ABSTRACT

The aim of the present research was to developing an indirect micropropagation protocol for African daisy (Gerbera jamesonii Bolus cv. Winter Queen) through callus induction from unopened flower buds with using some auxin types and concentrations, as well as improving the redifferentiation process of it by using some cytokinin types and concentrations. In addition, study the in vitro rooting and the acclimatization of this important pot and cut flower plant. As, the micropropagation protocols described by various scientists need to be improve, in addition to create a micropropagation protocol in line with Egypt conditions.

MATERIALS AND METHODS

This study was conducted during 2015/2016 in the Biotechnology Laboratory of Plant Cell and Tissue Culture, Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University, Egypt on Gerbera jamesonii Bolus cv. Winter Queen, plant. 

Explant source and sterilization

G. jamesonii cv. Winter Queen growing in a polyethylene greenhouse in the Nursery of Ornamental and Floriculture plants, Faculty of Agriculture, Mansoura University, was the source of the explants (Figure, 1a). Young unopened flower buds (approximately 0.8 cm in diameter, 10 days old) were cut from the donor plants (Figure, 1b) for use with their micropropagation. Likewise, flower buds were pre-treated under running faucet water for 60 min., then immersing in 1% pesticide solution of Rizolix 50% (Tolclofos-methyl) was used for 30 min. After that 1% pesticide solution of Rizolix 50% (Tolclofos-methyl) was used for 30 min and rinsing 4-5 times (3 min each) by sterilized distilled water to remove all residuals of disinfection materials. The explants were then moved to the laminar air flow cabinet for sterilization. The main

INTRODUCTION

African daisy or Transvaal daisy (Gerbera jamesonii Bolus) is an important pot plant and cut flower with attractive colors. It is native to the Family Asteraceae and takes the fifth positions in the global cut flower exchange (FloraHolland produces and keeps up the genetic purity and uniformity, but plants because of its magnificence, colors and long shelf life. Gerbera is propagated through the sexual or the vegetative propagation (Winarolt and Yufdy, 2017). On the other side, rhizomes division as a vegetative propagation produces and keeps up the genetic purity and uniformity, but it is arduous and tedious with less outcomes (Son et al., 2011). Along these lines clonal proliferation through plant tissue culture works are imperative to tend a large vigorous plants number, pathogen free and uniform (Debergh and Maene, 1981; Van Den Dries et al., 2013). As of late, micropropagation has been perceived as the most effective way for the commercial propagation scale of the important plants within a short period in restricted space with all the advantages of the vegetative propagation which prompting methodical advancement of the floriculture business.

Several protocols for micropropagation of gerbera with differed results were performed by many researchers through using different explants of gerbera jamesonii cultivars; like using the capitulum (Ray et al., 2005; Akter et al., 2012), shoot tip (Gantait et al., 2010; Cardoso and Teixeira da Silva, 2013; Nazari et al., 2014), petal (Kumar and Kanwar, 2006), leaf (Kumar et al., 2004; Altaf et al., 2009), seeds (Nazari et al., 2014), apical meristems and vegetative buds (Naz et al., 2012), auxillary bud (Kadu, 2013). This research was achieved in the Laboratory of Plant Tissue Culture, Vegetable and Floriculture Department, Mansoura University, Egypt during 2015/2016 on Gerbera jamesonii Bolus cv. Winter Queen for studying the indirect micropropagation of this important pot and cut flower plant in Egypt. For callus induction, unopened flower buds(10 days old) were cultured after preparation on MS medium fortified with either 2,4-D or NAA (0.5, 1.0, 2.0, 4.0 and 6.0 mg/l) each alone. The calli were transferred on media supplemented with either BAP or meta-topolin (0.0, 0.3, 0.6, 1.2 and 2.4 mg/l) each alone with 0.5 mg/l NAA as a fixed concentration for redifferentiation. Moreover, shoots were transferred to media supplemented with NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) with 0.3 mg/l meta-topolin for the in vitro rooting. Results showed that 6.0 mg/l 2,4-D produced the heaviest callus fresh weight (7.22 g), and decreased the callus induction time (17.50 days), but the callus texture was compact and creamish to brown in color. In addition, the lower concentrations from either 2,4-D (1.0 and 2.0 mg/l) or NAA (0.5 to 1.0 mg/l) produced a nodular growth and reduced the roots initiation time. Finally, well rooted plantlets were transferred to a mixture of peatmoss+perlite+clay (2:1:1 v/v) produced a survival percentage of 92.90% with a plantlets height of 4.61 cm and 4.50 leaves / plantlet on average.

