of colors and forms, this flower is one of the most significant cut and pot flowers in the world (Shafiei *et al*., 2019).

Conventional methods for propagating chrysanthemums, such as root suckers or terminal cuttings, are slow processes (Teixeira da Silva *et al.*, 2013). Tissue culture techniques can improve plant propagation efficiency, allowing for more efficient reproduction and the development of superior genotypes (Shahzad, 2017). Tissue culture is also an important tool of chrysanthemum biotechnology (Teixeira da Silva and Kulus, 2014).

Callus (or tissue) cultures are a type of culture that involves the growth and maintenance of mostly unorganized cell masses resulting from the uncoordinated and disorganized proliferation of small plant organs, plant tissue, or previously cultivated cells. Tissue cultures are formed from entire plant parts. The small organs or pieces of tissue that are used are called explants (Cassells, 1997). Explants can be placed on a growth-supporting media under sterile conditions to induce callus formation *in vitro*. Although many components of a plant have the capacity to multiply *in vitro*, it is usually discovered that callus cultures are easier to produce from certain organs than others. Young meristematic tissues are best; however callus can form in meristematic sites in older portions of a plant, such as the cambium. Callus growth can be achieved from zygotic embryos, germinating seeds, seed endosperm or the seedling mesocotyl, and very young leaves or leaf sheaths in most cereals, but never from mature leaf tissue (Green and Phillips, 1975; and Dunstan *et al.*, 1978). 'Primary callus' is the callus that forms on an original explant. Pieces of tissue removed from primary callus are used to start secondary callus cultures. Subculture can then be sustained for

several years, although the longer the callus is kept, the greater the chance of genetic alteration in the cells. When a callus is produced from an explant that contains multiple types of cells, variability is more likely. For this reason there is often merit in selecting small explants from only morphologically homogenous tissue, keeping in mind that callus production usually required a minimum size of explant (George *et al.,* 2008).

Numerous explants have been successfully induced to produce callus. The success of callus initiation, on the other hand, is determined by the explant source and the culture medium's composition. Especially, culture medium which supplemented with auxins induces quickly cell division. Callus development is influenced by the size of the explants and, in some situations, the mode of culture or polarity of the explants in the media. Smaller explants are more likely to form callus while the larger ones maintain greater morphogenetic potential (David *et al*., 1981). A callus is an amorphous mass of loosely organized thin-walled parenchyma cells that develops from the parent tissue's proliferating cells (Dodds and Roberts, 1985).

The aims of the present investigation were to optimize the type of explants and concentrations of growth regulators in culture media for callus induction on chrysanthemum (*Dendranthema* × *grandiflorum*).

MATERIALS AND METHODS

Plant material and culture establishment

Plants of *Dendranthema x grandiflorum* cultivar 'Zembla yellow' were obtained from a private nursery in Dakahlia, Egypt. They were then grown in the nursery of the Floriculture, Ornamental Horticulture and Landscape Gardening, Faculty of Agriculture, Alexandria University. They were used as primary explant source in the plant tissue culture laboratory. For primary culture initiation nodal buds were taken from the middle portion of the chrysanthemum shoots, washed in running tap water for 30 min and immersed in 15% sodium hypochlorite with 2 drops/100 ml of Tween 20 for 15 min with continuous shaking. They were then rinsed 3 times with autoclaved distilled water, explants were sectioned to 2 cm segments containing two to the three nodes and cultured in jars, each containing (250-ml and 375-ml), jars containing 25 ml and 50 ml of MS media (Murashige and Skoog,1962) with 3% (w/v) sucrose and 2 mg/l^{-1} 6-benzylaminopurine (BAP) and 0.1 mg/l^{-1} ȧ-naphthalene acetic acid (NAA) and 7 g/l^{-1} agar to induce axillary buds to from new axillary shoots. The pH of all tested media was adjusted to 5.7 before autoclaving at 121°C and 118 kPa, a pressure of 15 psi for 20 minutes. Explants were cultured in 250 ml jars containing 25 ml of the tested media. All cultures were incubated between 4-8 weeks at 25±1°C under continuous fluorescent light (1000 lux) with (16 h/8 h/d) light/dark cycle.

Callus Induction

Internodes and leaves, established from plantlet of *D. x grandiflorum*, were used for induction of callus. The internodes were sectioned from the small sections each section was 1 cm segments. Five explants were cultured in 250-ml jars containing 25 ml of the tested media. MS media (Murashige and Skoog, 1962) supplemented with 30 g/l^{-1} sucrose, 0.1 mg/l^{-1} NAA and solidified with 7 g/l^{-1} agar. Different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1} were added to the media before autoclaving. MS basal media without plant growth regulators (PGRs) was used as a control. Five replicates were used per each treatment. The internodes and leaves cultured were incubated at 25±1°C under continuous fluorescent light (1000 lux) with (16 h/8 h/d) light/dark cycle. The fresh and dry weight (g) was recorded after 60 days of culture.

