PROPAGATION OF GERBERA PLANTS THROUGH TISSUE CULTURE
I –ESTABLISHMENT

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ABSTRACT

Soaking seeds of *Gerbera jamesonii* cv. Festival (hybrid F1) in water for 24 h before inoculation on MS medium lessened, to a significant extent the number of days needed for germination to take place. Using a syringe as a tool for sterilization procedure resulted in a significant higher survival percentage compared to that obtained by putting seeds in a gauze or cloth mesh.

The highest significant survival percentage was obtained by treating seeds with clorox at 1% active ingredient (a.i.). 8-hydroxyquinoline sulphate (HQS) at 1% gave the lowest values. The effect of sterilization treatment on contamination was statistically insignificant. The highest significant percentage of dead seeds occurred after applying HQS at 1%.

Key Words: *Gerbera jamesonii*, Seed soaking, Syringe, Clorox, HQS, Mercuric chloride.

INTRODUCTION

Gerberas came from Asia (Bali, Indonesia), South Africa (the Transvaal and Cape Provinces) and Tasmania. *Gerbera jamesonii* Bolus ex Hooker F., is the most important genetic contributor to our modern varieties. Gerberas commonly cultivated nowadays can be grouped under their official name of *Gerbera jamesonii* hybrids. These are hybrids created from the two species known as *Gerbera jamesonii* and *Gerbera viridifolia*.

Gerbera belongs to the Family Asteraceae (Compositae) of flowering plants. What is thought to be a flower is in fact many small, individual florets clustered in an inflorescence.

Originally, growers depended on seed for propagation. Later, they switched to cuttings, but the technique considered best nowadays is tissue culture. With tissue culture, not only we can grow good varieties, but also vigorous plants that produce long-lasting, high-quality flowers (**Perry, 1973** and **Everett, 1981**).

The use of seeds as a starting point in tissue culture technique is a well-known approach. When these seeds are permitted to germinate in aseptic conditions *in vitro* they gave rise to seedlings that can be exploited as a source of explants as mentioned by many workers. **Attard and Attard (2002)** claimed that seeds from immature fruits of *Ecballium elaterium* (Fam. Cucurbitaceae) were removed and placed on MS basal medium. Two weeks

from germination, node explants were sectioned and inoculated on MS medium containing different growth regulators. **Goncalves *et al* (2003)** stated that seeds of *Drosophyllum lusitanicum* (Fam. Droseraceae) were germinated *in vitro* on MS medium supplemented with 0.5 mg BA/l and 0.1 mg gibberellic acid/l. The obtained shoots were used in several proliferation assays. **Lucchesini *et al* (2003)** mentioned that seeds of *Passiflora incarnata* were germinated under sterile conditions and embryo tissues were used to start *in vitro* culture of this species. **Pawlowska and Bach (2003)** maintained that a micropropagation method for the endangered species *Gentiana pneumonanthe* from seeds was developed. Shoot tips and one-nodal cuttings of seedlings grown *in vitro* were used as explants. **Stamenkovic *et al* (2003)** stated that shoots of *Alyssum montanum* L. subsp. *pluscanescens*, that originated from aseptically germinated seeds, were used for culture initiation.

Workers in the field of tissue culture are always encountered by the major problem of contamination. A great percentage of failure in the establishment stage could be attributed to this factor. **Mendes (1999)** showed that establishment of cultures of *Thymus mastichina* (Fam. Lamiaceae) from nodal segments was difficult due to the high incidence of contaminations. When **Silva *et al* (2003)** cultured shoot apices and lateral buds of 3 rootstocks of *Prunus* on Lepoivre medium, they found that explant contamination rates were 14.8% and 29.8% for shoot apices and lateral buds, respectively.

To avoid this obstacle, some chemicals such as sodium hypochlorite, mercuric chloride and 8-hydroxyquinoline sulphate are used for surface sterilization of explants.

(1). Sodium hypochlorite:

Mendes (1999) stated that soaking the explants of *Thymus mastichina* in a solution of sodium hypochlorite 20% (v/v) for 30 min. was the most effective method, both in terms of efficiency and growth viability. **Pellegrino *et al* (1999)** stated that seeds of *Artemisia annua*, *Calendula officinalis* and *Helianthus annuus* were surface disinfected with 25% NaOCl solution for 15 min. **Hasnida *et al* (2001)** claimed that the seeds of *Eurycoma longifolia* and *Aquilaria malaccensis* were surface sterilized by 20% clorox. The seeds were germinated *in vitro* in basal MS medium. Germinated seedlings were used as a source of explants for the multiplication of shoots. **Bertsouklis *et al* (2003)** ascertained that well-developed seeds of *Globularia alypum* (an evergreen small shrub) were disinfested in 10-15% commercial bleach.

