Microbiology, Metabolites and Biotechnology
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The effects of culture media, various NAA, and sucrose concentrations on *in vitro* propagation of *Gerbera jamesonii*

Morteza Fayeghi Mohammadi[1], Mousa Torabi[2], Batool Hosseinpour*[3]

[1] *Department of Horticulture Sciences, College of Agriculture and Natural Resources, University of Mohagheh Ardabili,Iran*
[2]*Department of Horticulture Sciences, College of Agriculture and Natural Resources, University of Mohagheh Ardabili,Ardabil, Iran*
[3]*Department of Agriculture, Iranian Research Organization for Science and Technology, Tehran, Iran. *Corresponding Author:*

Article Info

Document Type:
Research Paper

Received 16/08/2023
Received in revised form
09/10/2023
Scientific-Acceptation
21/10/2023
Final-Acceptation
06/11/2023

Published 03/06/2024

Keywords:
Gerbera jamesonii,
carbon source,
micropropagation

Abstract

Gerbera jamesonii Bolus is one of the most popular ornamental plants worldwide and its micrpropgation allows to produce a large number of true to type plants with good quality in a short time. In vitro propagation of gerbera requires culture media supplemented with specific concentrations of minerals, organic supplements and energy sources. The aim of this study was to determine suitable culture medium and evaluate different concentrations of sucrose and Naphtalic Acetic Acid (NAA) for *in vitro* development of gerbera explants. Micro shoots of two gerbera cultivars (Artist and Brilliance) were cultured on four different culture media and the effect of various concentrations of sucrose (20 and 30 g L^{-1}) as well as $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ (0.5X, 1X and 1.5X) on *in vitro* propagation properties were examined. MS medium provided better shoot development and application of lower concentration of sucrose improved the efficiency of gerbera micropropagation. In Artist cultivar, the highest number of shoots was obtained by using 20 g L^{-1} of sucrose and with or without NAA application (9.08 micro shoots per explant). In contrast, Brilliance produced the highest number of shoots by using 20 g L^{-1} of sucrose and 0.1 mg L^{-1} of NAA (7.4 micro shoots per explant). In addition, the increase or decrease of $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ did not change propagation efficiency. The results can contribute to optimize bioreactor systems for large-scale production.

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1. Introduction

Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.), also known as Transvaal daisy or Barberton Daisy, is one of the important commercial cut flowers in global flower business. It has a wide range of attractive flower colors which makes it a more valuable ornamental species. Colors include white, yellow, orange, red, and pink. They vary greatly in shape and size. Gerbera is widely and commercially produced by the floral industry both as cut flower and potted plant. The flowers are hardy and can withstand vigorous transportation. It stands among the top ten cut flowers of the world (Sujatha et al., 2002).

*Corresponding author. Batool Hosseinpour. Department of Agriculture, Iranian Research Organization for Science and Technology, Tehran, Iran, E-mail address: bhosseinpour@gmail.com; hosseinpour@irost.ir
DOI: 10.22104/MMB.2023.6434.1119
Please cite this article as: M. Fayeghi Mohammadi, Microbiology, Metabolites and Biotechnology (MMB), https:// DOI: 10.22104/MMB.2023.6434.1119

Gerbera is propagated by means of seeds, cuttings of clumps with buds and from tissue cultured plants. For commercial cultivation under protected environment, seedlings developed by tissue culture technique are preferred to traditional propagation. Traditional propagation methods are too slow with low efficiency due to soil-borne diseases by transmitting to new propagated plants. Tissue culture enables a million-fold expansions per year of a desired plant (Aswath & Choudhary, 2002).