Experimental design and data analysis

Experiment was set up split-plot in randomized complete block design (RCBD) and each treatment had five replications. Each replicate was represented by culture jars containing one segment rendering a group of five segments per treatment. The means and ANOVA were calculated using SAS program, version 6 (1985) statistical software. The mean comparison was carried out using least significant difference tests (LSD) and significance was determined alpha= 0.05 level of significance.

RESULTS AND DISCUSSION

1. Effect of type of explants on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/1^{-1}. The effect of explant type on chrysanthemum performance of *in vitro* callus induction cultures was tested. Callus induction cultures were significantly influenced by type of explants. Analysis of variance presented in (Table 1 and Fig. 1) clearly indicated that, type of explants had significant effect on callus induction in both fresh and dry weight parameters. Callus tissues derived from leaf explant produced highest callus in both fresh (3.41g) and dry weight (0.18 g) parameters. Generally, callus tissues derived from leaves origin recorded highest callus in both fresh biomass and dry weight parameters compared to which derived from internode explant on all parameters.

Table 1: Effect of type of explants on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture.

Type of explants	Fresh weight (g)	Dry weight (g)
Leaf	3.41a	0.18a
Internode	1.88b	0.15a
LSD	0.27	0.03

*Means within column with the same letter(s) are not significantly different at P= 0.05 according to t-test.

a

b

Fig 1: a. Callus formation from Leaf explant; b. Callus formation from Internode explant.

The results are in correlation with the work of Sutter and Langhans (1981) who, compared 9 years old leaf callus of *Chrysanthemum morifolium* with those of one month old callus derived from leaf explants to assess the regeneration ability of long term cultures. Some related research indicated that, callus induction in chrysanthemum cultivar 'Zipri' exhibited greater callus induction than cv. 'Shyamal Dark Pink'. In both cultivars, leaf disc explants produced more calluses than stem disc explants (Wankhede *et al.*, 2000). Jin *et al.*, (2017) observed that the effects of several plant growth regulating compounds on different concentrations callus induction were explored using stem segments, leaves, and petioles as explants in *Dendranthema indicum* var. aromaticum. The results showed that, the percentage of callus formation, callus hardness, growth potential and shoot differentiation was the highest with the leaf as explants. The results also are in correlation with the work of (Hassan *et al.*, 2019) who reported that the callus derived from leaf explant on MS medium containing 0.25 mg/l^{-1} 2,4-D gained the highest fresh weight in *Ocimum basilicum.*

2. Effect of BAP concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1}to figure out the optimum conditions for callus induction cultures. The effect of BAP concentrations on chrysanthemum performance of *in vitro* callus induction cultures was tested. Callus induction cultures were significantly influenced by different BAP concentrations. Analysis of variance presented in Table (2). Results summarized that; callus in both fresh and dry weight parameters was significantly variable according to BAP concentrations. The highest means in the both fresh (2.80 g) and dry weight (0.18 g) parameters collected on BAP concentration 0.2 mg/l^{-1}, whereas, the lowest fresh and dry weight parameters were obtained at low BAP concentrations. These results related with the results obtained by Xue *et al.*, (2003) who stated that, in *Chrysanthemum morifoliu*m the media supplemented with NAA 0.1 mg/l^{-1} and 6- BAP (0.1-1.0 mg/l^{-1}) were much better than others when callus were induced.

Table 2: Effect of BAP concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture.

BAP conc. (mg/l^{-1})	Fresh weight (g)	Dry weight (g)
0.1	2.49b	0.15b
0.2	2.80a	0.18a
LSD	0.17	0.02

*Means within column with the same letter(s) are not significantly different at P= 0.05according to t-test.