(2). Mercuric chloride:

Kumar *et al* (2003) mentioned that treatment with 0.1% $HgCl_2$ for 5-7 minutes gave the best response for surface sterilization of nodal segments of field-grown male sterile African marigold plants that suffer from high microbial contamination (100%). **Ying *et al* (2003)** ascertained that dipping branch explants of *Betula alnoides* (Fam. Betulaceae) in solution of 1:1000 $HgCl_2$ for 3 minutes was the best way for disinfection.

However, the use of mercuric chloride has some restrictions. In this respect, **Miranda *et al* (2000)** mentioned that the establishment of an initial disinfestation protocol for stem nodal segments of potato (*Solanum tuberosum* L.) cv. Chiquita explants revealed that using $HgCl_2$ resulted in a high rate of abnormally developed plantlets.

(3). 8-hydroxyquinoline sulphate:

Abdel-Kader (1987) mentioned that 8-HQS has strong inhibitory effects on fungi, yeasts and bacteria. The growth of these microorganisms was reduced at 10 ppm, almost eliminated at 100 ppm and completely inhibited at 300 ppm concentration. The effects of 8-hydroxyquinoline compounds are related to their strong chelating properties. They act both as antimicrobial agents and as enzymatic inhibitors. **Machado *et al* (1991)** reported that the application of 0.2 ml. of a 0.1% solution 8-HQS (an antimycotic and bactericidal) as an overlay cover for 24 h, greatly reduced losses of explants of 16 apple cultivars from unidentified infection. After 7 days, yields of 50-90% sterile explants could be obtained, compared with 100% losses of untreated shoot tips. **Doorn and Van-Doorn (1998)** stated that 8-HQC is an antimicrobial compound when added to vase water for rose (Rosa hybrida) cv. Sonia flowers.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory of the Horticulture Department, Faculty of Agriculture, Ain Shams University in 2001-2002. The aim of this study was to establish an applicable protocol for the rapid micropropagation of *Gerbera jamesonii* cv. Festival (hybrid F1).

Glass jars of 11.5 cm height x 6.5 cm diameter with their polypropylene caps were used during establishment. Jars were filled with 40 ml of the **Murashige and Skoog (1962)** medium (MS), and autoclaved at 121°C for 20 minutes under 1.05 kg/cm^2 pressure, left to cool and stored at 25±2ºc for one week before being used.

After inoculation, jars were incubated at 25/20°C (day/night) ±2°C, 70% relative humidity. Two fluorescent tubes/shelf were installed at 30 cm above explants to provide light intensity of 2200-2400 lux at explant level.

Exp. 1:

Seeds of gerbera cultivar Festival (hybrid F1) were obtained from "Norcom S.P.A." Milano, Italy. They were brought to the tissue culture lab. where they were subjected to a number of experiments. In the first experiment, seeds were divided into two groups. Seeds of the first group were surface sterilized with NaOCl solution (52.5 g Cl/l) at 1% a.i. (20 cm^3 clorox diluted with distilled water to 100 cm^3 final volume) for 15 min., rinsed by sterile distilled water for three times and inoculated on MS medium under aseptic conditions using a laminar air-flow cabinet. Seeds of the second group were soaked in distilled water for 24 h. before sterilization, rinsing and inoculation as the first one. Each group consisted of 6 replicates (jars), with 3 seeds in each jar. The two treatments were arranged in a completely randomized design. Number of days for these seeds to germinate was recorded in this experiment.

Exp. 2:

To avoid the loss of seeds during sterilization by NaOCl and rinsing, two approaches were examined in order to achieve these processes.

a- Using a 60 ml medical syringe supplied with a cotton stopper.

b- Using a piece of gauze.

Seeds of each one of these two treatments were inoculated in 6 replicates (jars), with 3 seeds each. The two treatments were arranged in a

completely randomized design. Data obtained in this experiment were percentages of survival, contamination and death.

