

Impact of cytokinin BA and chemical stress Sorbitol on Callus growth and adventitious micro shoots regeneration of the plant *Gerbera jamesonii* Bolus" Malibu" cultivar grown In vitro

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Abstract

The response of *Gerbera jamesonii* (Malibu) leaf bases to callus induction in media prepared with different concentrations of 0.0, 17.76, 26.64, 35.52, and 44.40 $\mu\text{mol L}^{-1}$ of BA, with a constant concentration of 4.30 $\mu\text{mol L}^{-1}$ of auxin (NAA), and the reaction of primary callus to varying concentrations of 24.0, 36.0, 48.0, and 60.0 g m L⁻¹ of the sugar alcohol sorbitol in inducing osmotic stress, encompassing aspects such as callus growth (measured by fresh weight, dry weight, and water content percentage) and adventitious shoot formation. The study results indicated that the media containing BA at a concentration of 44.40 $\mu\text{mol L}^{-1}$ and NAA at 4.30 $\mu\text{mol L}^{-1}$ had a significant impact on the induction percentage compared to all other treatments. Callus and the largest diameter of the loose callus mass reached 100%, 3.23 cm respectively and over a period of 6.33 days. It was noted that with increasing the concentration of BA in the nutrient medium, the percentages gradually decreased with decreasing its concentration, while no response was shown in the percentage of callus induction in the BA-free medium, which reached 0.0%. The results showed that reducing the sorbitol concentration in the nutrient medium for the growth of primary callus to 24.0 g.L⁻¹ led to a significant increase in the average fresh and dry weight of callus and the percentage of its water content, which reached 8.517 g, 1.202 g, 85.857% respectively. The same concentration also achieved the highest average number of adventitious shoots and leaves, the longest shoots and leaf width, as micro-shoot per 100 mg reached 11.20, 9.60 leaf per shoot, cm 6.88, 0.94 cm, and all the characteristics decreased. (Callus growth and adventitious shoot generation) with a gradual increase in concentration in the medium.

Keywords: BA, Sorbitol, *Gerbera jamesonii* L, in vitro

Introduction

Gerbera jamesonii belongs to the Asteraceae family. The genus *Gerbera*, named after the German traveler Traugott Gerber, includes 40-45 species, the most important of which is *jamesonii*, named after the English scientist Jameson. This genus includes all commercial varieties that grow in most parts of the world (Rangnamei *et al.*, 2019). It has a high marketing value as cut flowers in many countries, including the Netherlands, Germany, Japan, and the United States. *Gerbera* is a perennial herbaceous flower, reaching a height of 24.5-9.8 cm, suitable for long-distance shipping without compromising its shape, color, or freshness. Its flowers are distinguished by their strength, durability, long flower stalks, and long flower life. *Gerbera* is ranked fourth among the top ten cut flowers in the world (Maitra *et al.*, 2020). Thus, ornamental plants, such as gerbera plants, have garnered significant focus in the realm of plant

tissue culture Propagation, following the discontinuation of many established traditional propagation methods due to the achievements realized in this area, as micropropagation is the main system used to propagate them outside the living body and obtain millions of plantlets within a year due to the increasing demand for them as commercial cut flowers (Darras, 2021). Plants are exposed to various types of environmental stresses such as drought or high osmotic pressure. These stresses include morphological changes and development in the protein pattern, such as physiological and chemical processes. During these processes, some solvents accumulate, formed by osmosis, classified as hydroxy compounds, such as polyhydric alcohols, such as sorbitol (Yu *et al.*, 2023). In addition to its role in the manufacture of hormones (auxins, cytokines, kinins) that stimulate cell division and elongation and thus activate growth (Awuchi, 2017). Carbohydrates are essential for the growth of any part of

