Medium Term Preservation of Wild Crocus (*Crocus Hyemalis* and *Crocus Moabiticus*) Embryogenic Callus

Savinaz H. Baghdadi, Rida A. Shibli*, Maha Q. Syouf, Mohammad A. Shatnawai, Arabia Arabiat, Ibrahim M. Makhadmeh

**ABSTRACT**

Slow growth *in vitro* preservation using different osmotic agents (sucrose, mannitol and sorbitol) at different concentrations (0.0, 0.1, 0.2, 0.3 and 0.4 M) and at reduced temperatures (4, 8 and 24 °C) was investigated. Embryogenic calli of crocus species were used. Preservation of calli on a medium supplemented with 0.2 M sucrose, mannitol or sorbitol under complete darkness was able to decrease the growth rate and maintain explant quality for 12 weeks. Calli preservation at 8 °C in the dark was also able to decrease the growth and maintain explants in a better condition for 12 weeks before the next subculture. Also, the appearance of some physiological disorders like browning and tissue death was observed as the concentrations of osmotic agents increased, the temperature lowered and the preservation period extended.

**Keywords**: Embryogenic callus, *in Vitro* Preservation, Manitol, Sorbitol, Sucrose, Wild Crocus.

**INTRODUCTION**

*Crocus* is one of the genera of the *Iridaceae* family including about 83 species with a range from the Mediterranean region and Asia Minor through S. Russia to Iran (Jellito and Schscht, 1990). *Crocus sativus* L. is the most common *Crocus* species, also known as saffron. Due to its yellow dye; crocin (Himeno et al., 1988), saffron is commonly used in dyeing and flavoring of foods (Chichiricco and Grilli-Caiola, 1987). There is also a long practice of saffron use in the traditional medicine of many cultures (Molina et al., 2005). It has been demonstrated that some of *Crocus* components have cytotoxic, anti-carcinogenic and anti-tumor properties (Molina et al., 2004). It has also been reported that saffron has a uterine sedative property, which is useful in dysmenorrhoea diseases and the premenstrual syndrome (Nazzal, 2007). In addition, saffron has also properties as anticholesterol and atherosclerosis reducer (Dhar et al., 1997). Common saffron is an autotriploid (2n=3x=24, x=8) (Chichiricco and Grilli-Caiola, 1987).

*Crocus hyemalis* Bioss. and Blanche. is used to be named with the common name of I'hlian in Ajloun as its corms are edible. While *Crocus moabiticus* Bornm. and Dinsmore ex Bornm. is a close relative to common saffron and it is one of the two crocus species endemic to Jordan along with *C. naqabensis*. Both species underwent several studies for specifying growth locations, characterization, utilization, multiplication and
preservation by different cryopreservation based techniques (Baghdadi et al., 2010; Nazzal, 2007; Shibli et al., 2009).

The term "slow growth" is used to cover "growth limitation", "inhibition" or "minimal growth" and other similar terms which imply a modification of the culture conditions (Botau et al., 2005). Depending on species, materials preserved under slow growth in vitro techniques can be held for 1-15 years with periodic sub-culturing (Rao, 2004). They depend on reducing growth rate through the use of growth retardants such as abscisic acid, low light intensity, low temperature, low pressure/low oxygen (Bajaj, 1991), manipulating the nutrient contents in the growth medium or the addition of osmotic agents such as sucrose, mannitol or sorbitol to the culturing medium.

Storage under low temperature is one of the major tissue culture techniques used for the conservation of PGR (Moges et al., 2003). It enhances accumulation of unsaturated fatty acids in the cell membrane causing cell membrane thickening, thus retarding cell division and elongation (Hopkins, 1999). In most cases, a low temperature often in combination with low light intensity or even darkness is used to limit growth (Rao, 2004).

The addition of osmotic agents to culture media was found to be efficient in reducing or retarding growth and increasing the storage life of many in vitro tissues of different plant species (Gopal and Sukh-Chauhan, 2010; Shibli et al., 2005; Tahtamouni et al., 2001). High levels of osmotic agents in the in vitro culture medium would act against the creation of a critical turgor pressure, which must be established before cell expansion can occur (Moges et al., 2003).

Sucrose is a major component of most tissue culture media. It functions as both a carbon/energy source and as osmotic agent (Shibli et al., 2006). In date palm callus culture, 0.1 M sucrose gave 86.7% survival after 12 weeks of preservation (Shibli et al., 2005). Moges et al. (2003) found complete survival of preserved African violet microshoots at all sucrose concentrations except on 0.35 M sucrose for 12 weeks of preservation where only 66.7% survival was obtained.

