Effect Of Cytokinin BA And Different Concentrations Of Chemical Stress Treatments On The Multiplication Of Vegetative Shoots Of The Plant Antirrhinum Magus L. Grown In Vitro

Lamyaa Hussein Mousa Mazene[1], Shaymaa Hussein Mousa Mazeni[1]

[1]University of AL-Qadisiyah/Environmental Research and Pollution Prevention Unit, College of Science, University of AL-Qadisiyah, AL-Qadisiyah, Iraq

KEYWORDS

Shoot tip, Mannitol, PEG8000, water stress, In vitro, Antirrhinum magus L

ABSTRACT

The study was conducted on the multiplication of Antirrhinum magus L. plant using the micropropagation technique, represented by culture vegetative shoots on MS nutrient medium prepared with different concentrations of cytokinin BA and different levels of chemical stress treatments. The treatment of 4.44 µmol.L–1 BA with a NAA concentration of 0.54 µmol.L–1 gave the highest significant increase in the average number of vegetative shoots per plantlet, shoot length, number of leaves per plantlet, and leaf width, which amounted to 6.40 shoot explant-1, 4.00 cm, 20.60 shoot leaves -1, 0.68 cm, respectively, while a significant decrease in the average vegetative traits was observed when the concentration of BA in the medium was increased in the two treatments (8.88, 11.10) µmol.L–1 BA, which did not differ significantly between them. The results of chemical stress showed that reducing the level Mannitol to 15gm L-1 in multiplication medium led to a significant and inverse increase in the average number of vegetative shoots per plantlet, shoot length, number of leaves per shoot, and leaf width reached 4.80 shoot explant-1, 3.50 cm, 11.40 shoot explant1-, 0.44 cm in succession, and their averages decreased. By increasing the concentration in the middle. It was also observed that there was an inverse relationship between the average vegetative traits and the levels of PEG8000, where the control treatment achieved the highest significant increase in the average of all vegetative traits, amounting to 6.60 shoot explant-1, 4.06 cm, and 7.60 shoot explant-1, 0.92 cm, respectively, compared to the other averages of water stress whenever the level of PEG8000 increased. In the nutrient medium down to the level of 45g. L-1.

1. Introduction

Antirrhinum magus L. is a perennial herbaceous plant that is treated as a winter plant in temperate regions. It is known by many names such as fish mouth, dragon nose, or mouth. The English name of the plant is Snapdragon, and the scientific name is *Antirrhinum majus* L. It belongs to the genus of fish mouth from the Lamiales order and goes back to the family is scrophulariaceae (Ahmed, 2020.) The Snapdragon flower is a pink spike, and its flowers are found in a simple spikelet inflorescence in many colors and diverse shapes (Seo *et al*, 2020). The Snapdragon plant gains an important advantage when arranging flowers, and therefore the demand for it for this purpose increases as it A major harvest flower, its flowers vary between red and yellow in color. It is grown for ornamental purposes in gardens, orchards, on the edges of public roads, and in private parks (Al-Ealayawi *et al*., 2020)in plant tissue culture, the morphological growth process is also controlled by the type and concentration of growth regulators used in the nutrient medium, as well as the interaction between these regulators (Al-Bayati, 2019). Many studies have confirmed the necessity of adding cytokinins to the nutrient media for the purpose of micropropagation, where they play a role. It is important in the process of emergence and stimulating cell division in in vitro culture of plant tissues in the presence of auxin, and the process of generating shoots ex-vivo is mainly based (Ibrahim, 2017). Water stress is also known as one of the biological environmental stresses that has the ability to cause damage to biological activities. Which negatively affects the growth and development of plant productivity because environmental factors are not suitable for growth, increase or decrease, such that the amount of absorbed water that is insufficient for the plant's need to perform its vital activity is known as WaterStress (Gercek *et al*., 2017). Exposure of plant cells to some water stress compounds, such as the sugar alcohol mannitol and the compound PEG8000, involves changes in the physiological and chemical processes through the accumulation of some osmotic solvents, causing a decrease in the water stress outside the cells without causing specific toxicity. One of the easy methods to evaluate the effects of drought on growth and development is Simulating drought stress under controlled conditions in the laboratory using chemical reagents.