Keywords: callus dedifferentiation, callus redifferentiation, callus induction, multiplication, in vitro rooting.

Indirect Micropropagation of Gerbera jamesonii Bolus cv. Winter Queen through Callus Redifferentiation

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sterilizer was HgCl₂ at 0.1% solution for 5 min according to Kharrazi et al., (2018) with adding 5 drops of Tween 20 for 5 min, followed by washing for 4-5 times (3 min each) through sterile distilled water.

In vitro by using the following equations; by soaking 0.5 g of plantlets leaf tissues in 20 ml of 80% Kasem, M. M.

callus morphology characteristics (texture and color).

autoclave. Data were recorded for callus induction period (days), callus induction percentage, callus fresh weight and callus redifferentiation treatments

(1.0 and 2.0 mg/l 2,4-D or 0.5 and 1.0 mg/l NAA) were re transferred on MS free hormone medium for 4 weeks to induce any callus. So, it was omitted from the statistical analysis. All the media were fortified with 8 g/l agar and 30 g/l sucrose with a pH of 5.8 before sterilization in the autoclave. Data were recorded for callus induction period (days), callus induction percentage, callus fresh weight and callus morphology characteristics (texture and color).

Callus induction treatments

Sterilized flower buds explants were cut from all of its sides for enhancing callus cells formation to reach 0.5 cm³ approximately. Then, the treated flower buds were transferred on MS (Murashige and Skoog, 1962) basal medium fortified with either 2,4-D or NAA (0.5, 1.0, 2.0, 4.0 and 6.0 mg/l) each alone for callus induction. It is worth to mention that the hormone-free medium did not induce any callus. So, it was omitted from the statistical analysis. All the media were fortified with 8 g/l agar and 30 g/l sucrose with a pH of 5.8 before sterilization in the autoclave. Data were recorded for callus induction period (days), callus induction percentage, callus fresh weight and callus morphology characteristics (texture and color).

Callus redifferentiation treatments

Nodular calli obtained from the previous experiment (1.0 and 2.0 mg/l 2,4-D or 0.5 and 1.0 mg/l NAA) were transferred on MS free hormone medium for 4 weeks to eliminate any residual hormones from the previous experiment. Then, callus pieces of approximately 1 cm³ were transferred on MS solid medium fortified with either BAP or meta-topolin each alone at different concentrations (0.0, 0.3, 0.6, 1.2 and 2.4 mg/l), in addition to a fixed concentration from NAA (0.5 mg/l) as recommended by Gantait et al., (2010) on G. jamesonii cv. Scilla. Three subcultures were carried out after every 4 weeks interval. All the media were supplemented with 8 g/l agar and 30 g/l sucrose. Data were recorded after the third subculture for shoot initiation time (days), multiplication percentage, microshoots number/callus, microshoots length (cm) and microshoots total chlorophylls (mg/gfw.) according to Lichtenthaler (1987), since leaf chlorophylls was extracted by soaking 0.5 g of plantlets leaf tissues in 20 ml of 80% acetone. The absorbance of the extract was measured for chlorophyll a and b at 663.2 and 646.8 nm, respectively by a spectrophotometer. Then, total chlorophylls were calculated by using the following equations;

Chlorophyll a (mg/gfw) = 12.25 A₆₄₃.₂ - 2.79 A₆₆₄.₈
Chlorophyll b (mg/gfw) = 21.50 A₆₄₄.₈ - 5.10 A₆₆₃.₂
Total chlorophylls (mg/gfw) = 7.15 A₆₄₃.₂ - 18.71 A₆₆₄.₈

In vitro rooting

Microshoots with approximately 3 cm long which derived from the previous treatment (callus redifferentiation) were excised and subjected to ½ MS basal medium supplemented with various concentrations of NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) with a fixed concentration from meta-topolin (0.3 mg/l) for rooting. Data were recorded for the rooting initiation time (days), rooting percentage, roots number/plantlet and roots length (cm).