Different explants (shoot tips (Shabanpour et al., 2011), young leaves (Bhatt et al., 2015), petioles (Kumar & Kanwar, 2006) and petals (Ray et al., 2005) were selected and cultured on a specific culture medium containing different plant growth regulators. Micropropagation efficiency is affected by various factors such as the number of the regenerated micro shoots per explant, the length of micro shoots, the quality of micro shoots, root number and length per explant. Culture medium is one of the most important factors affecting the productivity of the tissue culture protocol. The medium contains a mixture of macronutrients, micronutrients, vitamins, and plant growth regulators such as auxins and cytokinins that are essential for the growth and development of plant cells and tissues under *in vitro* culture conditions. Murashige and Skoog medium (MS) (Murashige & Skoog, 1962) is the most commonly used for micropropagtion of gerbera as well as a wide range of plant species, including ornamental plants, fruit trees, and vegetables. Optimization of the growth medium based on the minerals, energy sources and growth regulators, is very challenging due to different requirements of various plants. Sucrose is carbon source in the culture medium. Its optimum concentration can affect the induction and growth of the shoots. Sucrose concentration of 2% and 3% are the most commonly carbohydrate source in plant tissue culture. Sucrose induces auxin levels in a phytochrome-interacting factors dependent way and contributes to auxin transport and signal transduction (Zahara et al., 2017). Moreover, it more efficiently affects root induction compared to glucose and fructose alone (Lastdrager et al., 2014).

Magnesium is an essential atom for chlorophyll pigments and plays important role in the transfer of phosphate. Calcium is involved in *in vitro* morphogenesis and its deficiency leads to shoot tip necrosis, poor root growth and curling leaves (George et al., 2008).

In this study, we studied the effects of various media culture, $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ and sucrose concentrations on micropropagation efficacy of two gerbera varieties to improve commercial production.

2. Material and methods

2.1 Explant preparation and sterilization

Young and immature capitulum explants of two *Gerbera jamesonii* cultivars (Brilliance and artist) were washed under tap water for 30 minutes and then immersed in 70% alcohol for one minute followed by thorough washing three times using sterilized distilled water. For final surface sterilization, explants were immersed in 20% commercial bleach with 0.01% Tween-20 for 20 minutes followed by washing for three times using sterilized distilled water.

Sterilized explants were cut into three pieces and were cultured on establishment culture medium. Cultures were kept in growth chamber with 25°C with a 16 hr photoperiod with cool white fluorescent bulbs (PPFD = 35 µmol m^{-2} s^{-1}) and 8 hr darkness. After two months, regenerated shoots were transferred to multiplication medium. Regenerated shoots were used for next experiments.

2.2 Experiments

The objective of the first experiment was to study the effects of four various culture media on in vitro multiplication parameters of two gerbera varieties. Three culture media including MS (Medium 1), MS supplemented with 128.4 mg L^{-1} NaH_2PO_4 (Medium 2), MS supplemented with 0.5 mg L^{-1} thiamine, 0.05 mg L^{-1} biotin, 0.5 mg L^{-1} folic acid and 5 mg L^{-1} nicotinic acid (Medium 3 and MS supplemented with 0.1 mg L^{-1} $MnSO_4.4H_2O$, 0.1 mg L^{-1} H_3BO_3 and 0.1 mg L^{-1}

$ZnSO_4.7H_2O$ (Medium 4) were evaluated. Each culture medium was supplemented with 0.5 mg L^{-1} 6-benzyladenine (BA) and 0.1 mg L^{-1} naphtalic acetic acid (NAA) and 30 g L^{-1} sucrose.

The aim of the second experiment was to evaluate the effect of low level of sucrose concentration in presence or absence of NAA on *in vitro* propagation parameters of Gerbera. Therefore, MS medium was selected as the basal culture medium and supplemented with 30 and 20 g L^{-1} of sucrose in combination with 0 and 0.1 mg L^{-1} of NAA. All the treatments contained 0.5 mg L^{-1} BA.

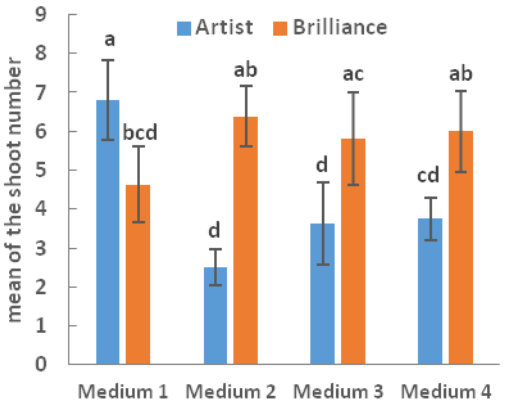
In the third experiment, we analysed the effect of three various concentrations (0.5X, 1X and 1.5X) of $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ (salts) on *in vitro* propagation parameters of Gerbera. The basal medium was MS and three treatments included 220 mg L^{-1} $CaCl_2.2H_2O$ and 185 mg L^{-1} $MgSO_4.7H_2O$ (T1), 440 mg L^{-1} $CaCl_2.2H_2O$ and 370 mg L^{-1} $MgSO_4.7H_2O$ (T2) and 660 mg L^{-1} $CaCl_2.2H_2O$ and 555 mg L^{-1} $MgSO_4.7H_2O$ (T3). All the treatments contained 0.5 mg L^{-1} BA, 0.1 mg L^{-1} NAA and 30 g L^{-1} sucrose.