(Superasana, 2010; Pradhan *et al*., 2020) Hamza *et al.,* (2013) indicated that the propagation of A. majus with this technology in MS nutrient medium supplemented with BA 0.5 and 1.0 mg.L^{-1} gave the best rate of number of shoots. Vegetative, and a study (Al-Taha and Mazine, 2021) also indicated that a concentration of 1.0 mg L^{-1} BA gave the maximum number of vegetative shoots and all vegetative characteristics when growing the shoot tips of *Chrysanthemum hortorum* Hort. on the nutrient medium MS, Bennett (2011). (Al-Jubouri) indicated that there was a reversible increase in the multiplication rates of vegetative shoots of *Euphorbia tirucalli* L. plant when the concentration of mannitol in the MS nutrient medium decreased to 30 g.L^{-1}, as a study (Al-Taha and Mazeni, 2020,) indicated that A significant increase in the averages of vegetative traits of Chrysanthemum plant when treated with the control compared to the rates of other water stresses whenever the level and compound of PEG8000 in the MS nutrient media increased. One of the main aims of plant breeding is to increase production during drought (Khadka *et al*., 2020). Therefore, this study was conducted to develop a protocol for the abundant production of this commercial plant in addition to traditional methods, and then produce plants free of viral and fungal diseases and determine the optimal combination of cytokinin BA as well as Chemical stress treatments include the compound PEG8000 and the sugar alcohol Mannitol, which are added to the nutrient medium prepared for the purpose of multiplying vegetative shoots and increasing their tolerance to water stress.

2. Methodology

The study was conducted in the Plant Tissue Culture Laboratory of the College of Agriculture - University of Basra. Explants represented by shoot tips were used in this study. These explants were excised and were (1.5-1.2) cm long (Fig. 1. A), from seedlings imported from One of the nurseries in Al-Diwaniyah Governorate planted it in 15 cm diameter anvils and a 6-8 shoot container (Figure 1. B). The plant parts were washed with water and liquid soap several times to get rid of the dust stuck to them. Then they were washed with distilled water several times, then immersed in a glass container containing the pesticide. Mycelium (Elsa) at a concentration of 500 mg L^{-1}, was stirred several times for (5) minutes, then washed with distilled and sterilized water several times, and all plant tissues were preserved in glass containers containing an antioxidant solution, which consists of 150 mg L^{-1} of citric acid and 100 mg L^{-1} of ascorbic acid and kept in the refrigerator at a temperature of 4% for 24 hours until the surface sterilization process is completed, by transferring it to a Laminar flow air cabinet, pre-sterilized with 70% ethanol and chlorine diluted with distilled and sterilized water several times, after that The lower parts of the explants were cut to a length of (0.7-0.6) cm in order to get rid of the sterile material that had penetrated the plant tissues after sterilizing them with ethyl alcohol. Then they were immersed in a 40% sodium hypochloride solution (volume:volume) (prepared from a commercial minor solution containing... 1.05% active ingredient (sodium hypochloride) with the addition of (2-3) drops of Tween-20 diffuser for 30 minutes with shaking and stirring from time to time. After that, the plant parts were extracted separately and washed with distilled and sterile water several times .A nutrient medium consisting of salts (MS) (Murashige and Skoog, 1962) was used by taking a weight of 4.33 g.L^{-1} and organic materials, which are sucrose at a concentration of 30 g.L^{-1}, adenine sulphate at a concentration of 40 mg.L^{-1}, and mesioinositol 80 mg.L^{-1} and acidic sodium phosphate 170 mg.L^{-1} with the addition of some vitamins and glycin at a concentration of 1 mg.L^{-1}. Then cytokinins and auxins were added. The pH of the nutrient medium was adjusted within the range of 5.7-5.8, then Agar was added at a concentration of 6 g.L^{-1}. Heat The nutrient medium reached a temperature of 91-90°C, then it was distributed into tubes measuring (18 x 2.5) cm, with 20 ml for each tube. Then the nozzles of the tubes were plugged with cotton and closed with aluminum foil. Each treatment included ten test tubes (replicates), then they were sterilized with a device. Auto clave under a pressure of 1.04 kg.cm^2 and a temperature of 121 °C for 20 minutes. After that, the plants were incubated in the growth chamber at a temperature of 25±2 °C and under a lighting intensity of 1000 foot-candles for 16 hours of light per day.

This study included several inclusion experiments

1-The first experiment: The effect of different levels of cytokinin BA on the multiplication of shoot tips in the plant *Antirrhinum magus* L.