Exp. 3:

This experiment was conducted to find out the best sterilization treatment by comparing three sterilizing agents:

(1) Clorox: NaOCl solution (52.5 g Cl/l) at 1% a.i. for 15 min.
(2) HQS: HQS for 1 h. at 0.25 %, 0.50 %, 0.75 % and 1%.
(3) MC: $HgCl_2$ at 500 ppm for 4, 6 and 8 min.

Few drops of liquid soap were added as a wetting agent. All these treatments were followed immediately by rinsing in sterile distilled water for three times.

These eight treatments were arranged in a completely randomized design. Data obtained in this experiment were the same as those of experiment 2.

Data of these experiments were statistically analyzed using SAS 1995 computer program, and means were compared by L. S. D. method, and all percentages were transformed according to **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

1- Effect of germination pretreatment on number of days to germinate (Table 1)

Germination pretreatment had a significant effect on the number of days to germinate. Soaking seeds in water for 24 h. before inoculation on MS medium lessened, to a significant extent the number of days needed for germination to take place compared to the non-soaked seeds (9.33 and 17.11 days, respectively).

This result is in accordance with that of **Hashim *et al* (2001)** who reported that the pretreatment of soaking seeds of both *Drosera capensis* and *Drosera rotundifolia* in water for 6 hours before sowing on MS medium increased percentages of germination in vitro compared to the non-soaked seeds. However, this increment was insignificant.

Table (1). Effect of germination pretreatment of *Gerbera* seeds on number of days to germinate

Germination Pretreatments	Days to germinate
Soaking in Water for 24 h	9.33 b
No Soaking	17.11 a
LSD at 5%	6.51

2 - Effect of sterilization method on survival percentage (Table 2):

The method used in sterilization process exerted a significant influence on the percentage of seeds that survived sterilization. Using a syringe as a tool for sterilization procedure resulted in a significant higher survival percentage compared to that obtained by putting seeds in a cloth mesh (80.30 and 66.21, respectively).

Table (2). Effect of sterilization method on survival percentage

Sterilization Methods	Survival %
Syringe	80.30 a
Mesh	66.21 b
LSD at 5%	8.67

The efficacy of using a syringe as a tool for sterilization might be attributed to the relative vacuum created inside the syringe when drawing up the sterilizing solution. This vacuum help in getting rid of the micro-air bubbles present in the seed coats that may provide a protection to the microorganisms on the seed surface and between its coats. The removal of these air bubbles allows for a good infiltration of the sterilizing solution and hence a direct contact with microorganisms. In this concern, **Hammerschlag *et al* (2000)** reported that vacuum infiltration with the antibiotic cefotaxime at 5 ppm reduced contamination by about 20-30%

3 - Effect of sterilization methods on contamination percentage (Table 3):

Percentage of contaminated seeds when using a syringe in sterilization was 7.38. This was significantly lower than the corresponding percentage by using the other method, i.e. the mesh method that was 19.79.

Table (3). Effect of sterilization methods on contamination percentage

Sterilization Methods	Contamination %
Syringe	7.38 b
Mesh	19.79 a
LSD at 5%	8.29

4 - Effect of sterilization methods on death percentage (Table 4):

Percentage of dead seeds was significantly higher (10.45) by using a mesh in sterilizing the seeds, compared by the same character after using a syringe. In the later case death percentage was only 3.83.

Table (4). Effect of sterilization methods on death %

Sterilization Methods	Death %
Syringe	3.83 b
Mesh	10.45 a
LSD at 5%	5.97

5 - Effect of sterilization treatments on survival % (Table 5):

The material used in sterilizing seeds proved to have a significant effect on survival percentage. The highest record (72.37%) was obtained by treating seeds with clorox, followed, without any significant difference, by those obtained by using HQS at 0.25% and 0.50% (50.88 and 45.00%, respectively). Using HQS at 0.75% yielded a survival percentage of 26.75, significantly not different from percentages obtained with HQS 0.25% and HQS 0.50% from one side, and with MC for 4, 6 and 8 min and HQS 1.00% (39.12, 42.37, 32.63 and 20.88%, respectively) from the other side. Result of the last treatment, HQS at 1.00%, however, was the lowest at all.