3. Effect of 2,4-D concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1} to figure out the optimum conditions for callus induction cultures. The effect of 2,4-D concentrations on chrysanthemum performance of *in vitro* callus induction cultures was tested. Callus induction cultures were significantly influenced by different 2,4-D concentrations. Analysis of variance presented in Table (3). Results clearly demonstrated that, callus in the same fresh and dry weight parameters was significantly variable according to 2,4-Dconcentrations. The use of MS medium containing 0.5 mg/l^{-1} 2,4-D produced the highest callus induction for fresh weight (3.40 g) although it did not differ significantly from the 1 mg/l^{-1} 2,4-D concentration (3.16 g), the high 2,4-D concentrations 2 mg/l^{-1} also performed better for dry weight (0.21 g) although it did not differ significantly from the 1 and 1.5 mg/l^{-1} 2,4-D concentration . Similarly, the least fresh (1.42 g) and dry weight (0.10 g) were obtained from the control which was devoid of 2,4-D. The results are in correlation with the work of Sherman *et al.,* (1998) they noticed that, when leaf explants were cultured on an embryogenesis-type medium containing a high concentration of 2,4-D, promoted callus formation. Obukosia *et al.,* (2004) they discovered that, MS medium supplemented with 2 mg/l^{-1} 2,4-D was best for chrysanthemum callus induction. Thomas and Maseena (2006) they investigated that, a given range of 2,4-D concentrations (0.1-2.0 mg/l^{-1}) is essential for embryogenic callus formation from leaf and nodal explants in *Cardiospermum halicacabum*. Also these results are in correlation with the work of (Wongsen *et al.,* 2015) who reported that the concentration of 2,4-D particularly 0.5 mg/l^{-1} in the medium of callus culture of sweet basil was very important for callus proliferation and antioxidant activity that mainly may be due to Rosmarinic acid content. On the other hand, Jin *et al.,* (2017) observed that in *Dendranthema indicum* var. aromaticum the optimal induction mediums were MS supplemented with 1.0 mg/l^{-1} 2.4-D and 0.2 mg/l^{-1} 6-BAP. The results also showed that, the suitable inoculum size was 2 g and the suitable cell suspension culture medium was MS supplemented with 0.2 mg/l^{-1} BAP and 0.5 mg/l^{-1} 2,4-D.

4. Effect of type of explant and BAP concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1}to figure out the optimum conditions for callus induction cultures. The effect of type of explant and BAP concentrations on chrysanthemum performance of *in vitro* callus induction cultures was tested. Application of type of explant and BAP concentrations resulted in not significant differences in the measured parameters. Analysis of variance presented in Table (4).

Table 3: Effect of 2,4-D concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture.

2,4-D conc. (mg/l^{-1})	Fresh weight (g)	Dry weight (g)
0	1.42d	0.10c
0.5	3.40a	0.16b
1	3.16ab	0.18ab
1.5	2.90b	0.18ab
2	2.34c	0.21a
LSD	0.27	0.04

*Means within column with the same letter(s) are not significantly different at P= 0.05according to t-test.

Table 4: Effect of type of explant and BAP concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture.

Type of explants	BAP conc. (mg/l^{-1})	Fresh weight (g)	Dry weight (g)
Leaf	0.1	3.30	0.16
Leaf	0.2	3.52	0.20
Internode	0.1	1.68	0.15
Internode	0.2	2.08	0.16
LSD		n.s.	

*****Means within column with the same letter(s) are not significantly different at P= 0.05 according to DMRT.
n.s: non-significant.

The results are in contrast with the results obtained by Xue *et al.,* (2003) they reported that, to determine the best conditions for *Chrysanthemum morifolium* leaf tissue culture in Anhui Province. All of the media used were capable of inducing callus, but the effects of callus redifferentiation were very different. When callus was induced, the media with NAA 0.1 mg/l^{-1} + BAP (0.1-1 mg/l^{-1}) performed significantly better than the others. This contrast may due to the auxin: cytokinin concentration.

5. Effect of type of explant and 2,4-D concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1} to figure out the optimum conditions for callus induction cultures. The effect of type of explant and 2,4-D concentrations on chrysanthemum performance of *in vitro* callus induction cultures was tested. Callus induction cultures were significantly influenced by type of explant and 2,4-D concentrations. Analysis of variance presented in (Table 5). In case of callus induction culture, Results of analysis of variance were clearly demonstrated that type and 2,4-D concentrations have a great influenced on the same fresh and dry weight parameters. The use of leaf explant and 0.5 mg/l^{-1} 2,4-D excelled on all 2,4-D concentrations for fresh weight (5.31 g) than internode explant, while the use of leaf explant and 2 mg/l^{-1} 2,4-D excelled on all 2,4-D concentrations with respect to dry weight (0.27 g) than internode explant. Similarly, the least fresh weight (1.53 g) was obtained from the control which was devoid of 2,4-D of leaf and control of internode explants (1.31 g) and 0.5 mg/l^{-1} 2,4-D (1.50 g) in the both concentrations (0 and 0.5 mg/l^{-1}) 2,4-D, while in dry weight the least was obtained from the control which was devoid of 2,4-D of leaf explant. Thomas and Maseena (2006) they investigated a given concentrations of 2,4-D in a wide range (0.1-2.0 mg/l^{-1}) is essential for embryogenic callus formation from leaf and nodal explants in *Cardiospermum halicacabum*.