the plant, as they provide energy and the basis for growth processes. Various. Many plant tissues contain levels of free carbohydrates. These levels are noticeable even when signs of aging appear. Carbohydrates, including sugar alcohols, are important components of any culture medium and are among the compounds formed by osmosis. Low molecular weight osmolality is found in plants, and their effect on the culture explant is determined by their role as a source of energy and carbon, as well as their role in regulating osmosis (Twaij *et al.*, 2022; Khaliq *et al.*, 2023). Sorbitol also participates in the metabolism of essential carbohydrates and stimulates many genes to produce substances that enhance the plant's ability to withstand adverse conditions and diseases and generate natural plant hormones like abscisic acid (ABA), salicylic acid, and jasmonic acid. It enhances the plant's ability to withstand biotic and abiotic stresses and promotes vegetative growth. Sorbitol has gained great importance in the study of moisture stress to stimulate and improve the quality and quantity of callus produced. It also plays a role in creating and regulating osmotic stress in the cell, making it more negative (Jahnke *et al.*, 2020). Benoit (Muñoz *et al.*, 2019) induced osmotic potential using the osmotically active compound sorbitol, which was added at different concentrations to the MS medium to determine its effect on the growth averages of plants. The researchers confirmed a gradual decrease in the vegetative growth averages with increasing sorbitol concentrations from 20-60 g L⁻¹, and their average decreased when the concentration was increased to 60 g L⁻¹, the average height of the vegetative group. Mazene (2020) also showed that inducing osmotic potential using the sugar alcohol sorbitol in the callus medium at different concentrations significantly affected the growth of the primary callus of *Chrysanthemum hortorum* Hort. cv. Dwarf, which led to a gradual reduction in the average fresh and dry weight and water content of callus, and this is what the researchers (Mazene and Mazeni, 2024), water treatment can save water from being polluted (Alsaifi *et al.*, 2024; Bozókí, B., *et al.*, 2025), indicated when they studied effect of the sugar alcohol mannitol in reducing the water stress on the growth of the seven-palate plants, which resulted in a noticeable reduction in the relative growth rate when the stress was induced from 15-60 g L⁻¹. Given the commercial importance of the Malibu gerbera plant, which is one of the varieties that require specific environmental conditions and protected areas for its growth, it has been tissue cultured to expand its culture in Iraq, especially in the Middle Euphrates regions, in addition to its propagation using

traditional methods. and the response of primary callus to different concentrations (24.0, 36.0, 48.0, and 60.0 g m L⁻¹) of the sugar alcohol sorbitol in creating osmotic stress, including factors like callus growth (assessed through fresh weight, dry weight, and water content percentage) and adventitious shoot development. The findings of the study showed that media with BA at a concentration of 44.40 µmol L⁻¹ and NAA at 4.30 µmol L⁻¹ significantly affected the induction percentage in comparison to all other treatments. inducing callus and treating callus with different levels of the sugar alcohol sorbitol to determine the best concentration for callus growth and the generation of adventitious shoots from it, and to increase its and the plants generated from it's tolerance to water stress, thus enabling the production of plants that are tolerant to environmental stress.

Materials and Methods

The research was carried out in the Plant Tissue Culture Laboratory at the University of Basra's College of Agriculture. Seedlings imported from a Diwaniyah province nursery were utilized, and were planted in pots with diameters of 0.5-0.7 cm. Plant parts (explants) were taken from these plants, represented by the bases of young leaves, which represent the leaf bases, which appear as an elongated triangle with dimensions of (0.5-0.7) cm (Fig. 4-A). In order to eliminate any accumulated dust, the components of the plant were washed multiple times using water and liquid soap. Subsequently, they underwent several washings with distilled water before being submerged in a glass vessel containing the fungicide (Elsa) at a concentration of 400 mg.L⁻¹. The container was stirred several times for (5) minutes and then washed multiple times with distilled and sterilized water. Glass containers containing an antioxidant solution of 150 mg were used to store all plant tissues. L⁻¹ of citric acid and 100 mg.L⁻¹ of ascorbic acid were refrigerated at 4°C for a duration of 24 hours, until they were surface sterilized by being moved to a laminar flow air cabinet that had been sterilized beforehand with a solution of 70% ethanol and chlorine diluted with distilled and sterilized water. Subsequently, the lower sections of the plant parts were trimmed to lengths of (0.4-0.6) cm in order to remove the sterilizing material that had penetrated the plant tissues during ethyl alcohol sterilization. Subsequently, they were placed in a shaking 40% v:v sodium hypochlorite solution (made from a commercial bleach product with 1.05% sodium hypochlorite) for 20 minutes, to which (2-3) drops of Tween-20 had been added. The plant components were then individually extracted and washed multiple times with distilled, sterile water while stirring from time to time.