Manitol is a sugar-alcohol which is produced as a primary photosynthetic product by some plant species, and can be metabolized by them (Shibli et al., 2006; Tahtamouni et al., 2001). It reduced shoot growth of Chrysanthemum morifolium (Shibli et al., 1992); increasing concentrations of mannitol decreased both survival and regrowth, irrespective to preservation period. Physiological disorders increased with increasing mannitol concentration and extension of preservation period in wild pear (Tahtamouni et al., 2001), and African violet (Moges et al., 2003).

Sorbitol is another sugar alcohol, which when added at high concentrations to the proliferation medium has been found to inhibit shoot growth of Chrysanthemum morifolium (Shibli et al., 1992). The death of tissues under high concentrations of sorbitol may be due to the toxicity which resulted from continuous accumulation of carbohydrates as a response to osmostressing (Moges et al., 2003; Shibli et al., 2005; Tahtamouni et al., 2001).

Due to crocus species susceptibility to wide range of pathogens and environmental stresses (Plessner et al., 1990); those species can not be effectively stored for long time using conventional conservation methods. In vitro preservation provides an important and effective preservation method for such plant species. This study was conducted to provide new information about the most efficient procedure for medium term storing of wild Crocus (C. hyemalis and C. moabiticus) via slow growth; which included the optimum concentration for each osmotic agent and optimum temperature.
MATERIALS AND METHODS

Plant Material

The corms and seeds of both species were collected from their natural habitat in Jordan: C. hyemalis was collected from Rhaba region in Irbid district; at longitude of 35°48′33.3 E, latitude 32°24′30.1 N and elevation of 943 m above sea level, while C. moabiticus was collected from Al-Khanasri region in Mafraq district; at longitude of 36°03′44.5 E and latitude of 32°24′45.6 N with elevation of 576.40 m above sea level (Nazzal, 2007). Both species where identified according to Flora-Palestina (Zohari and Feinburn-Dothan, 1986).

In Vitro Culture of Plant Material

Callus of Crocus hyemalis and C. moabiticus were originally obtained from the Plant Tissue Culture Laboratory/National Center for Agricultural Research and Extention (NCARE) (Baqa, Jordan).

Crocus seeds only were forced to callus form. The induction and subculturing medium used for multiplication of mother cultures consisted of solid Murashige and Skoog (1962) [MS] medium supplemented with 0.1 M sucrose, 4.4 µM 6-benzylaminopurine (BA), 5.4 µM α-naphthaleneacetic acid (NAA) and 0.5 g/L PVP. Subculturing was performed every three weeks using a fresh growth media to establish sufficient callus stock for experimentation. The cultures were incubated in the growth room at 24 ± 2 ºC, with complete darkness.

In vitro Slow Growth Preservation

Preservation Using Osmotic Agents

Embryogenic calli were cultured on MS hormone-free medium for three days. Each piece of callus were then transferred to 250 ml Erlenmeyer flasks containing 40 ml of MS hormone-free medium supplemented with 0.1 M sucrose and incubated at 4, 8 and 24 ºC in the dark. Data were collected after 12 weeks of preservation for weight change, survival, viability index and regrowth.

TTC Test

Tetrazolium salt solution is reduced to formazen by hydrogen ions released with respiration of viable plant cells and results in a diagnostic red color formation (Lutts et al., 1996). TTC test was performed by incubating calli in test tubes with 5 ml of 0.5 % (w/v) TTC salt dissolved in 50 mM K₂HPO₄ at pH 7.0 for 15 h at 30 ºC in complete darkness (Lutts et al., 1996). The calli fragments were then rinsed in de-ionized water and
formazan was extracted with 5 ml ethanol 94 % for 5 min at 80 °C. After removal of calli, absorbance was measured at 487 nm and viability index of the calli was calculated as the following formula: Viability index ($I_v$) = Absorbance / 50 mg fresh weight.

**Experimental Design**

Treatments were arranged in a complete randomized design (CRD). Each treatment was replicated three times with three callus clumps per replicate. The collected data were statistically analyzed using SAS (Statistical Analysis System, Cary, NC, 2001). Means were separated according to the least significance difference (LSD) at 0.05 probability level.