Incubation condition

All experiments were incubated at 25 ± 3°C and were exposed to a photoperiod of 16/8 hours light and dark cycling under 2000 Lux intensity provided by cool day light fluorescent tubes with 70% ± 3% relative humidity maintained in the culture room.

Acclimatization of the in vitro rooting plantlets

Plantlet acclimatization was prepared by pulling out well-rooted plantlets from the culture jars gently using forceps. Plantlet roots were put under running faucet water to remove the media and agar residual attaching them. The plantlet roots were then immersed in 1% Rizolix 50% (Tolclofos-methyl) for 5 min and air-dried on paper, then cultured in pre-sterilized 5 cm plastic pots containing a mixture of peat moss + perlite + clay (2:1:1 v/v). The cultured pots were watered with sufficient amount of ¼ strength MS salts medium, and then placed in a glass box covered with a polyethylene transparent for 15 days. In addition, after the first week, the polyethylene sheet was exposed gradually every day during the second week. These plantlets were then transferred to a polyethylene greenhouse in the nursery.

Statistical analysis

A completely randomized design (one way) was used with all the experiments. Numbers in each table represent the mean of twice repeated experiments of 4 replicates each contains 4 jars. COSTAT v.63 statistical software was used for analysis of variance (ANOVA) and subsequently Least Significant Differences (LSD) method according to Steel and Torrie (1980) was done for means comparison at P ≤ 0.05.

RESULTS AND DISCUSSION

Callus induction

No callus was induced in hormone-free medium (control treatment), so it was omitted from the statistical analysis

Callus induction period (days)

As for auxin type and concentration on the callus induction period, data in Table (1) showed a relationship between increasing the concentrations of the two tested auxins (2,4-D and NAA) and the required time for starting callus induction. Since the least significant periods for the callus induction (17.50 and 18.75 days) were recorded for MS media supplemented with either 2,4-D or NAA at 6.0 mg/l each alone, respectively. Moreover, media fortified with either 2,4-D or NAA at the lowest concentration of 0.5 and 1.0 mg/l significantly retarded the time for the callus induction (42.75, 36.00, 45.25 and 33.50 days, respectively). In general, 2,4-D was superior for reducing the required number of days for starting callus induction comparing with the NAA one. This result was in the same trend which obtained by Akter et al., (2012) on G. jamesonii who revealed that callus initiation could appeared within 22 to 25 days from culturing the flower buds, but in case of our findings the required time was shorter.
more less and this might be according to the different in gerbera cultivars, growth regulators types and concentration. Subsequently, Arumugam et al., (2009) confirmed that the callus induction be based on many factors such as the culture environment, explant nature, and the growth regulator.

Table 1. Impact of auxin type and concentration on callus initiation characters from unopened bud flowers of G. Jamesonii cv. Winter Queen after 6 weeks.

<table>
<thead>
<tr>
<th>Auxin type and Conc.(mg/l)</th>
<th>Callus induction period (days)</th>
<th>Callus fresh weight (g)</th>
<th>Texture</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>42.75</td>
<td>4.17b</td>
<td>friable</td>
<td>Creamish white</td>
</tr>
<tr>
<td>1.0</td>
<td>36.00b</td>
<td>4.41c</td>
<td>nodular</td>
<td>Greenish white</td>
</tr>
<tr>
<td>2.0</td>
<td>30.00c</td>
<td>5.74b</td>
<td>nodular</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>4.0</td>
<td>22.00d</td>
<td>5.85b</td>
<td>compact</td>
<td>Creamish green</td>
</tr>
<tr>
<td>6.0</td>
<td>17.50e</td>
<td>7.22b</td>
<td>compact</td>
<td>Creamish brown</td>
</tr>
<tr>
<td>NAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>45.25e</td>
<td>3.16c</td>
<td>nodular</td>
<td>Brownish green</td>
</tr>
<tr>
<td>1.0</td>
<td>33.50e</td>
<td>3.09c</td>
<td>nodular</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>2.0</td>
<td>29.75e</td>
<td>3.67e</td>
<td>compact</td>
<td>Greenish white</td>
</tr>
<tr>
<td>4.0</td>
<td>20.50f</td>
<td>4.59f</td>
<td>compact</td>
<td>Greenish white</td>
</tr>
<tr>
<td>6.0</td>
<td>18.75f</td>
<td>5.34e</td>
<td>compact</td>
<td>Greenish brown</td>
</tr>
</tbody>
</table>

Data in table represent means of twice repeated experiments and each number have the same letter in the same column indicate statistically non-significant difference at p < 0.05.