The culture medium consisted of 4.33 g L⁻¹ Murashige and Skoog (1962) salts (MS) and organic components, such as sucrose at 30 g L⁻¹, adenine sulfate at 40 mg L⁻¹, meso-inositol at 60 mg L⁻¹, and sodium phosphate at 100 mg L⁻¹. At a concentration of 1 mg L⁻¹, some vitamins and glycine were added. Subsequently, cytokinins and auxins were incorporated, the culture medium's pH was fine-tuned to 5.7–5.8, and agar was introduced at a concentration of 5.6 g L⁻¹. The culture medium was heated to 90–91°C and then dispensed into 18 x 2.5 cm tubes at a rate of 100 g L⁻¹, with 20 ml per tube. The tubes were subsequently plugged with cotton and covered with aluminum foil. Every treatment consisted of ten test tubes (replicates). After that, these tubes underwent sterilization with an autoclave at a temperature of 121°C and a pressure of 1.04 kg/cm² for 20 minutes. The cultures were incubated in a growth chamber at a temperature of 25 ± 2°C, with an illumination intensity of 1000 foot-candles for 16 hours each day.

Callus induction and regeneration of adventitious shoots (indirect)

Effect of cytokinin (BA) on the induction of primary callus

Cuttings from the bases of young leaves were planted horizontally in a triangular shape on a medium composed of MS salts and the above-mentioned materials, supplemented with cytokinin (BA) at concentrations of 44.40, 35.52, 26.64, 17.76, 0.0 µmol L⁻¹ with a constant concentration of 4.30 µmol L⁻¹ of auxin NAA in inducing primary callus from young leaf bases. The culture process was carried out inside a laminar air flow table. The surfaces of the table and all the tools used in culture were sterilized with 70% ethyl alcohol and diluted chlorine. Young basal leaf pieces were planted at a rate of one piece in each test tube with dimensions of 2.5 x 18 cm and 15 replications of each treatment for each plant part for the purpose of inducing and multiplying callus. The cultures were incubated in a growth chamber at a temperature of 25±2°C in the dark for eight weeks, and then the following measurements were taken:

1 - Percentage of cutting response =

$$\frac{\text{number of callus} - \text{induced cuttings}}{\text{total number of culture cuttings}} \times 100$$

2 - Time required for callus emergence (days)

3 - Diameter of callus mass (mm)

4 - Callus density

Reculture of callus induced from the basal parts of young leaves, characterized by its brittle appearance and milky white color, was carried out on a culture medium supplemented with the optimal concentration of cytokinin BA (44.40 µmol L⁻¹) and NAA (4.30 µmol L⁻¹) for

eight weeks in the dark for the purpose of propagation and use in the sorbitol stress experiment.

Effect of different levels of sorbitol on callus growth and adventitious shoot generation.

This experiment utilized callus that had been induced and grown from the basal regions of young leaves. A callus weighing around 120 mg was taken and cultivated in a culture medium enriched with MS salts, the previously mentioned components of the culture medium, and the ideal concentration of the growth regulator BA, while maintaining NAA at a constant level of 4.30 µmol L⁻¹. Different concentrations of sorbitol were added, at concentrations of 60.0, 48.0, 36.0, and 24.0 g L⁻¹, to callus growth in glass bottles measuring 4.5 x 15 cm, at a volume of 25 cm³ per glass bottle. The openings were then sealed with cotton wool and covered with aluminum foil and screw-on metal caps. The cultures were incubated in an incubator at 25 ± 1°C for eight weeks in the dark, while the other part of the growing callus was incubated in the same conditions. Treatments under light for the purpose of generating adventitious shoots.

Measurement of fresh and dry weight of callus and percentage of water content (%)

1- The fresh and dry weights of the callus were measured using a sensitive balance. Pieces of fresh callus were extracted and placed on filter paper sterilized in an electric oven. Remains of the media adhering to the callus were removed using scalpels, and the fresh weight was calculated using a sensitive balance. The dry weight of the callus was then calculated by placing it in an electric oven at 40°C until the weight was stabilized using a sensitive KERN balance, according to the following equation: $W_0/W_1 - W_0 = FwGc$.

