

COST REDUCTION IN TISSUE CULTURE I – SAVING OF WATER AND POWER

Saadawy, F. M.

**Dept. of Ornamental Plant Researches and Landscape Design,
Horticulture Research Institute, Agriculture Research Center, Guiza,
Egypt.**

ABSTRACT

Using tap water in preparing media instead of distilled water gave significant higher number of shoots and leaves, in addition to an insignificant increase in shoot fresh weight, shoot length and total chlorophyll content of both *Gerbera* and *Lilium* explants at multiplication stage.

At the rooting stage, in both plants the number of leaves and root length were higher in plantlets when using tap water than when using distilled water in media preparation. However, the effect was statistically significant on root length in case of *Gerbera* only, and on leaf number in case of *Lilium* only. Insignificant increments in plantlet fresh weight, shoot length, root fresh weight, root number and chlorophyll content of *Gerbera* and *Lilium* plantlets were found when using tap water than distilled water.

Using tap water instead of distilled water in the approximately 30 tissue culture labs in Egypt may save yearly an amount of 13635 m³ of tap water intended originally for human potable purposes, 1350000 kilowatt of electric power and L.E. 822476 as a total cost, taking into consideration that these figures may change from lab to lab, from time to time and from a country to the other. Details of these figures are mentioned in the feasibility study.

Keywords: tap water, distilled water, rare earth elements, cost, media preparation.

INTRODUCTION

Chemically pure water does not exist for any appreciable length of time in nature. It is a must in almost all tissue culture labs to have one or more of the distillation units to provide them with the pure water needed to prepare the different types of media.

However, distilled water, because it is essentially mineral-free, is very aggressive, in that it tends to dissolve substances with which it is in contact. Notably, carbon dioxide from the air is rapidly absorbed, making the water acidic and even more aggressive.

The distillation process needs a lot of power (electrical energy), which is high in cost. Maintenance of distillation units can be a problem, depending on the design of the units. Minerals left behind in the boiling chamber can build up, interfering with the operation of the unit. Hard water can quickly clog a distiller. Because water is used also as a coolant liquid, waste of water during the distillation process will be very high.

This study aims to prove that using tap water instead of distilled water makes no difference either in the multiplication stage or the rooting one in two plants belonging to different classes, i.e. Dicotyledonae (*Gerbera*

jamesonii) and Monocotyledonae (*Lilium longiflorum*). Proving this will help to:

- 1-Reduce the monthly costs of the tissue culture technique through cutting down electric power bills.
- 2-Save the big sum of money needed to buy water distillers especially for the commercial tissue culture labs. Price of these units ranges from about L.E. 2000 for the Chinese unit to 4000 for the Polish and 15000 for the German ones.
- 3-Stop wasting huge amounts of potable tap water that cost a lot to prepare and that will be more and more scarce in the future.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory, Zohriya Garden, Agricultural Research Center, Cairo, during the period from July to December 2004.

In vitro-produced explants of *Lilium longiflorum* (basal parts of the leaves) and *Gerbera jamesonii* cv. Festival (shoots) were used as a starting material.

Glass jars of 11.5 cm height and 6.5 cm diameter with their polypropylene caps were used. These jars were filled with 40 ml of the Murashige and Skoog (1962) (MS) supplemented with either a cytokinin for explant multiplication or an auxin for rooting of the resultant shoots.

Multiplication media were supplemented with benzyl adenine (BA) at 5 ppm for *Gerbera* explants or 3 ppm for *Lilium*. Rooting media were supplemented with IAA at 2.5 ppm for *Gerbera*, or IBA at 7 ppm for *Lilium*.

For each plant, both types of media were prepared by using either distilled or tap water. These two treatments were arranged in a completely randomized design. Each treatment comprised 10 replicates (jars).

Explants were inoculated on the media under aseptic conditions using a laminar airflow cabinet. Jars were incubated for four weeks at 25/20°C (day/night) $\pm 2^\circ\text{C}$, 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 for 16 h. daily.

Data obtained in the multiplication stage were: shoot fresh weight (g), shoot number, shoot length (cm), leaf number and shoot content of total chlorophyll (mg/g fresh weight). Data obtained in the rooting stage were: shoot fresh weight, shoot length, leaf number, root number, root length, root weight and shoot content of total chlorophyll (according to Moran, 1982).

Data were statistically analyzed using SAS 1995 computer program, and means were compared by L. S. D. method according to Snedecor and Cochran (1980).

A feasibility study was conducted to assess costs of water distillation and amount of water wasted for cooling. A German distiller was used in this study.