**RESULTS AND DISCUSSION**

**Preservation Using Osmotic Agents**

**Sucrose**

Weight change, survival and regrowth percentages were decreased significantly in both *Crocus moabiticus* and *C. hyemalis* (Table 1) as the concentration of sucrose increased in the medium. As reported by Al-Ababneh (2001), explant growth in the presence of sucrose depends on its concentration. Survival and regrowth of the date palm callus decreased significantly as the concentration of sucrose increased in the medium (Shibli et al., 2005). Moreover, slow shoot growth rate resulting from using sucrose supplemented media was reported for African violet (*Saintpaulia ionantha*) (Moges et al., 2003), wild pear (*Pyrus syriaca*) (Tahtamouni et al., 2001) and bitter almond (*Amygdalus communis*) (Shibli et al., 1999).

The effect of high concentration of osmotic agents in a medium would result in negative water potential (Hopkins, 1999), which could reduce the turgor pressure needed for cell division and this might inhibit growth (Subaih, 2005). Embryogenic cultures of carrot could be conserved on a medium without sucrose for two years and would re-proliferate if a sucrose solution was supplied (Withers, 1979). Starch accumulation due to increased sucrose concentration and decreased availability of simple sugars needed for cell division and elongation might account for the decrease in shoot growth proliferated from tobacco callus (Brown et al., 1979).

Physiological disorders were developed in some of the preserved calli of both *C. moabiticus* and *C. hyemalis*. Browning increased with increasing sucrose concentration and with longer preservation period (Fig. 1, 2). Physiological disorders were escalated with increased concentration of sucrose and extended preservation period during *in vitro* preservation of African violet (Moges et al., 2003), date palm (Shibli et al., 2005), and wild pear (Tahtamouni et al., 2001). High nutrient accumulation at sites of nutrient uptake (basal-end) with prolonged preservation period and production of phenolic substances might account for browning (Tahtamouni et al., 2001).

A high survival (88.9 %) obtained when callus clumps of *C. moabiticus* were preserved on 0.1 M sucrose medium after 12 weeks (Table 1). Similar results were obtained for date palm (Shibli et al., 2005) and wild pear (Tahtamouni et al., 2001). Survival of 100 % was obtained when *C. hyemalis* (Table 1) callus was preserved with 0.1 M sucrose after 12 weeks. Survival was 100 % in preserved African violet microshoots irrespective of the concentration of sucrose except on 0.35 M sucrose for 12 weeks of preservation where only 66.7 % survival was obtained (Moges et al., 2003). The continuous accumulation of carbohydrates, which might have reached a toxic level in the plant cells due to turnover in the degradative enzymes as an osmostressing response, could account for the death of some explants (Tahtamouni et al., 2001).

Sucrose could be recommended for preservation of
crocus embryogenic callus preferably at lower concentration less than 0.2 M for 12 weeks to maintain quality of explants and survival and regrowth capacities.

Table 1. Weight change, survival, and regrowth percentages and viability index ($I_v$) of in vitro preserved Crocus embryogenic callus as influenced by sucrose concentration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sucrose (M)</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>LSD</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. moabiticus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. hyemalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight change %</td>
<td>10 e</td>
<td>540 a</td>
<td>397 b</td>
<td>278 c</td>
<td>127 d</td>
<td>44.5</td>
<td>55 b</td>
<td>365 a</td>
<td>126 b</td>
<td>102 b</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Survival %</td>
<td>33.3 cd</td>
<td>88.9 a ab</td>
<td>66.7 bc</td>
<td>50.0</td>
<td>22.2 d bc</td>
<td>24</td>
<td>44.4</td>
<td>100 a</td>
<td>66.7 b</td>
<td>16.7 c</td>
<td>31.5</td>
<td></td>
</tr>
<tr>
<td>$I_v$</td>
<td>0.061 c</td>
<td>0.176 a b</td>
<td>0.109 b c</td>
<td>0.102</td>
<td>0.061</td>
<td>0.024</td>
<td>0.070</td>
<td>0.189</td>
<td>0.082</td>
<td>0.024</td>
<td>0.104</td>
<td></td>
</tr>
<tr>
<td>Regrowth %</td>
<td>11.1 c</td>
<td>83.3 a</td>
<td>55.6 b</td>
<td>44.4 b</td>
<td>11.1 c</td>
<td>22.4</td>
<td>16.7 c</td>
<td>83.3 a</td>
<td>50.0 b</td>
<td>5.7 d</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* Means within row, for each Crocus spp., having different letters are significantly different according to LSD at $P \leq 0.05$.