2- Percentage of water content of callus =

$$\frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100$$

(Al-Bahrany, 2002) The following measurements were taken for the process of adventitious shoot propagation from callus:

1. Average number of vegetative shoots/plant segment.
2. Average length of vegetative shoot (cm).
3. Average number of leaves/explant .
4. Average leaf width (cm).

After the culture process was completed, the plants were incubated in a growth chamber at 25±2°C in a light incubator.

Statistical Analysis

The study used a completely randomized design (C.R.D.) for its studies. Analysis of variance was used to look at the results, and the Revised Least Significant Difference (R.L.S.D.) test was used to compare the treatment means at a 0.01 probability level (Al-Rawi and Khalaf Allah, 2000). The results were obtained using the previously developed statistical analysis tool Genestat. (2008) ausgewertet.

Results and Discussion

Effect of cytokinin BA on the induction of primary callus from young leaf bases of Gerbera jamesonii (Malibu)

The results shown in **Figure 1** demonstrate effect of different concentrations of benzyl adenine (BA) – 144.40, 35.52, 26.64, 17.76, and 0.0 $\mu\text{mol L}^{-1}$ with a constant concentration of auxin NAA – 4.30 $\mu\text{mol L}^{-1}$ on the induction of primary callus from young leaf bases of *Gerbera jamesonii* after eight weeks of incubation in the dark. Panel (Fig. 4-B) shows the horizontal culture of leaf bases on the surface of the MS medium, with the formation of transparent, shiny, granular callus characterized by its fragility, green color, loose, and clustered around the leaf bases (leaf blade). Then the callus mass began to grow and divide, forming loose granules that covered the All leaf surfaces with varying callus production depending on the type and concentration of cytokinin and auxin (Al-Taha and Mazene, 2020). The results in the same figure showed that the percentage of callus induction showed a high significant effect among all BA treatments and a significant superiority was recorded for the highest average percentage of callus induction over all BA treatments, reaching 100% when the BA concentration in the nutrient medium increased to 144.40 $\mu\text{mol L}^{-1}$. The percentages gradually decreased with the decrease in the BA concentration in the nutrient medium to 86.67, 68.89, 53.33% at the three concentrations of 35.52, 26.64, 17.76 $\mu\text{mol L}^{-1}$, respectively, while no response was shown in the percentage of callus induction in the medium free

of cytokinin (control), with the appearance of white roots directly from the base of the leaf blade until the end of the incubation period (Fig. 4-C). The reason for the lack of response of the leaf pieces to callus induction in the medium free of cytokinin (comparison), with the observation of an increase in the size of the leaf pieces and the appearance of direct roots from The leaf blade base may be attributed to the role of cytokinins in stimulating cell division and the balance between cytokinins and auxin is necessary for callus induction (Kazmi et al., 2015). The interaction of cytokinins with auxins in callus induction and growth was greatly encouraged compared to the addition of auxin alone, which stimulates roots and stimulates the formation of RNA necessary for building enzymes and proteins affecting growth (Kamruzzaman et al., 2015).

The results in the figure above also demonstrate a variable response in the average diameter of the callus mass and the time required for callus formation. It was observed that the high concentration of BA (144.40 $\mu\text{mol L}^{-1}$) showed a significant superiority in recording the largest callus diameter of 3.23 mm, and within the shortest time required for callus formation, 33.6 days. A gradual decrease in the average callus diameter was observed, along with an increase in the average time required for callus induction, as the BA concentration in the medium decreased. The three concentrations (35.52, 26.64, and 17.76 $\mu\text{mol L}^{-1}$) showed a significant increase in the average callus mass of 2.47, 1.90, and 1.57 mm, respectively, with significant differences between them. During the time period required for callus induction at these concentrations, which amounted to 17.33, 12.67, and 22.00 days, respectively, which did not show significant differences in the average time period for callus induction, the results agreed with (Hamza et al., 2013), as they concluded when studying the callus induction process from the culture of the plant *Antirrhinum majus* L. that the nutritional medium consisting of BAP 5.0 mg L^{-1} + NAA mg L^{-1} - 1.0 mg L^{-1} , the callus mass volume was 2.35 cm^3 , and this was confirmed by many researchers (Abd-, Elaleem, 2009, Al-Taha and Mazene, 2016, Al-Taha and Mazene, 2020), as the addition of cytokinin BA with the presence of auxin is the most effective in callus induction, and it was noted that the callus mass increased with the increase in the concentration of BA in the medium. Nutritional results were consistent with (Ibrahim and Yassin, 2025), who showed how to induce callus from young *Gerbera* leaves and the considerable advantage of interacting the cultivar with a high concentration of 5.0 mg L^{-1} BA. The outcomes also concurred with (Kumari