6. Effect of type of plant growth regulators and concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1} to figure out the optimum conditions for callus induction cultures. The effect of type of explant and 2,4-D concentrations on chrysanthemum performance of in vitro callus induction cultures was tested. Application of different types of plant growth regulators and concentrations resulted in significant differences in fresh weight parameters but in dry weight parameters proved to be not significant differences. Results in (Table 6) clearly demonstrated that, similarly, the use to 0.1 mg/l^{-1} BAP with 0.5 mg/l^{-1} 2,4-D (3.51 g) and 0.2 mg/l^{-1} BAP with (0.5, 1, and 1.5 mg/l^{-1}) 2,4-D (3.30, 3,34, and 3.18 g respectily) excelled all other treatments with respect to fresh weight, while the best is to use 0.1 mg/l^{-1} BAP with 0.5 mg/l^{-1} 2,4-D. The least fresh weight (1.42 g) was obtained from the control which was devoid of plant growth regulators. The previous studies indicated that, the best response for induction of callus was produced by combining BA and 2,4-D, which was likely owing to a difference in endogenous levels of growth regulators in chrysanthemum plants or a difference in sensitivity. (Trewavas and Cleland, 1983). On the other hand, Aribaud *et al.,* (1994) who found also that, BA plus 2,4-D caused callus formation and proliferation in leaf explant of chrysanthemum.

Table 5: Effect of type of explant and 2,4-D concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture.

Type of explants	2,4-D conc. (mg/l^{-1})	Fresh weight (g)	Dry weight (g)
Leaf	0	1.53e	0.05e
	0.5	5.31a	0.20b
	1	3.92b	0.19bc
	1.5	3.62b	0.20b
	2	2.67c	0.27a
Internode	0	1.31e	0.15cd
	0.5	1.50e	0.12d
	1	2.40cd	0.18bc
	1.5	2.18d	0.17bc
	2	2.02d	0.15cd
LSD		0.38	0.04

*****Means within column with the same letter(s) are not significantly different at P= 0.05 according to DMRT.

Table 6: Effect of type of plant growth regulators and concentrations on performance of *in vitro* callus induction cultures of *Dendranthema x grandiflorum* after 8 weeks in culture.

BAP conc. (mg/l^{-1})	2,4-D conc. (mg/l^{-1})	Fresh weight (g)	Dry weight (g)
0.1	0	1.42e	0.10
	0.5	3.51a	0.14
	1	2.98bc	0.16
	1.5	2.61c	0.15
	2	1.92d	0.23
0.2	0	1.42e	0.10
	0.5	3.30ab	0.18
	1	3.34ab	0.20
	1.5	3.18ab	0.22
	2	2.77c	0.20
LSD		0.38	n.s.

*Means within column with the same letter(s) are not significantly different at P= 0.05 according to DMRT.

n.s: non-significant.

Wankhede *et al.,* (2000) who reported that, the callus induction and growth in chrysanthemum were most pronounced in cv. 'Zipri' grown in the MS medium containing either 1 mg/l^{-1} 2,4-D, 0.2 mg/l^{-1} BAP plus 0.2 mg/l^{-1} NAA, or 0.5 mg/l^{-1} BAP, and in cv. 'Shyamal Dark Pink' grown in the MS medium containing either 2 mg/l^{-1} 2,4-D, 0.1 mg/l^{-1} BAP plus 0.1 mg/l^{-1} NAA, or 0.25 mg/l^{-1} BAP. In both cultivars, leaf disc explants produced more calluses than stem disc explants.

7. Effect of type of explants, plant growth regulators and concentrations on performance of *in vitro* callus induction cultures on fresh weight (g) of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1}to figure out the optimum conditions for callus induction cultures. The effect of type of explant, plant growth regulators and concentrations on chrysanthemum performance of *in vitro* callus induction cultures was tested. Application of type of explants, plant growth regulators and concentrations resulted in not significant differences in the measured parameters. Analysis of variance presented in Table (7). The results are in contrast with the results obtained by Bhattacharya *et al.,* (1990) within two weeks of culture, they obtained good green calluses from both leaf and stem segments on MS basal salts supplemented with 2 mg/l^{-1} 2,4-D. They also found that a mixture of 0.1 mg/l^{-1} IAA and 0.2 mg/l^{-1} BAP was best for callus formation from nodal segments, shoot apices, and leaf, as well as for shoot regeneration from callus. On the other hand, Gul (2001) reported that, in chrysanthemum on MS medium supplemented with BAP and 2,4-D callus was induced. Callus was obtained from the nodal segments of the stem as well as shoot tips. A single flask containing callus produced up to 60 plantlets. This contrast may due to the type of explant.