The pH of all media was adjusted at 5.7. One liter of culture medium was solidified with 0.7% agar and autoclaved at 121°C for 30 minutes. Each treatment was included four jars and each jar was considered as a replicate. Three shoots were cultured in each jar. After four weeks, the number of shoots per explant, shoot length, number of roots per explant and root length were recorded. Experiments were organized as a completely randomized design and data from the experiments were subjected to analysis of variance using SAS 9.4 software.

3. Results and Discussion

Data analysis of the first experiment showed culture medium×cultivar significantly affected shoot number (Fig 1). However, shoot length was significantly affected by cultivar and culture medium effect. As a result, shoot length decreased by using MS medium (2.7 cm) and Artist produced longer shoots than Brilliance (3.5 cm and 2.6 cm, respectively). Based on the results, MS medium was the appropriate culture medium. MS is the common culture medium which is used for micropropagation of Gerbera (Akter et al., 2022; da Silva et al., 2020). Our analyses indicated that application of higher levels of vitamins or micronutrients did not improved micropropagation efficiency.

Figure 1: Comparison of the mean number of the shoots regenerated in explants of gerbera cultured *in vitro* on four different culture mediums supplemented with 0.5 mg L^{-1} BA.



In the second experiment, statistical analysis of shoot number data indicated that the single effect of sucrose and the triple interaction effect among cultivar, NAA and sucrose (cultivar×NAA×sucrose) were significant at 1% probability level. As a result, 2% sucrose increased the number of regenerated shoots per explant in both cultivars compared to 3% sucrose concentration (Fig 2). According to Fig 3 and 4, in Artist, the greatest number of shoots (9.08) were produced in MS medium supplemented with 0 mg L^{-1} NAA and 2% sucrose. However, in Brilliance, the greatest number of shoots were produced in MS medium supplemented with 0 or 0.1 mg L^{-1} NAA and 2% sucrose (7.4) or with 0 mg L^{-1} NAA and 3% sucrose (6.87).

Figure 2: The effect of different concentrations of sucrose (20 and 30 g L-1) on mean number of the shoots regenerated in explants of gerbera cultured in vitro on MS medium supplemented with 0.5 mg L-1 BA.

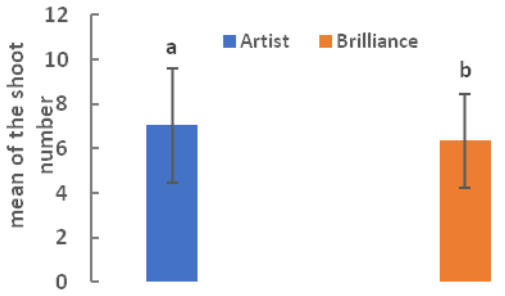


Figure 3: Mean number of the shoots regenerated in explants of two gerbera varieties cultured *in vitro* on MS medium supplemented with different concentrations of sucrose (20 and 30 g L^{-1}) and NAA (0 and 0.1 mg L^{-1}).

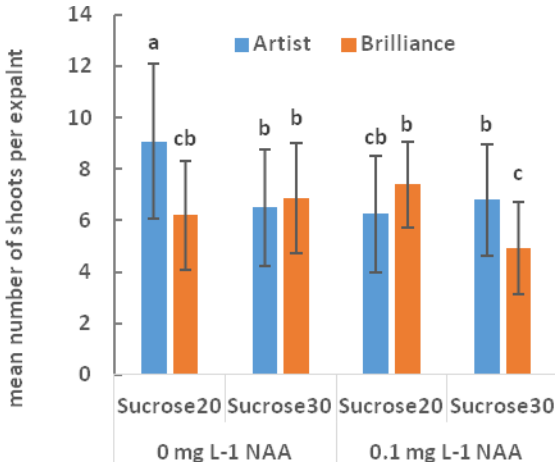


Figure 4: *In vitro* propagation of *Gerbera jamesonii* var. Artist (Left) and Brilliance (right).