In this respect, **Abdelnour *et al* (2002)** reported that disinfection of waterpear, *Sechium edule* (Cucurbitaceae) explants with 4% calcium hypochlorite allowed the highest percentage of aseptically established explants under *in vitro* conditions. **Beura *et al* (2003)** stated that in case of the axillary bud explants of gladiolus cv. American Beauty, the treatment of 1% NaOCl for 10 minutes was found the best, having 100% aseptic culture and survival of explants. **Nagaraju and Mani (2003)** on gladiolus cv. Ice Gold cormels concluded that a sterilization period of 8-10 minutes in mercuric chloride was optimum for the complete elimination of microorganisms. **Reddy and Reddy**

(2003) found that dipping explants *Trichosanthes dioica*, Fam. Cucurbitaceae for five minutes in 0.05% mercuric chloride ($HgCl_2$) resulted in the highest percentage of survival (96.1%). **Ribas *et al* (2003)** found that NaOCl 0.25% solution for 10 minutes resulted in 70% disinfection and survival of apical shoots of *Aspidosperma polyneuron* (Fam. Apocynaceae). However, $HgCl_2$ was more efficient than NaOCl in the disinfection of these apical shoots. Therefore 0.05% $HgCl_2$ during 10 minutes was recommended (84.1% survival). **XinPing *et al* (2003)** stated that when Andersson's modified MS medium was used as the basic medium in tissue culture of *Rhododendron simsii*, the supernatant liquid of 5% NaOCl aqueous solution was better in explant sterilization. **Arafa (2004)** found that using NaOCl (at 0.5% a.i.) to surface-sterilize explants of *Gerbera jamesonii* cv. Duolla gave a survival percentage of 35%.

Table (5). Effect of Sterilization treatments on survival %

Sterilization treatments	Survival %
Clorox	72.37 a
HQS 0.25%	50.88 ab
HQS 0.50%	45.00 abc
HQS 0.75%	26.75 bc
HQS 1.00%	20.88 c
MC 4 min	39.12 bc
MC 6 min	42.37 bc
MC 8 min	32.63 bc
LSD at 5%	28.46

A lot of reports are available on the efficiency of mercuric chloride in combating microorganisms. **Kolbanova and Koukharchik (2000)** stated that least infection was obtained by sterilizing explants of some cultivars of black currant (*Ribes nigrum*, Fam. Grossulariaceae) with 0.1% sublimate ($HgCl_2$) for 3 min. **Nagaraju and Mani (2003)** concluded that for proper sterilization of gladiolus cv. Ice Gold cormels, a sterilization period of 8-10 minutes by mercuric chloride was optimum for the complete elimination of microorganisms. **Kumar *et al* (2003)** mentioned that for surface sterilization of nodal segments of field-grown male sterile African marigold plants that suffer from high microbial contamination (100.00%), treatment with 0.1% $HgCl_2$ for 5 to 7 minutes gave the best response. **Ying *et al* (2003)** ascertained that dipping in solution of 0.1% $HgCl_2$ for 3 minutes was the best way for disinfection of branch explants of *Betula alnoides*.

6 - Effect of sterilization treatments on contamination percentage (Table 6):

The effect of sterilization treatments on contamination was shown to be statistically insignificant. However, the highest contamination percentage was a result of using MC for 4 min. (41.75%), while the lowest one (11.75%) was observed after using either clorox, HQS 1.00% or MC for 8 min.

Table (6). Effect of Sterilization treatment on contamination percentage

Sterilization treatments	Contamination %
Clorox	11.75 a
HQS 0.25%	32.63 a
HQS 0.50%	26.75 a
HQS 0.75%	26.75 a
HQS 1.00%	11.75 a
MC 4 min	41.75 a
MC 6 min	23.51 a
MC 8 min	11.75 a
LSD at 5%	**N.S.**