8. Effect of type of explants, plant growth regulators and concentrations on performance of *in vitro* callus induction cultures on dry weight (g) of *Dendranthema x grandiflorum* after 8 weeks in culture:

Leaf and internode explants were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with different concentrations of BAP (0, 0.1, and 0.2 mg/l^{-1}) and 2,4-D (0, 0.5, 1, 1.5, and 2 mg/l^{-1}) with NAA 0.1 mg/l^{-1} to figure out the optimum conditions for callus induction cultures. The effect of type of explant plant growth regulators and concentrations on chrysanthemum performance of *in vitro* callus induction cultures was tested. Application of different type of explants, plant growth regulators and concentrations of resulted in significant differences in dry weight. Analysis of variance presented in (Table 8). Data showed that, the use to leaf explant to 0.1 mg/l^{-1} BAP with 2 mg/l^{-1} 2,4-D excelled on all other treatments for dry weight (0.35 g). The least dry weight (0.05 g) was obtained from leaf explant control which was devoid of plant growth regulators (Table 8 and Fig. 2). The results are in contrast with the results obtained by Kumari and Varghese (2003) investigated that, the effects of 2,4-D (1 or 2 mg/l^{-1}), NAA (1 or 2 mg/l^{-1}) and Kinetin (0.5 and 1 mg/l^{-1}) on callus of node, young leaves and inter-nodal explant of chrysanthemum. Nodal explants had the highest fresh weight in two combinations, and internode and young leaf explants had the highest fresh weight in one combination. Dry matter accumulation was higher in nodal explant followed by young leaves and inter-node in both cultivars. Out of different media combinations, MS

supplemented with 2 mg/l^{-1} 2,4-D and 1.0 mg/l^{-1} Kinetin was superior in terms of fresh weight and dry matter accumulation in all explants. This contrast may due to the different type of explants and auxin concentrations.