After the completion of the process of sterilizing the explants and completing the preparation of the nutrient medium, the shoots of the vegetative shoots were excised from Snapdragon plant, with a length ranging between 0.8 and 1.0 cm inside the stratified air flow table. They were planted in tubes containing the nutrient medium MS and the components of the medium mentioned previously, at an average of one explant for each test tube. Using 10 replicates, taking into account placing the shoot tip vertically and dipping the basal part into the MS nutrient medium supplied with different concentrations of cytokinin BA in vegetative propagation of the septa plant, 2.22, 4.44, 6.66, 8.88, 11.10 µmol.L^{-1} with the presence of 0.54µmol.L^{-1} NAA. Glass tubes were used for this experiment, with 10 replicates for each treatment, and the following measurements were taken:

1- Average number of vegetative shoots/explants.

2 -Average length of vegetative shoot (cm)

3 -Average number of leaves/explants.

4- Average leaf width (cm)

After the completion of the culture process, the plantlet were incubated in the growth room at a temperature of 25±2°C with light inside the incubator and a lighting intensity of 1000 lux. The reculture process was conducted after eight weeks for the purpose of multiplication the vegetative shoots formed at the best concentration of BA and to begin conducting subsequent experiments. The reculture process was conducted every eight weeks, as the shoots multiplication process continued for 16 weeks and was placed in the incubator at a temperature of 2±25°C.

2- The second experiment: it studied the effect of Mannitol on vegetative multiplication in Snapdragon, with vegetative shoots with a length ranging from 2.5-2 cm, the number of nodes 2 cm, and the number of leaves 4-5, homogeneous in terms of thickness, as it was added in concentrations 15.0, 30.0, 45.0 60.0 , g L^{-1}, while PEG_{8000} was added at concentrations of 45.0, 30.0, 15.0, 0.0 g L^{-1}, with the best concentration of BA and NAA at a fixed concentration of 0.54 µmol L^{-1}. In addition, sucrose was added at a fixed concentration of 30 g L^{-1}, supplied with MS salts and the above-mentioned nutrient medium components, were used in test tubes for eight weeks, then transferred to glass bottles for another eight weeks, and the same measurements were taken in the previous experiment.

Statistical analysis:

1-The study experiments were designed according to a completely randomized design (C.R.D.) and the results of the study were analyzed using analysis of variance and the means of the coefficients were compared according to the Revised Least Significant Difference test (R.L.S.D.) at the probability level of 0.01 (Al-Rawi And Khalaf Allah, 2000)

2- The ready-made statistical analysis program Genestat (2007) was used to analyze the results.

3. Result and Discussion

Effect of different concentrations of cytokinin BA on the multiplication of vegetative shoots of *Antirrhinum magus L.* eight weeks after culture.

The results of the statistical analysis in Table (1) showed that there is a significant effect of the different BA concentrations (2.22, 4.44, 6.66, 8.88, 11.10 µmol.L^{-1}) on the unfolding and multiplication of the tips of vegetative shoots in the presence of a fixed NAA concentration of 0.54 µmol.L^{-1} when cultured. Vertically in the MS nutrient medium after eight weeks of incubation in continuous light (Figure.1.C), the MS medium prepared with a concentration of 4.44 µmol.L^{-1} BA was significantly excelled in the average number of vegetative shoots, shoot length, number of leaves, and leaf width at all concentrations. BA, which reached 7.80 shoot explant^{-1}, 5.80 cm, 29.60 leaf explant^{-1}, 0.86 cm, respectively, followed by a concentration of 2.22 µmol.L^{-1}, which significantly excelled on the higher