The failure of sodium hypochlorite to overcome contamination was encountered by some authors. **Craig *et al* (1994)** found that household bleach (10% sodium hypochlorite) for 1, 5, 15 or 30 minutes was ineffective in inhibiting growth of bacteria in sweet potato explants. **Vianna *et al* (1997)** mentioned that high contamination by bacteria was observed in papaya tissue cuttings introduced *in vitro* from plants grown in the field. The use of sodium hypochlorite did not guarantee good bacterial control. **Kolbanova and Koukharchik (2000)** stated that least infection was obtained by sterilizing explants of some cultivars of black currant (*Ribes nigrum*) with 0.1% sublimate ($HgCl_2$) for 3 min. **Meghwal *et al* (2001)** said that all cultures of guava (*Psidium guajava*) were contaminated in the treatments where NaOCl (10%, for 5 minutes) was used, either alone or with alcohol. **Mogor *et al* (2003)** disinfected rhizopore buds and shoot axillary buds of yacon (*Polymnia sonchifolia*, Fam. Asteraceae) by immersing in 20% aqueous solution of NaOCl for 20 or 40 minutes before culturing in MS media. Both treatments resulted in a high fungal and bacterial contamination of cultures after 20 days. **Pickens *et al* (2003)** reported that a sterilization protocol using 2.6% NaOCl for 40 minutes disinfested seeds of *Tillandsia eizii in vitro* and promoted seedling growth. **Waegel (2003)** stated that shining club moss, *Huperzia lucidula* (Fam. Lycopsida; Pteridophyta) can be propagated in tissue culture, but it is difficult to obtain sterile explants. When standard disinfestation methods were used, 100% of apical shoot-tips disinfested for 10-30 minutes with 1% NaOCl became contaminated or turned brown within 3 weeks. **Arafa (2004)** found that using NaOCl (at 0.5% a.i.) to surface-sterilize explants of *Gerbera jamesonii* cv. Duolla gave a contamination percentage of 56%.

7 - Effect of sterilization treatments on death percentage (Table 7):

A significant effect of sterilization treatment on the percentage of dead seeds was detected. Highest significant value occurred after applying HQS 1.00% (60.00%), while the lowest one was a result of using clorox (5.88%). It is worthwhile to notice the progressive increase in the death percentage as the HQS level increased from 0.25 to 0.50, 0.75 and finally to 1.00% (11.75, 23.51, 41.75 and 60.00%, respectively) or as the exposure to MC increased from 4 to 6 and finally to 8 min. (11.75, 26.75 and 50.88%, respectively), with no significant difference between the adjacent intervals.

Related to these findings, **Machado *et al* (1991)** remarked that 16 apple cultivars were successfully established *in vitro* from shoot tips. They were covered for 24 h with 200 ml of a 0.1% solution of 8-HQS before transferring to the medium. The application of 8-HQS (an antimycotic and bactericidal agent) greatly reduced losses of explants from unidentified infection and inhibited the browning of explants and media caused by oxidized polyphenolic compounds. **Arafa (2004)** found that using NaOCl (at 0.5% a.i.) to surface-sterilize explants of *Gerbera jamesonii* cv. Duolla gave a mortality percentage of 9%.

Table (7). Effect of Sterilization treatments on death percentage

Sterilization treatments	Death %
Clorox	5.88 d
HQS 0.25%	11.75 d
HQS 0.50%	23.51 cd
HQS 0.75%	41.75 abc
HQS 1.00%	60.00 a
MC 4 min	11.75 d
MC 6 min	26.75 bcd
MC 8 min	50.88 ab
LSD at 5%	25.32

Therefore, it is recommended to soak the seeds of *Gerbera jamesonii* cv. Festival (hybrid F1) in water for 24 h before inoculation and to use a syringe as a tool for sterilization. Clorox at 1% (a.i) should be used for sterilization.

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إكثار نباتات الجربيرا عن طريق زراعة الأنسجة
١ – إنشاء المزرعة

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أجريت هذه الدراسة فى معمل زراعة الأنسجة الخاص بالشتلات فى قسم البساتين بكلية الزراعة – جامعة عين شمس خلال ٢٠٠١ – ٢٠٠٢ .

أدى نقع بذور الجربيرا فيستيفال فى الماء لمدة ٢٤ ساعة إلى التقليل بدرجة معنوية من عدد الأيام اللازمة للإنبات . وعند إستعمال محقن (سرنجة) كآداة أثناء عملية التعقيم كانت النسبة المئوية لبقاء البذور حية أعلى مقارنة بهذه النسبة عند وضع البذور فى قطعة من الشاش .

تحققت أعلى نسبة بقاء للبذور بإستعمال الكلوروكس بتركيز ١% (مادة فعالة). أما سلفات الهيدروكسى كينولين بتركيز ١% فقد أعطت أقل نسبة . ولم يكن تأثير معاملات التعقيم معنويا على النسبة المئوية للتلوث . وكانت أعلى نسبة موت للبذور بسبب المعاملة سلفات الهيدروكسى كينولين بتركيز ١% .