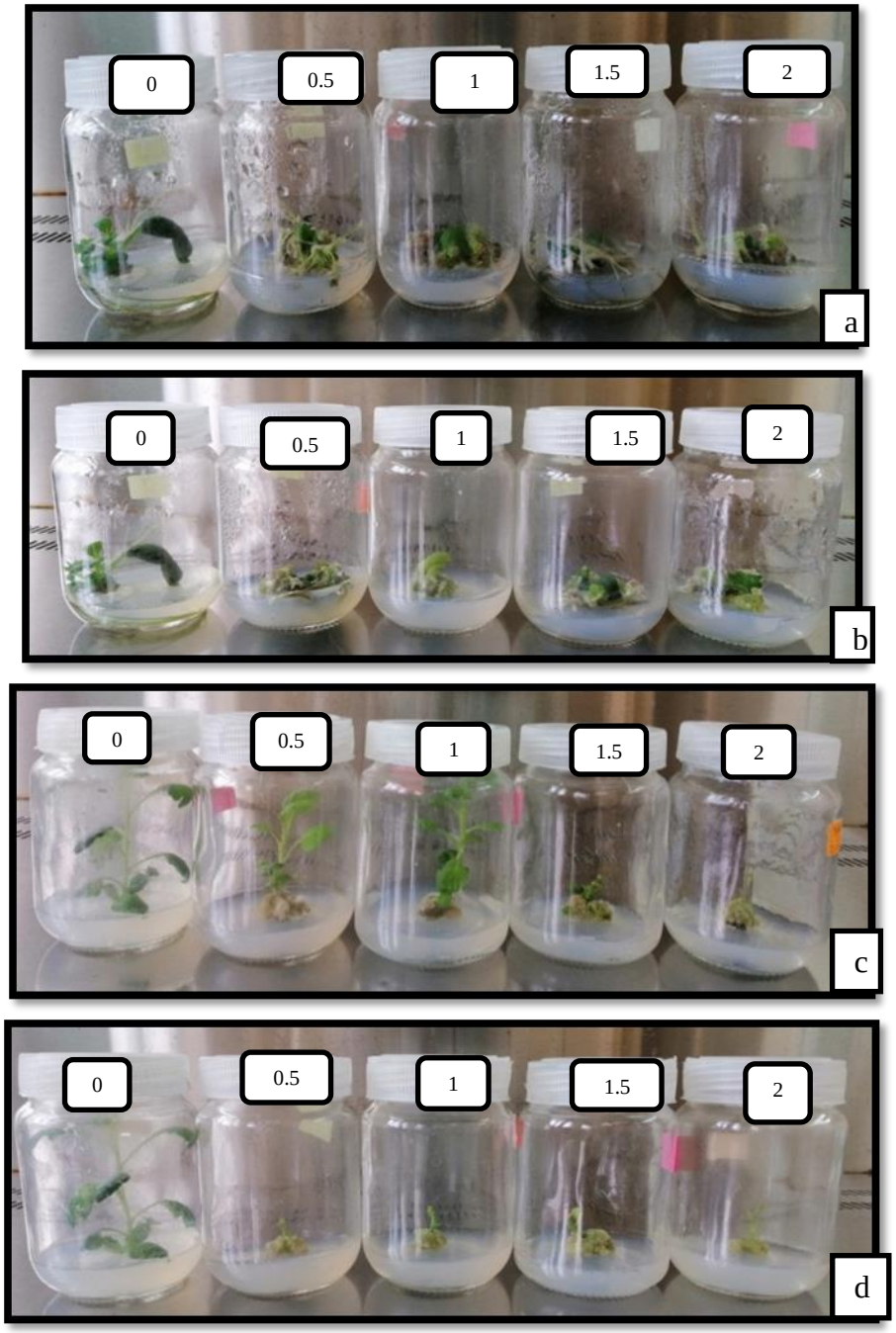

Fig. 2: a: Leaf explants grown in media contain different concentrations of 2,4-D with BAP 0.1 mg/l^{-1}; b: Leaf explants grown in media contain different concentrations of 2,4-D with BAP 0.2 mg/l^{-1}; c: Internode explants grown in media contain different concentrations of 2,4-D with BAP 0.1 mg/l^{-1}; d: Internode explants grown in media contain different concentrations of 2,4-D with BAP 0.2 mg/l^{-1}.

Table 7: Effect of type of explants, plant growth regulators and concentrations on performance of *in vitro* callus induction cultures on fresh weight (g) of *Dendranthema x grandiflorum* after 8 weeks in culture.

Type of explants	BAP conc. (mg/l^{-1})	2,4-D conc. (mg/l^{-1})				
		0	**0.5**	**1**	**1.5**	**2**
Leaf	0.1	1.53	5.50	3.70	3.40	2.36
Leaf	0.2	1.53	5.12	4.13	3.83	2.98
Internode	0.1	1.31	1.52	2.26	1.82	1.48
Internode	0.2	1.31	1.48	2.55	2.53	2.55
LSD				n.s.		

*Means within column with the same letter(s) are not significantly different at P= 0.05 according to DMRT.
n.s: non-significant.

Table 8: Effect of type of explants, plant growth regulators and concentrations on performance of *in vitro* callus induction cultures on dry weight (g) of *Dendranthema x grandiflorum* after 8 weeks in culture.

Type of explants	BAP conc. (mg/l^{-1})	2,4-D conc. (mg/l^{-1})				
		0	**0.5**	**1**	**1.5**	**2**
Leaf	0.1	0.05g	0.15def	0.13ef	0.16de	0.35a
Leaf	0.2	0.05g	0.26bc	0.24bcd	0.25bcd	0.19d
Internode	0.1	0.15def	0.13ef	0.19d	0.15def	0.11f
Internode	0.2	0.15def	0.11f	0.16de	0.18de	0.20d
LSD				0.05		

*Means within column with the same letter(s) are not significantly different at P= 0.05 according to DMRT.

REFERENCES

Aribaud, M., Carre, M., & Martin, J. T. (**1994**). Polyamine metabolism and *in vitro* cell multiplication and differentiation in leaf explants of *Chrysanthemum morifolium* Ramat. *Plant Growth Regulation*, **15**, 143-155.

Arora, J. S. (**1990**). Introductory Ornamental Horticulture. Kalyani Publishers, New Delhi.

Bhattacharya, P., Dey, S., Das, N., & Bhattacharyya, B. C. (**1990**). Rapid mass propagation of *Chrysanthemum morifolium* by callus derived from stem and leaf explants. *Plant Cell Reports*, **9**, 439-442.

Cassells, A. C. (ed.) (**1997**). Pathogen and Microbial Contamination Management in Micropropagation. Kluwer, Dordrecht.

David, A. E., Sharp, W. R. & Flick, C. E. (**1981**). Growth and behavior of cell culture, embryogenesis and organogenesis In: Plant Tissue Culture, Methods and Application in Agricuture. Thorpe, T.A., Ed. Academic Press, San Diego., 45-130.

Dodds, J. H. & Roberts, L. W. (**1985**). Experiments in plant tissue culture. 2nd Ed. Cambridge University Press, Cambridge.

Dunstan, D. I., Short, K. C. & Thomas, E. (**1978**). The anatomy of secondary morphogenesis in cultured scutellum tissues of Sorghum bicolor. *Protoplasma*, **97**, 251-260.

George, E. F., Hall, M. A., & De Klerk, G. J. (**2008**). Plant Propagation by Tissue Culture. 3rd Edition. Published by Springer, Netherland.

Green, C. E. & Phillips, R. L. (**1975**). Plant regeneration from tissue cultures of maize. *Crop Sci.*, **15**, 417-427.

Guan, Z, Wu D., Song, A., Chen, F., Chen, S., & Fang, W. (**2017**). A highly sensitive method for the detection of chrysanthemum virus. *B. Electr. J. Biotechnol.*, **26**: 64–68. https://doi.org/10.1016/j.ejbt.2017.01.003.