concentrations that followed it, which were µmol.L^{-1} 6.66, 8.88, 11.10. Given the highest average in the number of vegetative shoots, shoot length, number of leaves, and leaf width for all BA concentrations, as it reached 6.40 shoot explant^{-1}, 4.00 cm, 20.60 leaf explants^{-1}, 0.68 cm, respectively, while a significant decrease was observed in the average number of vegetative shoots, length and number of shoot. Leaves and leaf width when the concentration of BA in the MS medium increased in the treatment µmol.L^{-1} 11.10, as it reached 2.20 shoot. Explant $^{-1}$, 0.54 cm, and 6.40 shoot.explant $^{-1}$, 0.24 cm, respectively, which did not differ significantly between them from the treatment µmol.L^{-1} 8.88 in the average multiplication of all vegetative traits studied. The reason for the excelled of the 4.44 µmol.L^{-1}BA treatment may be due to the role of cytokinins in stimulating chloroplast development, stimulating the division process, encouraging lateral shoots, and inhibiting apical dominance. It is also due to the occurrence of a balance between the growth regulators BA and NAA within the plant tissues (Al-Aradi *et al*., 2017). Thus, it increases the process of gene expression and nutrient transfer quickly by pushing the plant part to grow vegetative shoots in the axils of the leaves, which in turn stimulates the development of organs and the formation of more than one well-developed vegetative shoot ((Abd-Al-Husssein *et.al.*, 2010), The reason for the decrease in the average of the vegetative multiplication characteristics studied in all of them when the concentration of BA increased to µmol L^{-1} 11.10 is due to the occurrence of toxic effects that reach an inhibitory level (Al-Khafaji, 2014), in addition to the cytokinins contained in this part, which cause the accumulation of growth regulators in plant tissues when Adding them in high concentrations, which in turn affects the growth processes and sometimes causes tissue discoloration and death (Mazeni, 2020), and this was confirmed by a study (Al-Taha and Mazeni, 2016) in an experiment on the emergence and multiplication of vegetative shoots of the Gardenia plant, that low concentrations of cytokinins were the best in achieving The highest average number of shoots and their length. These results agreed with what was indicated by researchers Hamza *et. al. (*2013) and (Al-Taha and Mazine, 2021) who showed that the best concentration for multiplying vegetative shoots from the process of growing shoot tips in the prepared medium is 1.0 mg L^{-1} BA on Snapdragon and Chrysanthemum plants, respectively, and it is shown in (Fig. 1. D, E) The process of reculture vegetative shoots on the MS nutrient medium prepared with the best concentration of 4.44 µmol L^{-1} BA after 16 weeks of incubation with continuous light to multiply the vegetative shoots and divide them for the purposes of using them in experiments on multiplication chemical stress.

Table 1. Effect of different concentrations of N6-benzyladenine (BA) on the shoot multiplication from shoot tips (*Antirrhinum magus* L.) after eight weeks of culture

Treatments BA µmol·L^{-1}) (	**Shoots/ No. of explants**	**Shoot length (cm)**	**No.of leaf Branch**	**Width of the cm leaf**
2.22	**6.40**	**4.00**	**20.60**	**0.68`**
4.44	**7.80**	**5.80**	**29.60**	**0.86**
6.66	**4.00**	**2.08**	**14.80**	**0.46**
8.88	**2.80**	**1.62**	**8.60**	**0.30**
11.10	**2.20**	**0.54**	**6.40**	**0.24**
RLSD p≥ 0.01	**1.589**	**1.386**	**3.357**	**0.243**

effect of different levels of menthol on the multiplication of vegetative shoots of Snapdragon, eight weeks after the beginning of culture in the light.

The results in Figure (1) and (Figure.1.F) show that adding the sugar alcohol menthol to the multiplication medium at low concentrations led to an inversely significant increase in the average vegetative multiplication of shoot tips grown on MS nutrient medium with the presence of (BA 4.44 µmol • L^{-1}). mg L^{-1} + 0.54 µmol L^{-1} NAA) at a fixed concentration after eight weeks of incubation in

the light. The level of 15 g.L^{-1} gave a gradual positive moral effect in achieving the best results for all multiplication traits of the studied vegetative shoots of Snapdragon. This is achieved by achieving the highest average in the number of vegetative shoots, vegetative shoot length, number of leaves, and leaf width, which reached 4.80 shoot explant^{-1}, 3.50 cm, 11.40 shoot explant^{-1}, 0.44 cm, respectively, while a gradual decrease was observed in the average vegetative multiplication traits when the sugar level increased. Mennitol in MS medium to 60g. L^{-1}, which reached the average number of vegetative shoots, vegetative shoot length, number of leaves and leaf width of 1.20 shoot explant^{-1} 0.6 cm, 3.00 leaf explant^{-1} 0.12 cm respectively, and was followed in effect by the level of 45 g.L^{-1} and For those who did not differ significantly morally between them. The results also showed that the reason for the inefficiency of the menthol sugar in stimulating the multiplication of vegetative shoots and the decrease in the average number and length of the multiplying shoots of Snapdragon plant when its level in MS medium was increased is that the Snapdragon plant was exposed to the negativity of the low water potential of the nutrient medium caused by the increase in menthol sugar, which affected the occurrence of Decreased metabolic processes through the decomposition of carbohydrates and proteins (Sikuku *et al.*, (2010), which in turn caused a decrease in the plant's dry matter expressed in weight as a result of a disturbance in vital activities resulting from a lack of water absorption from the growth medium, as dry matter is a summary of the plants' vital activities. sensitive to water stress (Soleimanzadeh *et al*., 2010). The results agreed with many researchers Mohamed, *et al*., (2000) who used mannitol sugar at several concentrations and found that most of them inhibited the process of formation and multiplication of vegetative shoots in various plants, and this was confirmed by a study that recorded all from Al-Rekaby, and Merza, (2015). Increasing the level of water stress in the growing medium of the shrubby plant Adhatoda vasica (L.) Nees caused a significant decrease in the average dry weight of the shoot and root system and leaf area, and similar results were also obtained from Anber (2010) noted that there was a gradual reduction in the average stem length and number of leaves when he studied the effect of mannitol on geranium plants. The results of the current study also agreed with Al-Jubouri (2011), as he confirmed the occurrence of a significant decrease in the average number of vegetative shoots and their length when increasing the level of mannitol from 30-70 g. L^{-1} for *Euphorbia tirucalli* plantlets. The study also agreed with Munoz *et al*., (2019), as the researchers confirmed that increasing the concentration of mannitol from 20-60 g.L^{-1} led to a gradual significant effect on the average vegetative traits of potato plantlets compared to the control treatment, which It led to a decrease in the mortality rate of plants produced by the influence of mannitol to 26.6%. This decrease was explained by (Elsahooki, 2013) that the increase in water stress in the growth medium caused differences in osmotic regulation and tissue elasticity due to the increase in solute concentration, which affected the water potential gradient as a result of changes in cell size, rupture of membranes, and loss of turgor, all of which resulted about the plant's cellular water deficit.