Figure 1. Browning developed on in vitro preserved Crocus moabiticus calli on a medium supplemented with 0.4 M sucrose after 12 weeks of preservation.

Figure 2. Browning developed on in vitro preserved Crocus hyemalis calli on a medium supplemented with 0.4 M sucrose after 12 weeks of preservation.
Manitol

Significant variations in weight change, survival and regrowth were obtained among the different manitol treatments, irrespective to preservation period in both *C. moabiticus* and *C. hyemalis* (Table 2). Increasing concentrations of manitol decreased survival and regrowth for both crocus species. High manitol concentrations cause negative water potential which would reduce the optimal turgor pressure needed for cell division and thus reduce growth (Moges *et al.*, 2003).

However, in the current study, using of manitol as osmotic agent in the preservation medium increased browning of calli for both species. At higher concentrations (0.3 or 0.4 M) of manitol, complete browning was occurred (Fig. 3, 4). High nutrient accumulation at sites of nutrient uptake and production of phenolic substances might cause browning (Tahtamouni *et al.*, 2001). Moreover, physiological disorders increased with increasing manitol concentration and extension of preservation period in date palm (Shibli *et al.*, 2005), African violet (Moges *et al.*, 2003). Shibli *et al.* (1992) also found a decline in shoot proliferation with the use of manitol in Chrysanthemum *in vitro* cultures.

No survival was obtained when the concentrations of manitol was 0.4 M after 12 weeks of preservation for both species (Table 1, 2). In addition, a complete loss of regrowth capacity was also obtained when the concentrations of manitol was 0.4 M for 12 weeks of preservation for both species (Table 2). Similarly, in African violet complete loss of regrowth capacity was also occurred when concentrations of manitol exceeded 0.16 M for 12 weeks of preservation period, respectively (Moges *et al.*, 2003).

Generally, preservation for an extended period on media containing elevated levels of manitol significantly reduced the growth of cultures. Manitol could be recommended for preservation of wild crocus species calli at a concentration of 0.2 M for 12 weeks to maintain the quality of explants, good survival and regrowth capacities.

### Table 2. Weight change, survival, and regrowth percentages and viability index (*I*<sub>v</sub>) of *in vitro* preserved *Crocus* embryogenic callus as influenced by manitol concentration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C. moabiticus</th>
<th>C. hyemalis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manitol (M)</strong></td>
<td>0 0.1 0.2 0.3 0.4 LSD 0 0.1 0.2 0.4 LSD</td>
<td></td>
</tr>
<tr>
<td>Weight change %</td>
<td>10 b 73 a 59 a 8 b 3 b 21.7 55 a 45 a 38 a 17 b 20</td>
<td></td>
</tr>
<tr>
<td>Survival %</td>
<td>33.3 a 44.4 a 33.3 a 16.7 ab 0.0 b 31.9 44.4 b 72.3 a 55.6 ab 0.0 c 17.9</td>
<td></td>
</tr>
<tr>
<td><em>I</em>&lt;sub&gt;v&lt;/sub&gt;</td>
<td>0.061 c 0.139 a 0.074 b 0.039 d 0.000 c 0.016 0.070 b 0.131 a 0.077 b 0.000 c 0.018</td>
<td></td>
</tr>
<tr>
<td>Regrowth %</td>
<td>11.1 bc 33.3 a 27.8 ab 11.1 bc 0.0 c 21.8 16.7 bc 44.4 a 22.2 b 0.0 c 20.7</td>
<td></td>
</tr>
</tbody>
</table>

* Means within row, for each *Crocus* spp., having different letters are significantly different according to LSD at *P* ≤ 0.05.
Sorbitol