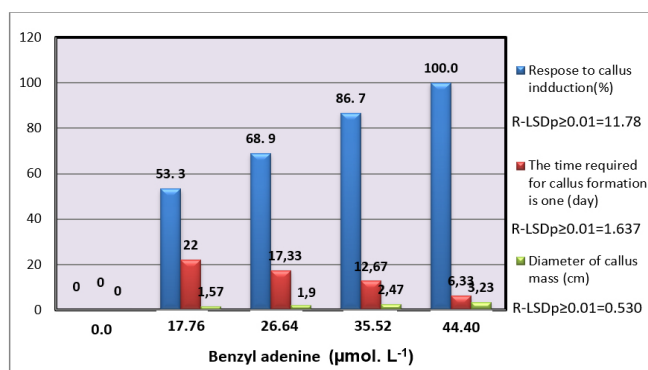


Figure 1. Effect of different concentrations of BA ($\mu\text{mol L}^{-1}$) on adventitious shoot formation from callus of *Gerbera jamesonii* after eight weeks of incubation in the dark.

et al., 2018), who developed an economic program for micropropagation of the Gerbera plant. On the other hand, the results of the study contradicted those of Mahindarkar, 2015, who showed that callus induced from Gerbera jamesonii seeds gave the lowest average duration of its emergence, reaching 25.87 days, in the MS medium supplied with a concentration of 1.0 for each of BA and 2,4-D, while the medium supplied with 5.0 mg L⁻¹ BA with the addition of 2,4-D at a concentration of 1.0 mg L⁻¹ gave the maximum duration of 33 days. Primary callus reculture was carried out by cultivating the basal parts of young leaves of Gerbera spp. in MS medium supplemented with 44.40 µmol L⁻¹ BA cytokinin and a constant concentration of 4.30 µmol L⁻¹ auxin NAA for eight weeks in the dark for propagation and use in experiments on chemical treatments (sorbitol) for callus growth. Callus induced from the leaf bases after reculture was characterized by its pale green color and brittle appearance after transforming into large granules at the end of the incubation period (Fig.4-D). In addition, the process of stimulating callus tissue division and expansion by adding the optimal combination provides strong evidence of plant cell specialization and the preservation of its latent energy (Cell Pectentialities) (Al-Sumaidaie, 2017).

Effect of different levels of osmotic stress factor sorbitol on the growth of primary callus of Gerbera oleifera.

The results of the analysis of variance related to effect of different levels of sorbitol (24.0, 36.0, 48.0, and 60.0 g m L⁻¹) on the fresh and dry weight and percentage of water content of callus growing in the MS medium containing 44.40 µmol L⁻¹ BA and a constant concentration of 4.30 µmol L⁻¹ auxin NAA after eight weeks of culture in the light are shown in **Figure 2**. It was observed that increasing the sorbitol concentration in the nutrient medium from 24.0 to 60.0 g.L⁻¹ caused a reduction in callus growth and a significant decrease in the average fresh and dry weights and percentage of water content of the primary callus upon gradual exposure to sorbitol levels in the nutrient medium until reaching the high concentration compared to other osmotic stress media. The nutrient medium prepared with the lowest level of sorbitol, 24.0 g.L⁻¹, gave the highest average fresh and dry weights and the highest percentage of water content of the developing callus, which reached 8.517 gm, 1.202 gm and 85.857%, respectively. While the lowest callus values were observed in the MS nutrient medium prepared with 60.0 g.L⁻¹ sorbitol, which reached 1.32 gm, 0.48 gm and 63.51%, respectively (Fig.4-E). The reason may be that the cells grown under the influence of tension may have more