Analysis of distilled and tap water was performed in the labs of the General Organization of Land Reclamation Project, Ministry of Agriculture, Dokki. Data of Nile water analysis were cited from Hamed (2003). (Table a)

Table a. Analysis of distilled, tap and Nile water

Parameter	Unit	Nile water *	Tap water **	Dist.water**
pH		7.2	7.1	6
E. C.	DS/m (mmohs/cm ³)	0.32	0.27	0.0001
Total soluble salts	Mg/l	204.8	172.8	0.064
Cations & anions				
Ca ⁺⁺	Meq/l	1.40	2.20	
Mg ⁺⁺	Meq/l	0.80	0.34	
Na ⁺	Meq/l	0.70	0.12	
K ⁺	Meq/l	0.10	0.04	
CO ₃ ⁼	Meq/l	0.00	0.00	
HCO ₃ ⁻	Meq/l	2.30	1.64	
Cl ⁻	Meq/l	0.59	0.02	
SO ₄ ⁼	Meq/l	0.11	0.86	
Elements concentration				
Fe	Mg/l	0.0400		
Mn	Mg/l	0.0030		
Zn	Mg/l	0.0200		
Cu	Mg/l	0.0010		
Cd	mg/l	0.0000		
Co	mg/l	0.0000		
Ni	mg/l	0.0002		
Pb	mg/l	0.0010		
Cr	mg/l	0.0000		

* = cited from Hamed (2003)

** = carried out in the General Organization of Land Reclamation Project, Ministry of Agriculture

RESULTS

1 – Effect of water quality at multiplication stage (Tables 1-a and 1-b):

1-1- Effect of water quality on shoot fresh weight:

No significant effect for water quality was detected on shoot fresh weight of both *Gerbera* and *Lilium* explants. However, in both cases shoot fresh weight of explants grown on media prepared by tap water was higher than that of explants grown on media prepared by distilled water.

Table 1-a - Effect of water quality on multiplication of Gerbera

Water quality	Shoot fresh weight (g)	Shoot No.	Shoot length (cm)	Leaf No.	Total chlorophyll (mg/g fresh weight)
Dist. Wat.	0.74	5.00	1.56	28.26	1.71
Tap Wat.	0.96	10.20	1.94	46.80	2.32
L.S.D. at 5%	N.S.	5.07	N.S.	8.25	N.S.

Table 1-b - Effect of water quality on multiplication of Liliium

Water quality	Shoot fresh weight (g)	Shoot No.	Shoot length (cm)	Leaf No.	Total chlorophyll (mg/g fresh weight)
Dist. Wat.	0.86	2.80	6.02	6.00	1.36
Tap Wat.	1.02	4.20	6.12	10.20	1.77
L.S.D. at 5%	N.S.	1.11	N.S.	2.39	N.S.

1-2- Effect of water quality on shoot number:

Shoot number was significantly influenced by water quality. Using tap water in preparing media gave higher number of shoots than distilled water. This significant effect was found on both *Gerbera* and *Lilium* plants.

1-3- Effect of water quality on shoot length (cm):

Although using tap water resulted in shoots longer than those produced in the presence of distilled water in both *Gerbera* and *Lilium*, differences were statistically insignificant.

1-4- Effect of water quality on leaf number:

In both *Gerbera* and *Lilium* explants, leaf number was significantly higher in number when tap water was used in preparing the media, compared with using distilled water.

1-5- Effect of water quality on total chlorophyll content (mg/g fresh weight):

The effect of water quality had an insignificant effect on the total chlorophyll content of *Gerbera* and *Lilium* shoots. Although the difference was insignificant, this record was higher in both plants when tap water was used.

2 - Effect of water quality at rooting stage (Tables 2-a and 2-b):**2-1- Effect of water quality on plantlet fresh weight (g):**

The effect of water quality on plantlet fresh weight of *Gerbera* and *Lilium* was found to be insignificant. Despite of this insignificance, this character was higher in case of tap water than that of distilled water.

Table 2-a - Effect of water quality on rooting of Gerbera

Water quality	Plantlet fresh weight (g)	Shoot length (cm)	Leaf No.	Root fresh weight (g)	Root No.	Root length (cm)	Total chlorophyll (mg/g fresh weight)
Dist. Wat.	0.32	3.34	5.80	0.08	4.20	7.24	1.36
Tap Wat.	0.34	3.60	6.40	0.10	4.80	8.42	1.43
L.S.D. at 5%	N.S.	N.S.	N.S.	N.S.	N.S.	1.06	N.S.