**Callus induction percentage**

Data illustrated in Figure (2) cleared that the highest callus induction percentages were obtained when pieces of closed flower buds were cultured on MS media fortified with 2,4-D at 4.0 and 6.0 mg/l or NAA at 6.0 mg/l, as it were 87.50, 93.75 and 81.25, respectively with non-significant differences between them. Similar results were obtained by Shabbir et al., (2012) on G. Jamesonii cv. Sunglow, since they cleared that among the tested auxins 2,4-D was more effective in producing the highest callus induction percentage (100%), followed by NAA which produced 83.33%. On the other hand, the lower concentration from 2,4-D or NAA (0.5 and 1.0 mg/l), minimized the callus induction percentages for a range from 31.25 to 43.75%. These results were in harmony with Mohlakola et al., (2017) on G. Jamesonii cv. Dasaxju who revealed that among the plant growth hormone 2,4-D was more effective in increasing and producing the highest callus induction percentage of 96.70%. Also, they indicated that 2,4-D at the lowest concentration, negatively affected the callus formation and when the 2,4-D concentrations gradually increased, there was a positive increase in callus formation with the different in the explant type (petiole) and 2,4-D concentration (1.5 mg/l), and this might be a cultivar dependent.

![Graph](image)

Figure 2. Impact of auxin type and concentration on callus induction % of G. Jamesonii cv. Winter Queen after 6 weeks. Columns with the same letters indicate statistically non-significant difference at p < 0.05.

Koroch et al., (2003) on Echinacea pallida compared between auxins and cytokinins in callus induction percentages and found that auxins like (2,4-D and NAA) was more effective, since auxins stimulate the metabolism of RNA and improve the mRNA transcription of which produce the required proteins for callus formation and cell proliferation. Moreover, the optimal concentrations of 2,4-D or NAA on Chrysanthemum morifolium improve the enlargement and cells division which increase the synthetic enzymes and the autolytic activities through synthesis of cell wall components and its effects on cell wall plasticity (Nahtid et al., 2007).

**Callus fresh weight**

From the data in Table (1), it was clear that 2,4-D at 6.0 mg/l significantly produced the heaviest callus fresh weight of 7.22g, when compared with all of the other treatments. This result emphasizes the result of Karimian et al., (2014) on Taxus Brevifolia, who found that 1.5 mg/l 2, 4-D was more effective on callus growth especially the callus fresh weight, but the 2,4-D optimal concentration varied with respect to explant types. In addition, media fortified with 2.0 and 4.0 mg/l 2,4-D or 6.0 mg/l NAA came in the second and the third order in that respect, since they were 5.85, 5.74 and 5.34g, respectively. The lightest callus fresh weight recorded for medium supplemented with NAA at concentrations of 0.5, 1.0 and 2.0 mg/l, as it were 3.16, 3.00 and 3.67g, respectively.

Auxins (NAA and 2,4-D) stimulate the cell elongation through activating the transportation process of the hydrogen ions away from the plant cells and decreasing the pH surrounding the cells. The acidification process surrounding the cell walls enhances and improves the cellulose micro-fibrils slippage and breaking the bonds of polysaccharides in the cell wall, which make the cell wall more flexible and then a vigorous callus growth will obtain (Taiz and Zieger, 2002). In addition, Can et al., (2008) stated that auxins increase the methylation of the nuclear DNA which makes the cells to enter in the redifferentiation process again and finally improve the cell division.

**Callus morphology**

Data in Table (1) and illustrated in Figure (3) cleared that a friable calli were formed with adding 2,4-D at the lowest concentrations of 0.5 mg/l. In addition, nodular calli were formed on MS medium fortified with 1.0 and 2.0 mg/l 2,4-D or 0.5 and 1.0 mg/l NAA. Moreover, the highest concentrations from either 2,4-D or NAA produced a
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As for callus color, it was noticed that the nodular calli which formed when 2,4-D or NAA were added to the growth media, have a greenish color mixed partially with another color as white, yellow or brown. Also, the friable or the compact calli which formed in case of using 2,4-D were creamish mixed with white, green and brown. Clearly, in most cases NAA concentrations a greenish calli contained some yellow, white or brown tissues were formed. This may be due to the findings of Shabbier et al. (2012) who reported that increasing 2,4-D concentrations turned the callus color of G. jamesonii cv. Sunglow to brown or dark brown when it pass the optimal level. In addition, Shirin et al., (2007) revealed that the browning of the formed callus might be as a result of 2,4-D high concentration which inhibit the cell division and suppress synthesis of protein. Also, Mohlakola et al., (2017) revealed that increasing the concentrations of 2,4-D derived a necrosis calli which made the calli tissues take a dark brownish color.