Gul, A. (**2001**). Micropropagation of chrysanthemum. M.Sc. thesis. Department of Botany, University of Peshawar, Pakistan.

Hassan, H. M., Abdelrahman, R. A., Eltorky, M. G., & Elmokadem, H. E. (**2019**). Experimental research on the production of rosmarinic acid from different *Ocimum basilicum in vitro* cultures. *Middle East Journal of Applied Sciences*, **9(2)**, 434-442.

Jin, L., Yang, Y., GAO, W., Gong, M., G., Wang, J., Anderson, N. O., A., & He, M. (**2017**). Establishment of Callus Induction and Cell Suspension Cultures of *Dendrathema indicum* var. Aromaticum a Scented Chrysanthemum. *Journal of Plant Studies*; **2(6)**, 38-44.

Kumar, S., Prasad, K. V., & Choudhary, M. L. (**2006**). Detection of genetic variability among chrysanthemum radiomutants using RAPD markers. *Current Science*, **90**, 1108-1113.

Kumari, M., & Varghese, T. M. (**2003**). Effect of different growth regulators on fresh and dry weight of callus and regeneration in chrysanthemum cultivars Miss Universe and Snow Ball. *Journal of Ornamental and Horticultural Plants*, **6**, 188-194.

Murashige, T., & Skoog, F. (**1962**). A revised medium for rapid growth and bio assays with Tobacco tissue cultures. *Physiologia Plantarum*, **15**, 473-497.

Nazeer, M. A., & Khoshoo, T. N. (**1983**). Variation in the chromosome complement of *Chrysanthemum morifolium* complex. *The Nucleus*, **26**, 22–29.

Obukosia, S. D., Kimani, E., Wathaika, K., Mutitu, E., & Kimani, P. M. (**2004**) Effect of growth regulators and genotypes on pyrethrum *in vitro*. *In vitro Cellular and Developmental Biology-Plant*, **11**, 162-166.

Padmadevi, K., & Jawaharlal, M. (**2011**). Induction of *in vitro* mutation in chrysanthemum (*Dendranthema grandiflora* Tzvelev) ray florets (var. Ravi Kiran) using gamma rays and EMS. *Floriculture and Ornamental Biotechnology*, 74-77.

Shafiei1, M. R., Hatamzadeh, A., Azadi, P., & Lahiji, H. S., (**2019**). Mutation Induction in Chrysanthemum Cut Flowers Using Gamma Irradiation Method. *Journal of Ornamental Plants*, **9 (2)**, 143-151.

Shahzad, A., Parveen, S., Sharma, S., Shaheen, A., & Saeed, T. *et al.*, (**2017**). Plant Tissue Culture: Applications in Plant Improvement and Conservation. In: Plant Biotechnology: Principles and Applications, Springer, Singapore, 37-72.

Sherman, J. M., Moyer, J. W., & Daub, M. E. (**1998**). A regeneration and agrobacterium-mediated transformation system for genetically diverse chrysanthemum cultivars. *Journal of the American Society for Horticultural Science*, **123**, 189-194.

Shinoyama, H., Anderson, N., Furuta, H., Mochizuki, A., Nomura, Y., Singh, R. P., Datta, S. K., Wang, B. C., & Teixeira da Sliva, J. A. (**2006**). Chrysanthemum biotechnology. In: Teixeira da Silva J.A. (ed) Floriculture, ornamental and plant biotechnology: advances and topical issues, 1st edn. Global Science Books Ltd, Isleworth; **1,** 140–163.

Sutter, E., & Langhans, R. W. (**1981**). Abnormalities in chrysanthemum regenerated from long-term cultures. *Annals of Botany*, 48, 559-568.

Teixeira da Silva, J. A, & Kulus, D. (**2014**). Chrysanthemum biotechnology: discoveries from the recent literature. *Folia Hortic*, **26(2)**, 67–77.

Teixeira da Silva, J. A., Shinoyama, H., Aida, R., Matsushita, Y., Raj, S. K. & Chen, F. (**2013**). Chrysanthemum biotechnology: Quo vadis? *Critical Reviews in Plant Sciences*, **32(1)**, 21-52.

Thomas, T. D., & Maseena, E. A. (**2006**). Callus induction and plant regeneration in *Cardiospermum halicacabum* (L.) an important medicinal plant. *Scientia Horticulturae*, **108**, 332–336.

Trewavas, A. J., & Cleland, R. E. (**1983**). Is plant development regulated by changes in concentration of growth substances or by changes in the sensitivity? *Trends in Biochemical Science*, **8**, 354-357.

Wankhede, K. N., Narkhede, M. N., Shivankar, R. S., & Rathod, T. H. (**2000**). Callus induction and micropropagation studies in chrysanthemum. *Annals Physiology*, **14**, 174-177.