Table 2-b - Effect of water quality on rooting of *Lilium*

Water quality	Plantlet fresh weight (g)	Shoot length (cm)	Leaf No.	Root fresh weight (g)	Root No.	Root length (cm)	Total chlorophyll (mg/g fresh weight)
Dist. Wat.	4.93	16.00	19.20	1.18	8.29	2.96	1.35
Tap Wat.	5.75	18.00	28.00	1.71	6.04	3.24	1.61
L.S.D. at 5%	N.S.	N.S.	5.15	N.S.	N.S.	N.S.	N.S.

2-2- Effect of water quality on shoot length (cm):

Water quality did not affect shoot length significantly. However, shoots grown on tap water media were longer than those on distilled water media.

2-3- Effect of water quality on leaf number:

In both *Gerbera* and *Lilium* the number of leaves was higher in plantlets provided with tap water than when using distilled water. However, this effect was statistically significant in case of *Lilium* only.

2-4- Effect of water quality on root fresh weight (g):

Water quality failed to affect root fresh weight significantly. However, an insignificant increase in this character was observed in case of both *Gerbera* and *Lilium* when tap water was used in media preparation.

2-5- Effect of water quality on root number:

In both *Gerbera* and *Lilium*, water quality did not affect root number significantly. Apart from this result, roots produced in the presence of tap water were greater in number than those of plantlets grown on distilled water media. However, this situation was reversed in case of *Lilium*, where number of roots was higher when using distilled water than when using tap water in media preparation.

2-6- Effect of water quality on root length (cm):

Roots of *Gerbera* plantlets were significantly longer as a result of depending upon tap water compared with when distilled water was used in media preparation. The same trend was detected also in case of *Lilium*, although the effect was statistically insignificant.

2-7- Effect of water quality on shoot total chlorophyll content (mg/g fresh weight):

Chlorophyll content of either *Gerbera* or *Lilium* shoots was higher in plantlets grown on media prepared with tap water than the same content in plantlets grown on media prepared with distilled water, although this influence was insignificant.

DISCUSSION

Among the many problems facing the tissue culture technique, if not the most important one, is the big sum of money needed to purchase the expensive equipments such as water distillers. The huge amount of water necessary for cooling distillation units represents a great loss of a very important resource that becomes more and more scarce in these days, i.e. potable water.

Using tap water instead of distilled one in order to cut down the already high expenses of tissue culture technique and to preserve the important factor of human life was tried by some researches. Ganapathi *et al* (1995) described a simple, low-cost method for the micropropagation of banana cv. Basrai. They remarked that tap water can be substituted for distilled water. The cheapest method for rooting and development of plantlets from shoot tips was on a medium containing tap water. Sharma and Singh (1995) investigated the effects of replacing distilled water with tap water in preparing the medium for culturing explants from *in vitro*-cultured shoots of ginger (*Zingiber officinale*) cv. Himachal Local. They found that the use of tap water gave good results. This technique can be used to reduce the cost of micropropagation. Belarmino and Gabon (1999) mentioned that to reduce the cost of producing *in vitro* plants of *Chrysanthemum morifolium*, non-distilled water can be used as a cheap substitute without affecting the quality of micropropagated plants. Tissue cultured chrysanthemum plants were normal, vigorous and flowered profusely in the field.

In addition, tap water proved to be more beneficial in some documented works. Punia *et al* (2000) studied the effects of using water from different sources on sugarcane (cultivars CoH92 and CoH99) shoot multiplication. They ascertained that ordinary tap water was better for the preparation of propagation media than distilled water from water purifiers.

Water content of rare earth elements (in very small amounts) may explain the effect of tap water in promoting some explant characters *in vitro* and crop yield *in vivo*. Shelton (2004) stated that while falling as rain, water picks up small amounts of gases, ions, dust, and particulate matter from the atmosphere. Then, as it flows over or through the surface layers of the earth, it dissolves and carries with it some of almost everything it touches, including that which is dumped into it by man.