Previous studies reported high number of produced shoots using MS medium but high concentrations of BA (2-7 mg L^{-1}) (Akter et al., 2022; Gantait & Mahanta, 2022; Talla et al., 2019). Although high levels of BA produce a greater number of shoots, there is no information about the quality of regenerated shoots during next multiplication stages because high levels of BA cause vitrification which significantly reduces the survival rate of the plantlets during adaptation procedure. In Blackberry, the higher number of shoots was produced by using 1.5% of sucrose (Ayub et al., 2019).

Shoot length was also significantly affected by sucrose concentration. Higher level of sucrose increased shoot length in both varieties (Fig 5). According to the results, root number and root length were significantly affected the triple interaction among cultivar, NAA and sucrose. High level sucrose significantly increased the length of the roots in both varieties (Fig 6 and 7). In accordance with our results, increase of sucrose produced longer shoots with longer roots in *Hylocereus polyrhizus* (Ng et al., 2020). Abbas *et al* also reported that increase of sucrose up to 9% increased root and shoot length in in vitro culture of potato (Abbas et al., 2020). Sucrose induces auxin in plants (Lastdrager et al., 2014). In wild-type seedlings of Arabidopsis, increasing exogenous sucrose increased lateral root formation and primary root elongation (Fukaki et al., 2007; Lee-Ho et al., 2007).

Figure 5: The effect of different concentrations of sucrose (20 and 30 g L^{-1}) on mean length of the shoots regenerated in explants of gerbera cultured *in vitro* on MS medium supplemented with 0.5 mg L^{-1} BA.

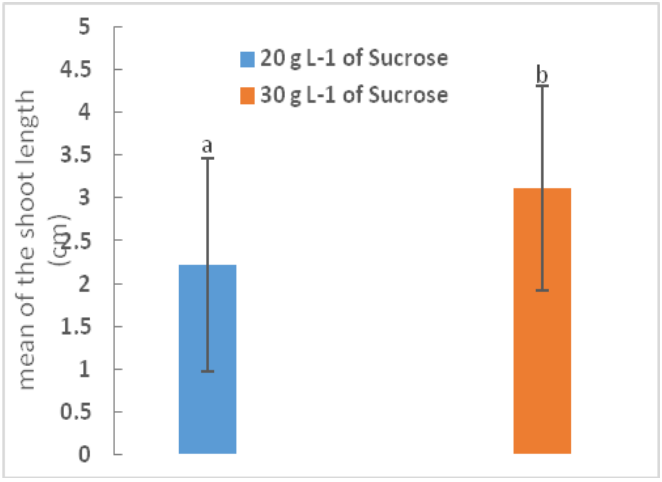


Figure 6: Mean number of the roots regenerated in explants of two gerbera varieties cultured *in vitro* on MS medium supplemented with different concentrations of sucrose (20 and 30 g L^{-1}) and NAA (0 and 0.1 mg L^{-1}).