Figure 3. Impact of auxin type and concentration on callus morphology of G. jamesonii cv. Winter Queen after 6 weeks, as figures from a to c; represent effects of 2,4-D concentrations (0.5, 1.0, 2.0, 4.0 and 6.0 mg/l, respectively), and figures from f to j; represent effects of NAA concentrations (0.5, 1.0, 2.0, 4.0 and 6.0 mg/l, respectively).

Callus redifferentiation

Micro-shoots initiation time (days)

Impact of BAP and meta-topolin on micro-shoots initiation time from the callus tissues were recorded in Table (2). It can be observed that meta-topolin had the upper hand in minimizing the required time for micro-shoots initiation with its different concentrations comparing with the concentrations of BAP. Moreover, supplying the redifferentiation media by meta-topolin at 1.2 and 2.4 mg/l or 2.4 mg/l BAP, came in the first order in that respect, since they recorded 14.75, 15.00 and 15.25 days, respectively. On the other side, media fortified with 1.2 mg/l BAP + 0.5 mg/l NAA or 0.6 mg/l BAP + 0.5 mg/l NAA delayed the required time for micro-shoots initiation to reach 24.50 and 23.25 days, respectively. Therefore, Shabanpour et al., (2011) indicated that the shoot regeneration process in gerbera is depended on plant growth regulators combinations and cultivars, so the regeneration procedure and the growth medium should be optimize for each cultivar.

Multiplication percentage

Data illustrated in Figure (4) showed that the highest multiplication percentages of 93.75% were recorded for MS medium fortified with 2.4 mg/l BAP + 0.5 mg/l NAA, followed by 87.50% for redifferentiation media supplemented with 1.2 or 2.4 mg/l meta-topolin. This finding was in a similar trend with Altaf et al., (2009) who crushed a germinated seeds of G. jamesonii and cultured it on MS medium fortified with BA at 3.0 mg/l and obtained the highest multiplication percentage. In addition, Koszeghi et al., (2014) on Ocimum basilicum cleared that fortifying the multiplication medium by BAP at 1.0 mg/l or meta-topolin at 0.5 improved the new shoots development characteristics, but meta-topolin still showing an increase comparing with the BAP one. Moreover, Bairu et al., (2006) on Aloe polyphylla used meta-topolin for inducing shoots multiplication and found that it increased the multiplication percentage and reduced the hyperhydricity. Also, in the current research adding 1.2 mg/l BAP + 0.5 mg/l NAA recorded 81.25% with non-significant differences between all the previous treatments. On contrary, the control medium (MS free hormone medium) failed to regenerate any micro-shoots from the cultured calli.

Figure 4. Impact of BAP and meta-topolin on multiplication % of G. jamesonii cv. Winter Queen after 6 weeks from culturing. Columns with the same letters indicate statistically non-significant difference at p < 0.05.

Micro-shoots number

As for the impact of BAP and meta-topolin on micro-shoots number derived from the redifferentiated calli of G. jamesonii Bolus cv. Winter Queen, data in Table (2) and illustrated in Figure (5) cleared that meta-topolin showed superiority than BAP especially with the higher concentrations (1.2 and 2.4 mg/l), when compared with all the other treatments. This result confirm the finding of Bairu et al., (2006) who found a positive relationship between increasing the meta-topolin concentrations and the shoots number of Aloe polyphylla. Moreover, the lowest concentrations from BAP (0.3 and 0.6 mg/l) derived a lower micro-shoots number of 4.00 and 5.75 micro-shoots, respectively.
increasing ethylene production in the cell which increases the inhibition of protein synthesis, giving a shortened shoots (Werner et al., 2001).