Significant reduction in terms of survival was occurred when sorbitol was used as an osmotic agent irrespective of its concentrations in both *C. moabiticus* and *C. hyemalis* (Table 3). When the calli was preserved on 0.1 M sucrose the survival was 100 % and 88.9 % for *C. hyemais* and *C. moabiticus*, respectively, while the use of 0.1 M sorbitol survival was 77.8 % for both species (Table 3). Shoot height of African violet decreased when sorbitol was used as an osmotic agent irrespective of its concentrations (Moges *et al.*, 2003). Tahtamouni *et al.* (2001) reported that all preserved microshoots of wild pear survived at the end of each preservation period at all osmostressing treatments except for those preserved on 12 % sorbitol for 12 weeks.
A complete loss of survival and regrowth were occurred when calli were preserved on 0.4 M sorbitol for 12 weeks in both Crocus species (Table 3). The death of calli might be due to the toxicity which resulted from continuous accumulation of carbohydrates as a response to osmostressing (Withers, 1979). Similarly survival reduction of explants with increased levels of sorbitol and prolonged preservation time was obtained in date palm (Shibli et al., 2005), Chrysanthemum (Shibli, 1991), wild pear (Tahtamouni et al., 2001) and African violet (Moges et al., 2003). A total of 77.8 % survival of C. moabiticus and C. heymalis calli was obtained when calli were preserved on a medium containing 0.1 M sorbitol, whereas, a complete loss of regrowth capacity was obtained after preservation of calli on 0.4 M sorbitol after 12 weeks for both species (Table 3). A total percentage of 93.3 % survival of date palm calli after 6 weeks were obtained when calli were preserved on a medium containing 0.16 M sorbitol, whereas, a complete loss of regrowth capacity was obtained after preservation of calli on 0.66 or 0.82 M sorbitol after 6 or 12 weeks, respectively (Shibli et al., 2005). Similarly, both Tahtamouni et al. (2001) and Moges et al. (2003) also found that regrowth of explants was decreased with increasing concentration of sorbitol and preservation time in wild pear.

Browning of both Crocus species calli was increased with increased concentrations of sorbitol and extended preservation period. All calli developed browning when calli were preserved on a medium containing 0.2 M sorbitol or higher concentrations for 12 weeks (Fig. 5, 6). Tahtamouni et al. (2001) reported that physiological disorders increased as the concentration of sorbitol increased and as the preservation period extended in wild pear. Similarly, Moges et al. (2003) found that leaves of African violet developed marginal necrosis and eventually turned brown with increased levels of sorbitol and as the preservation period was extended.

Generally, preservation for an extended period on media containing elevated levels of sorbitol significantly reduced the growth of cultures. Sorbitol could be recommended for preservation of wild crocus calli at a concentration of 0.2 M for 12 weeks to maintain the quality of explants, survival and regrowth capacities before subculturing to fresh medium.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sorbitol (M)</th>
<th>C. moabiticus</th>
<th>C. hyemalis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  0.1  0.2  0.3  0.4</td>
<td>0  0.1  0.2  0.4</td>
<td>LSD 0  0.1  0.2  0.4</td>
</tr>
<tr>
<td>Weight change %</td>
<td>10 c 46 a 42 a 21 b 17 bc</td>
<td>10.5 55 a 43 ab 31 ab 17 b</td>
<td>29.9</td>
</tr>
<tr>
<td>Survival %</td>
<td>33.3 b 77.8 a 44.4 b 22.2 bc 0.0 c</td>
<td>25.5 44.4 b 77.8 a 50.0 b 5.7 c</td>
<td>10.4</td>
</tr>
<tr>
<td>I_r</td>
<td>0.061 b 0.134 a 0.031 c 0.014 d 0.000 d</td>
<td>0.015 0.070 b 0.095 a 0.022 c 0.011 c</td>
<td>0.019</td>
</tr>
<tr>
<td>Regrowth %</td>
<td>11.1 c 66.7 a 33.3 b 16.7 bc 0.0 c</td>
<td>16.9 16.7 b 44.4 a 16.7 b 0.0 c</td>
<td>0</td>
</tr>
</tbody>
</table>

* Means within row, for each Crocus spp., having different letters are significantly different according to LSD at $P \leq 0.05$. 

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Preservation at Low Temperature

Weight change and regrowth of calli significantly decreased as storage temperature was decreased and storage period increased in in-vitro preserved calli of both *Crocus moabiticus* and *C. hyemalis* (Table 4). While there was no significant difference of survival in *C. moabiticus* calli as storage temperatures decreased, regardless to storage period, there was a significant difference in survival of calli of *C. hyemalis*.

Subaih (2005) found that both survival and regrowth of date palm (*Phoenix dactylifera*) embryogenic callus significantly decreased with decreasing the storage temperatures. Shoot height and callus formation significantly reduced in preserved shoot tips of African violet (*Saintpaulia ionantha*) with reducing storage temperature (Moges et al., 2003). Gopal and Sukh-Chauhan (2010) found that conserving potato microplants on MS medium having 20 g/L sucrose at

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Figure 5. Browning developed on *in vitro* preserved calli of *C. moabiticus* at media supplemented with various concentrations of sorbitol after 12 weeks. A: on a medium supplemented with 0.2 M. B: on a medium supplemented with 0.4 M.