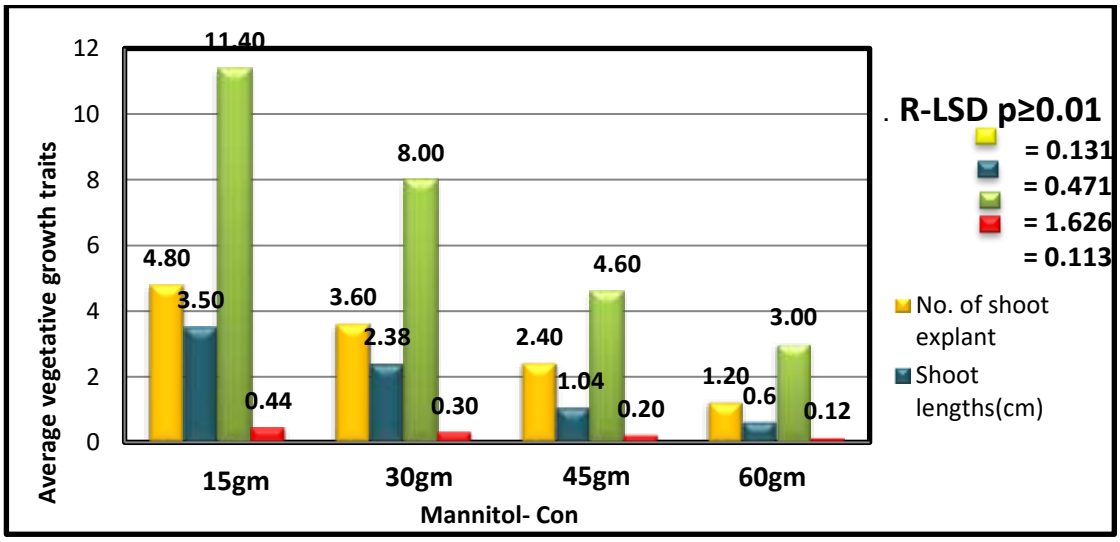

Figure (1) The effect of different levels of mannitol in the presence of (BA 4.44 µmol.L^{-1} mg L^{-1} + 0.54 µmol.L^{-1} NAA) on the multiplication of vegetative shoots of Snapdragon eight weeks after the start of culture in the light**.**

The effect of different levels of PEG8000 on the multiplication of vegetative shoots from Snapdragon, eight weeks after the start of culture in the light.