Wongsen, W., Bodhipadma, K., Noichinda, S., & Leung, D. W. M. (**2015**). Influence of different 2,4-D concentration on antioxidant contents and activities in sweet basil leaf-derived callus during proliferation. *International Food Research Journal*, **22(2)**, 638-643.

Xue, J. P., Yu, M., & Zhang, A. M. (**2003**). Studies on callus induced from leaves and plantlets regeneration of the traditional Chinese medicine *Chrysanthemum morifolium*. *Zhongguo Zhong Yao Za Zhi*.; **28(3)**, 213-216.

الملخص العربى

إنشاء مزارع الكالس لنبات الأراولا الصنف (Zembla yellow)

يسرى فيصل الشيخ بابكر[١]، محمد جمال الدين محمد التركى[١]، هدى السيد مصطفى المقدم[١]، هانى محمد عبد السلام النجار[١]، محمد أحمد محمد محيسن[٢]

[١]قسم الزهور ونباتات الزينة وتنسيق الحدائق– كلية الزراعة– جامعة الاسكندرية.

[٢]قسم علاج الأورام والطب النووى – كلية الطب– جامعة الاسكندرية.

Dendranthema x grandiflorum من أهم أنواع جنس *Dendranthema*, ولتطوير زراعات الكالس. في هذه الدراسة، تم أستخدام المنفصلات النباتية مثل الأوراق والسلاميات لتقييم تأثيرات الأنواع المختلفة من المنفصلات النباتية، والمواد المنظمة لنمو النبات بتركيزات مختلفة على إنتاج الكالس. أوضحت النتائج أن المنفصل الورقي أدى إلى زيادة معنوية في إنتاج الكالس مقارنة بمنفصل السلاميات حسب النسبة المئوية لتكوين الكالس ومعايير الوزن الطازج والجاف. بيئات الإنتاج المثلي هي MS (بيئة موراشيج وسكوج) مضاف إليها ٠,٢ مجم/لتر بنزيل أدنين (BAP) و٠,٥ مجم/لتر (2,4-D) حقق أعلي مستوى كفاءة من تكوين الكالس بعد شهرين من الزراعة. أظهرت النتائج أن زراعة الورقة أظهرت تحسين في إنتاج الكالس في نفس قياسات الوزن الطازج والجاف من زراعة السلامية. أن ٠,٢ مجم/لتر (BAP) حقق تحسين إنتاج الكالس بشكل كبير في نفس قياسات الوزن الطازج والجاف من ٠,١ مجم/لتر (BAP). تفوق أستخدام ٠,٥ مجم/لتر (2,4-D) علي جميع المعاملات الأخرى للوزن الطازج علي الرغم من عدم وجود فروق ذات دلالة إحصائية عن معاملة ١ مجم/لتر (2,4-D)، كما كان أداء التركيز العالية من (2,4-D) ٢ مجم/لتر أفضل من التركيزات المنخفضة للوزن الجاف. وبالمثل تم الحصول على أقل وزن طازج ووزن جاف من معاملة المقارنة الذي يخلو من (2,4-D) لكن بأستخدام زراعة الورقة مع ٠,٥ مجم/لتر (2,4-D) تفوق في جميع المعاملات الأخري للوزن الطازج مقارنة بزراعة السلامية، لكن أستخدام زراعة الورقة مع ٢ مجم/لتر (2,4-D) تفوق في جميع معاملات الوزن الجاف مقارنة بزراعة السلامية. وبالمثل، فإن زراعة معاملة المقارنة في منفصل الورقة وزراعة السلامية في كلا التركيزين (٠ و٠,٥ مجم/لتر) أنتج أقل وزن طازج، لكن أقل وزن جاف تم الحصول عليه من زراعة معاملة المقارنة. أستخدام ٠,١ مجم/لتر (BAP) مع ٠,٥ مجم/لتر (2,4-D) و٠,٢ مجم/لتر (BAP) مع (٠,٥، ١,٠، و١,٥ مجم/لتر (2,4-D) تفوقت في جميع المعاملات الأخري للوزن الطازج، ولكن الأفضل هو أستخدام ٠,١ مجم/لتر (BAP) مع ٠,٥ مجم/لتر (2,4-D).تم الحصول على أقل وزن طازج من معاملة المقارنة الذي يخلو من منظمات نمو النبات. إن أستخدام زراعة الورقة مع ٠,١ مجم/لتر (BAP) مع ٢ مجم/لتر (2,4-D) تفوق في جميع المعاملات الأخرى فيما يتعلق بالوزن الجاف. أقل وزن جاف تم الحصول عليه من معاملة المقارنة الذي يخلو من منظمات نمو النبات.