Figure 6. Browning developed on *in vitro* preserved calli of *C. hyemalis* at media supplemented with various concentrations of sorbitol after 12 weeks. A: on a medium supplemented with 0.2 M. B: on a medium supplemented with 0.4 M.
plus 40 g/L sorbitol at low (7 ± 1 °C) temperature for 18 months without sub-culturing with maximum survival (58.0 %) coupled with a microplant condition good enough to provide suitable nodes for sub-culturing.

In the current study there was no complete loss of regrowth even at temperature as low as 4 ºC. Tahtamouni and Shibli (1999) reported that low regrowth percentage was obtained from wild pear (*Pyrus syriaca*) preserved microshoots at 4 ºC, where at 8 ºC explants were able to resume growth. Also, a complete loss of survival and regrowth of date palm calli occurred at 4 ºC after 6 weeks and 4 ºC or 10 ºC after 12 weeks of preservation (Chawla, 2002).

Callus browning was less severe when calli was preserved at 24 ºC with increasing storage period while a slight browning was observed at 4 ºC on *C. moabiticus* preserved calli (Fig. 7), and sever browning occurred at 4 ºC in *C. hyemalis* preserved calli (Fig. 8). At 8 ºC, calli of both species gave dark yellow color for 12 weeks of storage. Complete callus color loss was observed at 4 ºC and 10 ºC preservation temperatures on date palm reserved calli (Shibli *et al*., 2005). Moges *et al. (2003)* observed less severe of leaf color loss when African violet microshoots were preserved at 10 ºC while a complete leaf browning observed on preserved microshoots at 2 ºC for 12 weeks. A similar finding was obtained when wild pear microshoots were preserved at 8 or 4 ºC, respectively. The color loss may be attributed to deceleration of metabolic activities at low storage temperature (Chawla, 2002).

The highest weight change percentage of both *C. moabiticus* (430 %) and *C. hyemalis* (274 %) (Table 4) calli was obtained at 24 ºC for 12 weeks of storage. The least weight change occurred at 8 ºC after 12 weeks of storage for *C. moabiticus* (129 %) with good quality while for *C. hyemalis* it was at 4 ºC (147 %) with poorer quality. Therefore, *in vitro* preserved calli of *C. moabiticus* and *C. hyemalis* embryogenic callus at 8 ºC for 12 weeks could be preferred as it decreased growth and maintained explants in better condition.

### Table 4. Weight change, survival and regrowth percentages and viability index (*I_v*) of *in vitro* preserved *Crocus* embryogenic callus as influenced by temperature.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature (ºC)</th>
<th><em>C. moabiticus</em></th>
<th>4</th>
<th>8</th>
<th>24</th>
<th>LSD</th>
<th>4</th>
<th>8</th>
<th>24</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight change %</td>
<td>272 b</td>
<td>129 b</td>
<td>430 a</td>
<td>153.8</td>
<td>147 b</td>
<td>180 b</td>
<td>274 a</td>
<td>57.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival %</td>
<td>100 a</td>
<td>89 a</td>
<td>100 a</td>
<td>22</td>
<td>44.4 a</td>
<td>77.8 a</td>
<td>83.3 a</td>
<td>40.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>I_v</em></td>
<td>0.115 a</td>
<td>0.099 a</td>
<td>0.133 a</td>
<td>0.087</td>
<td>0.079 b</td>
<td>0.112 b</td>
<td>0.247 a</td>
<td>0.100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regrowth %</td>
<td>61.1 a</td>
<td>7.78 a</td>
<td>83.3 a</td>
<td>31.8</td>
<td>16.7 b</td>
<td>22.2 b</td>
<td>77.8 a</td>
<td>31.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Means within row, for each *Crocus* spp., having different letters are significantly different according to LSD at *P* ≤ 0.05.
From this study it can be concluded that survival and regrowth rates of preserved crocus calli declined with increased concentration of osmotic agents or with reduced temperature and as the preservation period was extended. In addition, calli of crocus can be preserved for medium-term preservation (12 weeks) period on a medium supplemented with 0.2 M sucrose, manitol or sorbitol under complete darkness with reduced growth rate and maintain calli quality. Moreover, calli of crocus can also be preserved for medium-term preservation (12 weeks) at 8 ºC under complete darkness as it was able to slow the growth rates and maintain calli in a better condition. More research must investigate other osmoticums and temperature regimes to improve quality of the explants and to extend storage period.

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4. حدد نوعية الزراعة

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البحث

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