HongMei and XiaoPing (2001) found that $\text{La}(\text{NO}_3)_3$ at different concentrations (1-100 mg/litre) affected explant percentage of differentiation as well as the number of roots and dry matter contents of stems, leaves and roots. Media with 1-50 mg $\text{La}(\text{NO}_3)_3$ /litre increased the number and length of roots, and the percentages of dry matter of stems, leaves and roots, but that with 100 mg $\text{La}(\text{NO}_3)_3$ /litre recorded an inverse effect. Maheswaran *et al* (2001) remarked that the use of Rare Earth Elements (REE) in agriculture has been practiced in China since 1972. Increases in crop yield range between 8 and 50% and common responses in temperate crops of 8 to 10% were found. These responses are reported to be most probable when soils contain low available REE (<10 mg/kg, i.e. 5 and 10 kg/ha). FaDi and Bin

(2002) observed that La^{3+} could stimulate the callus formation, growth and bud differentiation of *Gerbera jamesonii* [*G. jamesonii*] cv. Sunbird at low concentration. The suitable concentration was 10 mg/litre in receptacle explants. Over this concentration, the bud initiation was inhibited and several explants turned brown. In leaf explants, the suitable concentration for callus formation and growth was 15 mg/litre, and 30 mg/litre for bud initiation and multiplication. WeiPing *et al* (2002) mentioned that the optimum concentration of $\text{La}(\text{NO}_3)_3$ at 1-3 $\mu\text{mol/litre}$ (0.33-1.00 ppm) in the rooting medium of loquat (*Eriobotrya japonica*) plantlets could increase the rate and the fresh weight of rooting, promote the length of root, and increase the activities of peroxidase and nitrate reductase.

Physiological and biochemical explanation for these effects might be found in the references. Hong and Chen (1996) cultured *Cymbidium sinense* rhizomes on 1/2-strength MS medium supplemented with BA at 1 ppm, NAA at 1 ppm and lanthanum (La) at 10 mg/litre. They stated that chloroplast development was enhanced by La; there were many more lamellae and osmiophilic granules in the stroma, and development was faster than in the control. Similarly mitochondrial development was enhanced by La, and mitochondrial volume was greater than in the controls. The chromatin content of the nucleus was increased by La treatment. Lopez-Serrano and Ros-Barcelo (1996) working on grape cv. Monastrell cell suspension cultures reported that the effectiveness of cations in lowering peroxidase activity followed the order: $\text{La} > \text{Cd} = \text{Hg} > \text{Ni} = \text{Zn} = \text{Co} > \text{Li}$, which is in accordance with their valence ($\text{C}^{3+} > \text{C}^{2+} > \text{C}^+$) and with ionic radius ($\text{La} (1.15 \text{ \AA}) > \text{Cd} = \text{Hg} (0.97-1.10 \text{ \AA}) > \text{Ni} = \text{Zn} = \text{Co} (0.72-0.74 \text{ \AA}) > \text{Li} (0.60 \text{ \AA})$). La^{3+} is the only cation together with Al^{3+} that belongs to class A metals, with a preference sequence for cellular ligands. XueFen *et al* (2003) conducted an experiment with 10th generation banana plantlets in MS medium with 4 mg BA and 0.2 mg NAA/litre. They added different concentrations of rare earth elements collected from a mine in Inner Mongolia. These consisted of 13.5% cerium nitrate, 7.33% lanthanum nitrate and 4.07% neodymium nitrate. They reported that treatment with 10-15 mg/litre of these rare elements promoted bud differentiation, and increased the leaf chlorophyll content, respiration rate and peroxidase activity. However, when the concentration of rare earth elements was increased to 30 mg/litre, inhibitory effects appeared.

Sometimes there was no difference between the two types of water. This also means a privilege in favor of tap water. Sunandakumari *et al* (2004) stated that an efficient protocol for the rapid multiplication of the herbal spice *Mentha piperita* through axillary bud multiplication and *ex vitro* rooting was established using MS medium. Media prepared with tap water and those prepared with double distilled water did not show significant difference in the *in vitro* induction of shoots/node and roots/shoot. Using tap water made the protocol economic.

FEASIBILITY STUDY

A German-made distilling unit that costed L.E. 15000 produced 7.5 l. of distilled water/h. This unit took 10 min to warm up, during which it wasted 15 l. of water. Given that a commercial tissue culture lab consumes 100 l. of

media/day, it took the unit 16.5 h. to produce 100 l. of distilled water every day. During this time this unit needed 1500 l. of water, wasted for cooling. As the unit consumes 9 kilowatt/h. of electric power, a total of 148.5 kilowatt will be needed to produce 100 l. of distilled water.

This could be summarized in the following points (Table b):

To get 100 l. of distilled water every day

Time needed = 16.5 h.

Water wasted during warming up = 15 l.

Water wasted for cooling = 1500 l.

Total wasted water = 1515 l.