**Total chlorophylls in micro-shoots**

Total chlorophylls in micro-shoots were analyzed as an indicator for the vitrification problem and the data was shown in Table (2). All over the tested concentrations from BAP and meta-topolin, it was obvious that all the meta-topolin concentrations still giving higher values for that respect as it ranged from 8.57 to 9.43 mg/gfw, comparing with BAP concentrations which ranged from 5.02 to 7.90 mg/gfw. Also, Mutui et al., (2012) sprayed various growth regulators on Pelargonium × hortorum and found that meta-topolin had significant effects in increasing the total chlorophylls and it is very active in reducing the leaf senescence. Moreover, Anuraj et al., (2017) stated that supplemented the in vitro growth medium with 2.5 mg/l meta-topolin improved the tolerance of Gracilaria corticata var. cylindrica to the salinity condition and increased the total chlorophylls and carotenoids. In general, our results showed that the superior treatment in increasing the total chlorophylls and then decreasing the vitrification in the micro-shoots was recorded for MS medium fortified with either 0.3 or 0.6 mg/l meta-topolin which showed a significant differences comparing with all the other cases.

**In vitro rooting process**

**Rooting initiation time (days)**

Impact of NAA concentrations with a fixed concentration from meta-Topolin (0.3 mg/l) in the rooting initiation time was recorded in Table (3). There was a relationship between NAA concentrations and the rooting initiation time, since increasing concentrations of NAA directly decreased the essential time (days) rooting initiation to 13.00 days comparing with the control which delayed the essential period to reach 93.75 days. Also, Mutui et al., (2012) sprayed various growth regulators on Pelargonium × hortorum and found that meta-topolin significantly minimized the required period for rooting initiation to 13.00 days comparing with the control which delayed the essential period to reach 93.75 days.

**Table 3. Impact of NAA concentrations with meta-topolin at a fixed concentration (0.3 mg/l) in half strength MS medium on rooting characters of G. jamesonii cv. Winter Queen after 4 weeks from culturing.**

<table>
<thead>
<tr>
<th>Rooting treatments (mg/l)</th>
<th>Rooting initiation time (days)</th>
<th>Rooting %</th>
<th>Roots number/shoot</th>
<th>Roots length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.75</td>
<td></td>
<td>2.25</td>
<td>4.55</td>
</tr>
<tr>
<td>NAA 0.5+ 0.3 meta-topolin</td>
<td>24.00</td>
<td>31.25</td>
<td>4.29</td>
<td>3.88</td>
</tr>
<tr>
<td>NAA 1.0+ 0.3 meta-topolin</td>
<td>17.25</td>
<td>50.00</td>
<td>5.30</td>
<td>4.13</td>
</tr>
<tr>
<td>NAA 1.5+ 0.3 meta-topolin</td>
<td>13.50</td>
<td>81.25</td>
<td>8.27</td>
<td>4.23</td>
</tr>
<tr>
<td>NAA 2.0+ 0.3 meta-topolin</td>
<td>13.00</td>
<td>93.75</td>
<td>9.75</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Data in table represent means of twice repeated experiments and each number have the same letter in the same column indicate statistically non-significant difference at p < 0.05.
Rooting percentage

Data illustrated in Figure (6) indicated that the highest rooting percentage of 93.75 and 81.25% resulted from supplying 2.0 mg/l NAA + 0.3 mg/l meta-topolin or 1.5 mg/l NAA + 0.3 mg/l meta-topolin into ½ strength MS medium. The least rooting percentage of 18.75% derived from the control medium (½ strength MS free hormone medium). Gentile et al., (2014) cleared that fortifying the rooting medium by meta-topolin produced a higher rooting percentage, so in the current research we combined meta-topolin with NAA for increasing the rooting percentage.

Gentile et al. (2014) cleared that fortifying the rooting medium by meta-topolin produced a higher rooting percentage, so in the current research we combined meta-topolin with NAA for increasing the rooting percentage. The least rooting percentage of 18.75% derived from the control medium (½ strength MS free hormone medium). Gentile et al., (2014) cleared that fortifying the rooting medium by meta-topolin produced a higher rooting percentage, so in the current research we combined meta-topolin with NAA for increasing the rooting percentage.