The results of the statistical analysis in Figure (2) showed at the 0.01 level that there were significant differences in the average vegetative multiplication trait of the shoot tips of the seven palanquin plants between different levels of $PEG8_{000}$ 15, 30, 45, g.L^{-1} and between the control treatment with the presence of (BA 4.44µmol.L^{-1} mg L^{-1} + 1NAA (0.54µmol .L^{-1} added to the MS nutrient medium after eight weeks of incubation in the light (Fig. 1. G), as the control treatment achieved the highest significant increase in the number of vegetative shoots, shoot length Vegetative, the number of leaves and leaf width reached 6.60 shoot explant^{-1}, 4.04 cm, 7.60 shoot explant^{-1}, 0.92 cm, respectively, in comparison with chemical stress treatments, as it was observed that there was a gradual decrease resulting from an inverse relationship between the average vegetative multiplication characteristics and the levels of PEG_{8000}, and whenever the level of $PEG8_{000}$ in the food medium increased to 45gm L^{-1} compared to their averages. Other water stresses. Upon gradual exposure to the compound PEG_{8000}, the average multiplication characteristics decreased, reaching 1.80 shoot explant^{-1}, 1.304 cm, 2.20 leaf seedlings 0.14-1 cm in succession. The reason for excelled of the control treatment at all levels of PEG_{8000} may be due to the division The cells and their expansion are a result of the state of water balance that the plant experiences without stress (Zidane and Hamza, 2014), and this was confirmed by the results of the study (Al-Taha and Mazeni, 2020,) when they studied the Chrysanthemum plant in vitro, as it caused the high level of 60.0 g.L^{-1} of PEG_{8000} low stress resulted in a reduction in the average multiplication traits compared to the control treatment, which outperformed all water stress treatments. While (Sharma *et al*., 2021) explained that the reason for the decrease in the average growth of vegetative growth traits in the presence of PEG_{8000} is that the high concentrations added to the MS nutrient medium caused osmotic stress and thus weakened the tissues' ability to absorb nutrients, causing an increase in the production of effective oxygen species (ROS). Which leads to inhibition of cell division, imbalance of nutrients, and cell shrinkage due to the decrease in water content of the cells. These results were also supported by (Cui *et al*., 2020), as they showed that adding high levels of the compound PEG to the MS nutrient medium negatively affected the vegetative growth of plants. Compared to low levels of it, which in turn caused decreased growth, damage to the cell membrane, and cell death as a result of the accumulation of radicals in the growth medium that led to protein decomposition and the incorporation of amino acids. Due to these oxidizing compounds, the protective mechanisms were unable to repair the damage depending on the type of tissue. (Dvořák *et al.,* 2021) This was confirmed by similar studies by researchers (Akbar-pour, 2017) that the high level of 7.0 gm L-1 PEG_{8000} led to a reduction in the growth of almond plants, and the results of (Anber, 2010) showed a decrease in the average vegetative characteristics of the cultivated geranium plants. In the laboratory, when increasing water stress using PEG, the same results were reached by researchers (Mengesha *et al.*, 2016) when growing aloe vera plants under the influence of water stress using PEG_{6000}. This causes stunted growth in the average shoots' multiplication and length when increasing its level from 0-40g.L^{-1}. The study also agreed with the results of an approach reported by (Alebidi *et al.,* 2024) when they studied reducing osmotic stress with the effect of different levels ranging from 2-10% (PEG) when growing four grape varieties on MS medium. The results of drought stress showed a negative effect. Gradually, it led to a decrease in the number of shoots and the plantlet survival rate.