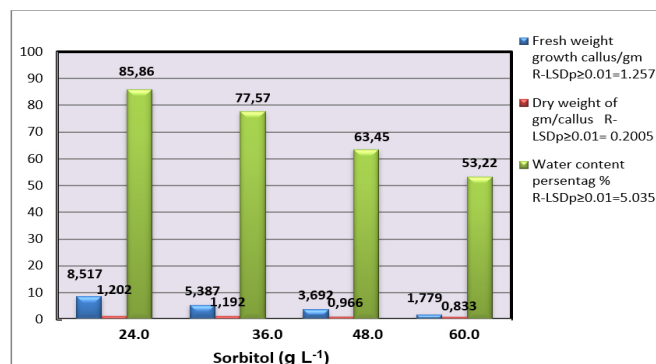


Figure 2. Effect of different concentrations of Sorbitol g.L⁻¹ on characteristics of callus Gerbera jamesonii After eight weeks of incubation in the light

additional energy than those that grow in the absence of a stress factor, it is likely that this additional energy is used to modify the osmotic potential, leading to a decrease in callus growth (Singh et al., 2015), or perhaps due to the decrease in callus growth associated with the presence of a chemical stress factor in the growth medium, which in turn affects the amount of internal growth regulators that respond to cell division, causing a significant decrease in the fresh and dry weight of the developing callus (Aliyah et al., 2017; Mazene et al., 2024). This was confirmed by many researchers (Mazene, 2020; Razavizadeh and Fatemeh, 2017; Al-Kaaby et al., 2013) through their study on effect of osmotic stress using sorbitol in callus tissue cultures, as they observed a significant decrease in the average relative growth of callus and a decrease in the water content of callus as expressed in the dry and fresh weight of callus. This decrease increased with increasing concentration in the medium, and the study was consistent with (Wu et al., 2015) showed that sorbitol participates in the metabolism of basic carbohydrates and stimulates many genes responsible for the production of substances that increase the plant's ability to withstand adverse conditions and diseases, and the production of abscisic acid (ABA), which increases plant senescence. This may be attributed to the role of sorbitol and mannitol in weakening the ability of callus tissue to absorb water after adding it at an appropriate concentration to the nutrient medium at a level similar to what occurs in nature under osmotic stress conditions, causing a reduction in the fresh and dry weight and water content of callus as a result of reducing the water potential in the growth medium, which causes a lack of water in the cells (Baan et al., 2022). Although adding sorbitol to callus culture media causes greater stress than the stress resulting from adding mannitol, supplementing callus culture media with either mannitol or sorbitol, alone or together, led to a

decrease in callus growth (Panwar and Guru, 2015).

Effect of osmotic stress using sorbitol on the indirect generation of adventitious shoots

The results in **Figure 3** indicate highly significant differences between sorbitol levels in the process of adventitious shoot generation from primary callus when it was added at different concentrations of 24.0, 36.0, 48.0, and 60.0 g.L⁻¹ to the MS medium after eight weeks in the light of the callus experiments. The callus turned green, characterized by its disintegration into hardened granules (Fig. 4-F). Many fine adventitious shoots appeared in the upper part and around the callus in all osmotic stress treatments induced by sorbitol, as shown in the microscopic panel (Fig. 4-G,H). New small green shootes appeared, covering the surfaces of the primary callus after eight weeks of incubation in the light (Fig. 4-I,J). Furthermore, it was noted that the low 24.0 g.L⁻¹ treatment From sorbitol, it was significantly superior in achieving the highest averages in the number of adventitious shoots and leaves, shoot lengths, and leaf width, as they reached 11.20 micro-shoot per 100 mg, 9.60 leaf per shoot, 6.88 cm, 0.94 cm, respectively, compared with the other levels of sorbitol, in which the average of the studied characteristics decreased gradually when its concentration was gradually increased in the medium until it reached the lowest limit of 4.40 micro-shoot per 100 mg, 3.20 leaf per shoot, 1.46 cm, 0.26 cm, respectively, in the treatment of 60.0 g.L⁻¹, panel (Fig.4-K), as it is noted that the adventitious shoots generated from the callus formed on the nutrient medium of 24.0 g.L⁻¹ after 16 weeks with the formation of many thin white roots, panel (Fig.4-L,M), may be attributed to the reason for the gradual decrease in the averages of organ generation from callus in Plants exposed to osmotic stress have a shortened lifespan of water in the cells, which leads to a decrease in cell turgor and eventual growth. The reason for the inefficiency of sugar alcohols in stimulating the growth of vegetative