Roots number

In a similar way, Figure (7) showed that the superior treatments in increasing the rooting percentage also increased the roots number per plantlet. As, medium fortified with 2.0 mg/l NAA + 0.3 mg/l meta-topolin significantly gave the maximum number of roots (9.75 roots/plantlet), followed by 8.27 roots/ plantlet for medium contained 1.5 mg/l NAA+ 0.3 mg/l meta-topolin. In addition, the control medium recorded the least value of 2.25 roots/ plantlet. A different result was obtained by Warar et al., (2008) who reported healthy roots of G. jamesonii cv. Sciella formed on MS medium fortified with 0.5 mg/l NAA, but Hasbullah et al., (2008) confirmed our findings, but with replacing the fixed concentration of meta-topolin by using 0.1 mg/l BAP in combination with 2.0 mg/l NAA, since this treatment increased the roots number value. In addition, Werbrouck et al., (1995) on Spathiphylum flouribundum found that micro-shoots which treated by meta-topolin significantly produced a higher roots number and length.

Roots length (cm)

As for roots length, data in Table (3) and illustrated in Figure (7) indicate that non-significant difference was shown between most of the tested NAA concentrations, but it was obvious that the control medium and media supplemented with 1.0 mg/l NAA + 0.3 mg/l meta-topolin and 1.5 mg/l NAA + 0.3 mg/l meta-topolin still improving the roots length, since they recorded 4.55, 4.73 and 4.23 cm, respectively. Clearly, the highest NAA concentration of 2.0 mg/l + 0.3 mg/l meta-topolin produced the shortest roots length of 3.02 cm. This result was agreed with the finding of Nazari et al., (2014) who showed non-significant differences between using NAA at 0.5 and 1.0 mg/l on roots length. In addition, higher concentration of NAA reduced the growth of roots as a result from increasing biosynthesis of endogenous ethylene (Salisbury and Ross, 2005).

Acclimatization process

Individual rooted plantlets were transferred to the mixture of Peatmoss + perlite + clay (2:1:1 v/v) gave a survival percentage of 92.90% with a plantlets height of 4.61cm and 4.50 leaves/plantlet (Figure, 8). This high response could be a result of the mixture ability to provide enough aeration and moisture to the plantlet. This confirm the findings of Weam et al., (2015) on Holmskioldia sanguinea who cleared that mixing several acclimatization growing medium is very important to produce a vigor plantlets with a good characteristics. In addition, they illustrated that the adaptation media must provide a good water balance with suitable water supply. Also, Ravanār et al., (2011) cleared that perlite is a vital commodity in the adaptation potting mixture if it mixed with peatmoss, since adding the perlite to the peatmoss improving the air amounts (oxygen) which held in the peatmoss beside the suitable water content in the peatmoss.

Figure 6. Impact of NAA concentrations with meta-topolin at a fixed concentration (0.3 mg/l) on the rooting % of G. jamesonii cv. Winter Queen after 4 weeks from culturing. Columns with the same letters indicate statistically non-significant difference at p < 0.05.

Figure 7. Impact of NAA concentrations with meta-topolin at a fixed concentration (0.3 mg/l) on in vitro roots number of G. jamesonii cv. Winter Queen after 4 weeks from culturing, as a; represent the rooting control medium (MS free hormone), figures from b to e; represent NAA concentration (0.5, 1.0, 1.5 and 2.0 mg/l, respectively).

Figure 8. Acclimatized G. jamesonii cv. Winter Queen plantlet cultured in a mixture of peatmoss + perlite + clay (2:1:1 v/v) after 4 weeks from the adaptation process.
CONCLUSION AND RECOMMENDATION

Indirect micropropagation procedure of *Gerbera jamesonii* Bolus cv. Winter Queen through callus induction and redifferentiation was achieved following the next stages. Unopened flower buds (10 days old) were taken from plants inside a polyethylene greenhouse and placed under running tap water for one hour, then soaked in 0.1% fungicide solution (Rizolix 50%, Tolecfos-methyl) for 30 min., the main sterilizer was HgCl₂ at 0.1% for 5min. For obtaining the highest green and nodular callus induction, we economically recommend to use MS full strength medium fortified with 1.0 mg/l meta-topolin for obtaining a higher multiplication percentage (87.50%) and micro-shoots with 1.2 mg/l of approximately 1 cm meta-topolin for a survival percentage of 92.90%, plantlet height of 4.61 cm and 4.50 leaves/plantlet. Finally, for further regulators may effect on the cultivar stability which could be a replacement for BA and zeatin?.

REFERENCES