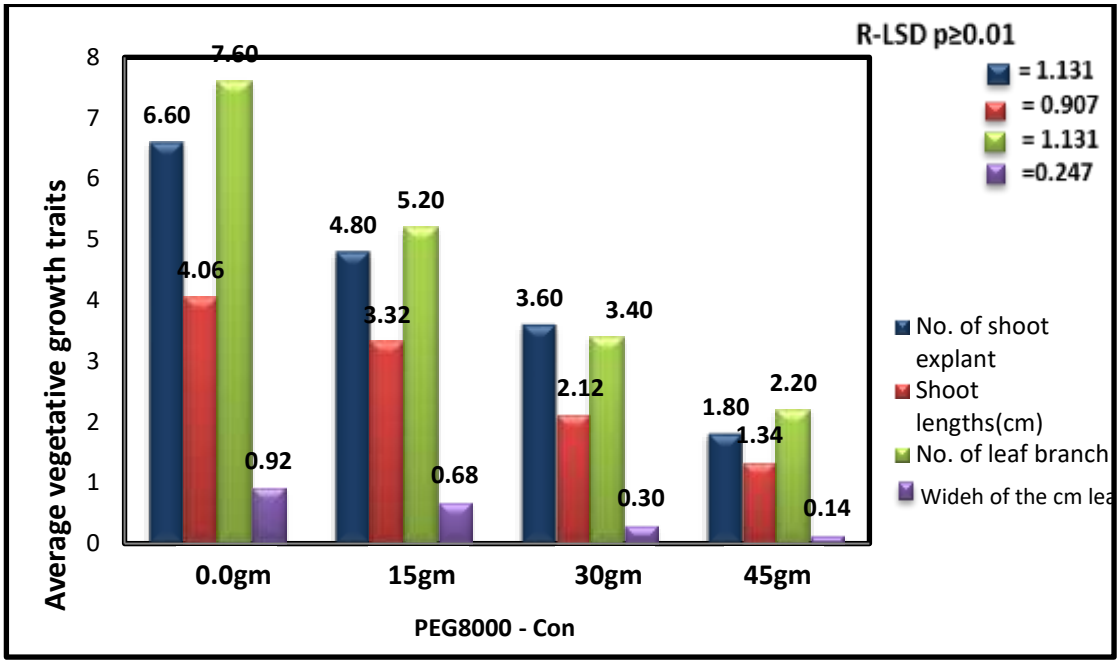

Figure (2) The effect of different levels of PEG8000 in the presence of (BA 4.44 µmol.L^{-1} mg L^{-1} + 0.54 µmol.L^{-1} NAA) on the multiplication of vegetative shoots of Snapdragon eight weeks after the start of culture in the light**.**

4. Conclusion and future scope

The possibility of determining the optimal combination of growth regulators (BA 4.44 µmol.L^{-1} mg L^{-1} + 0.54 µmol.L^{-1} NAA) and adding it to media that multiply chemical stress using a protocol that includes culture shoots, and the possibility of increasing the viability of Snapdragon plant In vivo environmental conditions, including the drought factor, by increasing the osmotic potential and regulation within plant tissues using the water stress compounds mannitol and PEG_{8000}. High levels of mannitol and PEG_{8000} also showed a significant negative decrease in the average of all vegetative growth characteristics of Snapdragon plant after eight weeks. In addition to that Selection of plantlets growing in high levels of mannitol and PEG_{8000} ex vivo may help improve the drought tolerance of this plant.

- Figure 1. A –*In vitro* Micropropagation of big-sage (*Antirrhinum magus* L.) mother plant

B –Shoots tips about 0.8-1.0 cm length explants

C –Shoot multiplication on MS medium supplemented with different concentrations of BA

D –Multiplication shoots on MS medium supplemented with 1.0 mg L^{-1} and 0.1 mg L^{-1} NAA

.

E –Mass on multipling shoots on apetri dish resulting from MS medium supplemented with 1.0 mg L^{-1} and 0.1 mg L^{-1} NAA after 16 weeks.

F –Shoot multiplication on MS medium supplemented with different concentrations Mannitol

G–Shoot multiplication on MS medium supplemented with different concentrations of PEG_{8000}

Reference

[1] Abdul Hussein, M. A. A; Aqeel K. H. Al-Tufaili and Siham, A. R.Al (2010). Effect of growth regulators The nutritional medium for micropropagation of bougainvillea climber. Bougainvillea glabra Kufa Journal of Agricultural Sciences, Vol (1)2: 180-100 .

[2] Ahmed, H.N. (2020). Effect of BA on initiation and multiplication of Atropa belladonna L. shoot tips produced in vitro and content protein. Int. J. Agricult. Stat. Sci., 16(Supplement 1), 1137-1141.

[3] Akbarpour, E., Imani, A., Ferdowskhah Yeganeh, S. (2017). Physiological and morphological responses of almond cultivars under in vitro drought stress. Journal of Nuts, 8(1). https://doi.org/10.22034/ jon.2017.530393.

[4] Al-Aradi, H. J. A; Ansam M. S; Khayoun A. M. and Jihad M. M. A. (2017). Propagation of water berbenine plants. Bacopa monnieri ex vivo Iraqi Journal of Aquaculture, Vol14(1)1-12.

[5] AL-Bayati, H.J.M. (2019). Growth and yield of Cauliflower as affected by boron and fertilizer type. Int. J. Agricult. Stat. Sci., 15(2), 595-599

[6] Al-Ealayawi, Z.A., K.W. Ibade and R.M.H. AL-Dulaimy (2020). The effect of spray dates with sitmplex seaweed extract and koffex and miteclean acaricides on some traits of date palm, Phoenix dactylifera L. Int. J. Agricult. Stat. Sci., 16(1), 211-218.

[7] Alebidi, A. , Adel M. A.*, Hosny F. A. A. , Gamal A. A. , Ibrahim A. E. , Abd El-wahed N. A., Mohammed H. F. , Ashraf E. H. (2024). In Vitro Drought Tolerance of Some Grape Rootstocks . Journal of Ecological Engineering 25(4), 184–202. https://doi.org/10.12911/22998993/184183.

[8] Al-Jubouri, S. A. S. (2011). The effect of different types and concentrations of sugars and benzyl adenine (BA) on the opening of vegetative branches of Euphorbia tirucalli L., tissue-grown ex vivo. University magazine Karbala Scientific Vol 9 (3) 138-143.