shoots and the decrease in the average number and length of duplicated shoots of the gerbera plant when its level increases in the MS medium is the exposure of the gerbera plant to the negative osmotic potential of the nutrient medium caused by the increase in sugar alcohols, which affected the occurrence of a decrease in metabolic processes through the decomposition of carbohydrates and proteins and their demolition by stimulating free radicals (ROS) (Martínez-Santos *et al.*, 2021; Omar, S. *et al.*, 2022). This, as the dry matter is a summary of the vital activities of plants sensitive to water stress, led to a decrease in the dry matter of the plant reflected by weight as a result of the disruption of essential activities brought on by the absence of water absorption from the growth medium.

. This can be explained by the fact that plants that survive under the influence of osmotic compounds are still able to absorb both water and nutrients from the culture medium due to the low water content in the medium (de Oliveira and Aloufa, 2022) The stressful effect of increased sorbitol concentration in the nutrient medium may also be attributed to a significant decrease in vegetative growth parameters (shoot length, leaf number) through its effect on hindering nitrogen metabolism, as it reduces the activity of ribonuclease and nitrate reductase enzymes. It also affects the formation of nucleic acids (DNA and RNA). Therefore, stressful conditions may cause plant necrosis and death due to nutrient deficiency after prolonged incubation, as it negatively affects plant growth by affecting cell division and elongation when nucleic acid levels are low (Petrova *et al.* 2021; Munthali *et al.* 2022). This was confirmed by (Gelmesa *et al.*, 2017; Kalve *et al.*, 2020) due to a decrease in growth parameters with increased sorbitol concentration in the nutrient medium, as well as a decrease in the efficiency of net photosynthesis, which is the basic process for plant growth when cells are exposed to water stress factors in response to water shortage conditions. As cell membranes lose their stability with the decrease in leaf water content, and plant pigments deteriorate when the internal CO₂ concentration decreases, this study is consistent with the results reported by (Ramirez-Mosqueda *et al.*, 2019) in their study on the plant *Laelia anceps* Lindl, where high concentrations of osmotic compounds in the culture medium led to a decrease in the survival of plantlets until the end of the incubation period. The results of these studies were supported by (Silva *et al.*, 2019), indicating that high levels of sorbitol in the culture medium significantly reduce the number of leaves in the plant *Poincianella pyramidalis*. This was

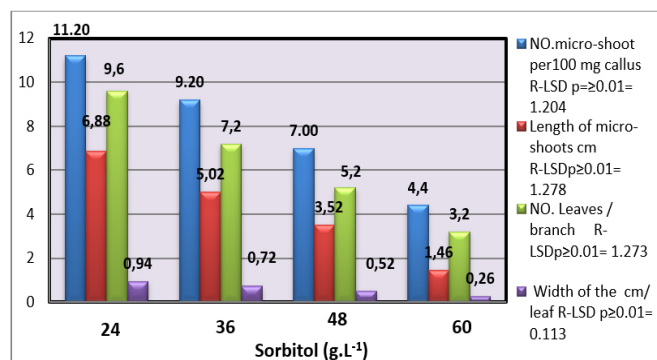


Figure 3. Effect of different concentrations of sorbitol g.L⁻¹ on adventitious shoot formation from callus of *Gerbera jamesonii*.