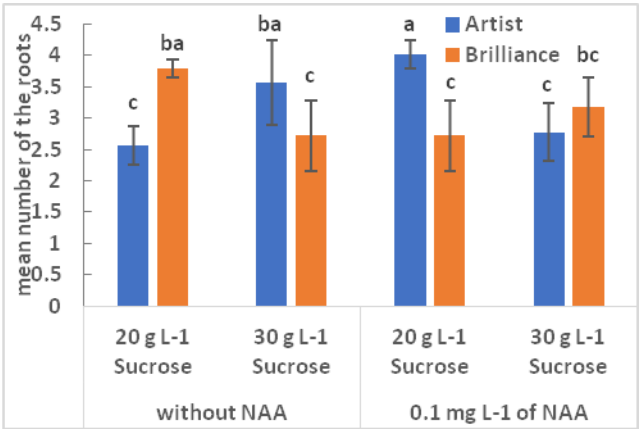
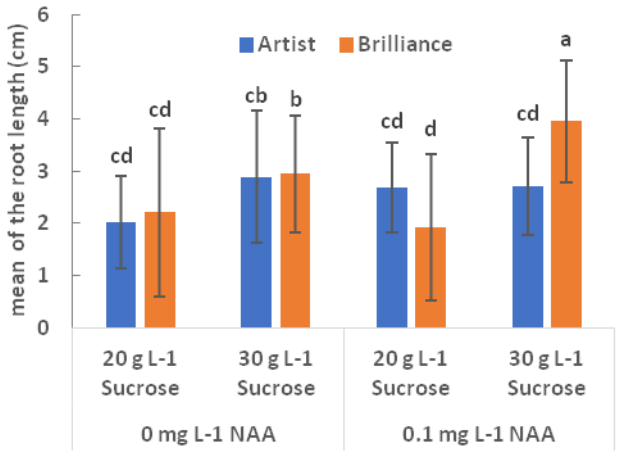


Figure 7: Mean number of the roots regenerated in explants of two gerbera varieties cultured *in vitro* on MS medium supplemented with different concentrations of sucrose (20 and 30 g L^{-1}) and NAA (0 and 0.1 mg L^{-1}).



Data analysis of the third experiment indicated that shoot number, root number and root length were significantly influenced by only cultivar effect and the increase or decrease of $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ levels did not changed those parameters (Table 1). As a result, Artist and Brilliance produced 5.58 and 6.46 shoots per explant respectively. The mean number of produced roots per explant was 1.6 in Artist and 2.4 in Brilliance. Artist produced longer roots than Brilliance (3.93 cm vs 3.35 cm). However, shoot length was affected by two single effects: cultivar and salt level. Artist produced longer shoots than Brilliance (3.21 cm vs 2.95 cm). As a result, 1X (T2) and 1.5X (T3) of salts increased shoot length (3.13 cm and 3.33 cm, respectively) but shoot length was decreased by using 0.5X of salts (2.76 cm). In tissue culture of melon, the highest level of magnesium occurred in direct somatic embryogenic cultures and the lowest level in callus cultures (Kintzios et al., 2004). Chloride toxicity happens when large quantities of calcium chloride are added to the culture medium and sometimes hyperhydricity occurs after several subcultures. Our results indicated that reduction of $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ levels did not affect propagation efficiency (George et al., 2008).

Table 1: Analysis of variance of the effect of three $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ levels on shoot number, shoot length, root number and root length of two gerbera varieties.

Trait	Source	DF	Mean Square	F Value	Pr > F
shoot number	variety	1	0.51	0.15	0.0426
	mesos	2	12.85	3.8	0.0581
	variety ×mesos	2	4.01	1.19	0.3344
shoot length	variety	1	0.49	1.86	0.0445
	mesos	2	1.15	4.3	0.0451
	variety ×mesos	2	0.65	2.43	0.1244
root length	variety	1	0.35	0.23	0.0377
	mesos	2	6.34	4.24	0.0562
	variety ×mesos	2	0.304	0.2	0.8182
root number	variety	1	0.32	1.32	0.0495
	mesos	2	2.75	11.35	0.0512
	variety ×mesos	2	0.48	1.97	0.1764

4. Conclusion

Optimizing sucrose and mineral concentrations for improving growth and development has been studied in many plant species. High levels of

sucrose and minerals in the culture medium are of the main causes of hyperhydricity as well as low rate of *in vitro* propagation of many herbal plants such as *Gerbera jamesonii*. In the current study, we demonstrated that 2% of sucrose in MS medium, promoted shoot multiplication and induced shoot regeneration. In addition, the reduction of calcium and magnesium did not affect the efficiency of gerbera micropropagation. These results are supposed to have significant contribution for more understanding about tissue culture of Gerbera by using bioreactor systems. Also, we propose further studies needs to be taken in account to obtain optimal concentrations of other macro nutrients for shoot multiplication of gerbera in solid and liquid culture media.

Author contribution

Conflict of interest

The authors declare no conflict of interest

Acknowledgment

Not Applicable.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Open access

Funding

Not Applicable.

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