Electric power for warming up = 1.5 kilowatt

Electric power for distilling= 148.5 kilowatt

Total electric power = 150 kilowatt

Given that there are about 300 working day/year,
and there are about 30 tissue culture labs in Egypt,
This means 9000 working day/year in Egypt

Total wasted water/year for 30 labs = 13635 m³

as 1 m³ of water costs L.E. 0.36 + L.E. 1.225 duties = L.E. 0.585

Cost of total wasted water/year for 30 labs = L.E. 7976

as 1 kilo watt/hour costs L.E. 0.27

Total electric power/year for 30 labs = 1 350 000 kilowatt

Cost of total electric power/year for 30 labs = L.E. 364500

Price of distilling unit = L.E. 15000

Supposing this unit will last for 10 years,

Cost of distilling unit/year = L.E.1500

For 30 tissue culture labs,

Total cost of distilling units/year = L.E. 450000

Table (b). Summary of the feasibility study

Item	
Total wasted water/year for 30 labs	13 635 m³
Cost of total wasted water/year	L.E. 7 976
Cost of total electric power/year	L.E. 364 500
Cost of total cost of distilling units/year	L.E. 450 000
Grand total costs	L.E. 822 476

SUMMARY AND CONCLUSION

1 – Effect of water quality at multiplication stage

No significant effects for water quality were detected on shoot fresh weight, shoot length and total chlorophyll content of both *Gerbera* and *Lilium* explants. However, in both plants these characters of explants grown on media prepared by tap water were greater than the corresponding characters of explants grown on media prepared by distilled water.

Shoot and leaf numbers of both *Gerbera* and *Lilium* were significantly influenced by water quality. Using tap water in preparing media gave higher number of shoots and leaves than when distilled water was used.

2 - Effect of water quality at rooting stage

The effect of water quality on plantlet fresh weight, shoot length, root fresh weight, root number and chlorophyll content of *Gerbera* and *Lilium* was found to be insignificant. Despite of this insignificance, records of these characters were higher in case of using tap water than that of using distilled water.

In both *Gerbera* and *Lilium* number of leaves and root length were higher in plantlets provided with tap water than those provided with distilled water. However, the effect was statistically significant on root length in case of *Gerbera* only, and on leaf number in case of *Lilium* only,

These positive effects of tap water might be attributed to its content of rare earth elements.

Using tap water in tissue culture labs may save yearly an amount of 13 635 m³ of tap water intended originally for human potable purposes, 1350000 kilowatt of electric power and L.E. 450000 as a cost for distilling unit.

RECOMMENDATIONS

It is recommended to depend upon tap water for media preparation in order to save a big deal of money and to preserve the very precious potable water.

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تقليل تكاليف زراعة الأنسجة

١ - توفير الماء والطاقة

فيصل محمد عبد العظيم سعادوى

قسم بحوث نباتات الزينة، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر

أدى إستعمال ماء الصنبور لتحضير البيئات بدلا من الماء المقطر إلى إنتاج عدد أكبر بدرجة معنوية من الأفرع والأوراق ، إلى جانب زيادة وإن تكن غير معنوية فى الوزن الطازج للأفرع ، طول الأفرع ، ومحتوى الكلوروفيل الكلى فى كل من المنفصلات النباتية للجربيرا والليليوم أثناء مرحلة الإكثار . وفى مرحلة التجذير لكل منهما كان عدد أوراق وطول جذور النبيتات أكبر عند إستعمال ماء الصنبور مقارنة بإستعمال الماء المقطر فى تحضير البيئات . ولكن التأثير كان معنويا على طول الجذور فى حالة الجربيرا فقط ، وعلى طول الأوراق فى حالة الليليوم فقط . كما كانت هناك زيادة وإن تكن غير معنوية فى الوزن الطازج للنبيتات ، وطول الأفرع ، والوزن الطازج للجذور وعدد الجذور ومحتوى الكلوروفيل الكلى لنبيتات الجربيرا والليليوم عند إستعمال ماء الصنبور مقارنة بإستعمال الماء المقطر .

وعلى ذلك فإن إستعمال ماء الصنبور بدلا من الماء المقطر فى حوالى ٣٠ معمل لزراعة الأنسجة فى مصر يمكن أن يوفر سنويا ١٣٦٣٥ متر مكعب من ماء الصنبور المخصص أصلا لأغراض الشرب الأدمى ، وطاقة كهربائية مقدارها ١٣٥٠٠٠٠ كيلو واط ومبلغ ٨٢٢٤٧٦ جنيه مصرى تكاليف كلية ، آخذين فى الإعتبار أن هذه الأرقام قد تختلف من معمل لآخر ، ومن وقت لآخر ومن بلد لآخر . وتبين دراسة الجدوى تفاصيل هذه الأرقام .