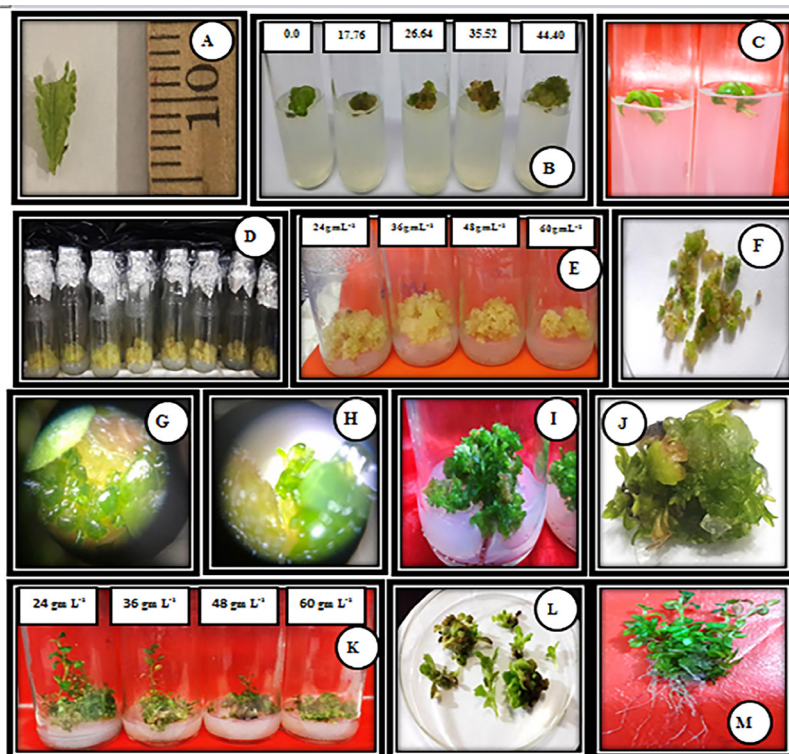


Figure 4. Induction of callus and regeneration of adventitious shoots from the primary callus of gerbera plant (*Gerbera jamesonii* Bolus Cv. "Malibu") (A) Leaf blade bases (B) Initiated callus from base of segment on MS medium+BA at 0.0, 17.76, 26.64, 35.52, 44.40 $\mu\text{mol}\cdot\text{L}^{-1}$ +4.30 $\mu\text{mol}\cdot\text{L}^{-1}$ NAA mg L^{-1} after 8 weeks.(C) Direct root growth from the end of the leaf blade in BA the free medium after 8 weeks (D) Reculture of callus on MS medium supplemented with 44.40 $\mu\text{mol}\cdot\text{L}^{-1}$ BA and 4.30 $\mu\text{mol}\cdot\text{L}^{-1}$ NAA after 8 weeks). E) callus grown on MS medium with sorbitol at 24.0, 36.0, 48.0, 60.0 $\text{g}\cdot\text{L}^{-1}$ after 8 weeks. (F,G,H,I,J) initiated small micro-shoot from callus on MS with 44.40 $\mu\text{mol}\cdot\text{L}^{-1}$ sucrose (microscope picture).(K) adventitious shoots grown on MS + Sorbitol at 24.0, 36.0, 48.0, 60.0 $\text{g}\cdot\text{L}^{-1}$ after 8 weeks. (L,M) micro-shoots grown on MS + 24.0 $\text{g}\cdot\text{L}^{-1}$.

confirmed by a study by (Al-Rekaby and Mirza, 2015; 30. Ali Al-Husseni et al, 2021), which stated that increasing the level of osmotic stress in the growth medium caused a significant decrease in the dry weight of the shoot and root system and leaf area of the plant *Adhatoda vasica* (L.) Nees. The results were consistent with many researchers. (Mazene and Mazeni, 2024) The researchers confirmed that increasing the concentration of mannitol from 15-60 $\text{g}\cdot\text{L}^{-1}$ led to a gradual significant effect on the average number of shoots, their lengths, and the number and width of leaves in *Antirrhinum magus* L. plants. The results were similar to those provided by the researchers (Moulin and Obaid, 2019), who explained that adding sorbitol at a concentration of 25 $\text{g}\cdot\text{L}^{-1}$ led to a significant increase in the average number of leaves and the area of one leaf in strawberry plants.

Conclusions

The drought-sensitive gerbera (Malibu) cultivar can be propagated in plant tissue culture by identifying the optimal combination of growth regulators for inducing primary callus and propagating it from leaf bases in MS medium supplemented with cytokinin (BA) at a concentration of 44.40 $\mu\text{mol}\cdot\text{L}^{-1}$, with a constant

concentration of auxin (NAA) at 4.30 $\mu\text{mol}\cdot\text{L}^{-1}$. This can be used to induce primary callus and then propagate it under environmental stress factors, generating complete plantlets resistant to environmental stress factors. Furthermore, the potential for increasing the tolerance of this cultivar to environmental conditions, including drought, can be increased by increasing osmotic potential and regulating it within plant tissues using the sugar alcohol sorbitol.

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