[9] Al-Khafaji, M. A. (2014). Plant Growth Regulators, Application And Utilizations In Horticulture. Bookstore For Printing, Publishing And Translating, University Of Baghdad. P: 132-138.

[10] Al-Rawi, K.M. And Khalaf Allah, A.M. (2000). Design And Analysis Of Agricultural Experiments. Dar Alkuteb For Press And Publishing, Mosul University, Iraq, 488 P. [In Arabic].

[11] Al-Rekaby, L. S. And Merza, T. K. (2015). Effect Of Water Stress And Organic Fertilizer (Azomin) On Some Growth Parameters Of Adhatoda Vasica L. (Nees) . Kufa, Journal Of Agricultural Sciences(7):2.

[12] Al-Taha, H.A. And -Mazine, H.M. (2020). Impact Of Osmotic Stress PEG And Sucrose On Callus Growth And Adventitious Micro Shoots Regeneration Of (Chrysanthemum Hortorum Hort. Cv. Dwarf) Via In Vitro Condition Plant Archives Vol. 20, Supplement 2. Pp 347-352.

[13] Al-Taha, H.A. And -Mazine, H.M. (2021). Rapid Micro Propagation Of (Chrysanthemum Hortorum Hort.) Cv. Dwarf White Dutch Through Shoot Tips And Nodal Segment As Explants. Indian Journal Of Ecology48 Special Issue (17): 169-173.

[14] Al-Taha, H.A. And -Mazine, H.M.(2016). Effect Of Source Of Explants And TDZ, IBA On Regeneration Plantlets Of Gardenia Jasminoides Ellis. Cv. Raicans Via Tissue Culture. Basrah Journal Of Agricultural Sciences Vol. 29(1), 239-256.

[15] Anber, M .(2010). Establishment of efficient in vitro method for drought tolerance evaluation in pelargonium. J. of Hort. Sci. and Ornam.plants ,.2(1):08-15.

[16] Cui, K., Shu, C., Zhao, H., Fan, X., Cao, J., Jiang, W. (2020). Preharvest chitosan oligochitosan and salicylic acid treatments enhance phenol me- tabolism and maintain the postharvest quality of apricots (Prunus armeniaca L.). Scientia Horti- culturae, 267, 109334. https://doi.org/10.1016/j.

[17] Dvořák, P., Krasylenko, Y., Zeiner, A., Šamaj, J., Takáč, T. (2021). Signaling toward reactive oxygen species-scavenging enzymes in plants. Frontiers in Plant Science, 11, 618835. https://doi.org/10.3389/ fpls.2020.618835

[18] Elsahookie, M.M . (2013) . Breeding Crops for Abiotic Stress-A Molecular and Epigenetic Approach, Coll of Agric., Univ. of Baghdad, pp.244.

[19] Gercek, C.; Kourtiche, D.; Nadi, M.; Magne, I.; Schmitt, P. A. And Souques, M. (2017). Computation Of Pacemakers Immunity To 50 Hz Electric Field: Induced Voltages 10 Times Greater In Unipolar Than In Bipolar Detection Mode. Bioengineering [Online]. DOI: 10.3390/Bioengineering4010019. ISSN 2306-5354.

[20] Hamza, A. M; Abd El-Kafie, O. M.; Helaly, A. A. And M. S. EL-Mongy.(2013). IN Vitro Propagation Methodes Of Snapdragon (Antirrhinum Majus.L) Plant. J. Plant Production, Mansoura Univ., Vol. 4 (11): 1621 – 1637.

[21] Ibrahim . K. M. (2017). Applications in plant biotechnology. Part One. Ministry of Higher Education and Scientific Research. Al-Nahrain University. Baghdad, Iraq.

[22] Khadka, K., Earl, H.J., Raizada, M.N., Navabi, A. (2020). A physio-morphological trait-based approach for breeding drought tolerant wheat. Frontiers in Plant Science, 11, 715. https://doi. org/10.3389/fpls.2020.00715.

[23] Mazeni, L. H.M.A. (2020). Propagation Of The Chrysanthemum Plant (Chrysanthemum Hortorum Hort.) Via Tissue Culture Technique And The Effect Of Some Water Stress Compounds. Doctoral Thesis, Plant Tissue Culture, The

College Of Agriculture, University Of Basrah, Basrah, Iraq. P: 90-127.

[24] Mengesha, B.; Mekbib, F. and Abraha, E. (2016). In vitro Screeningof Cactus [Opuntia ficus-indicia (L.) Mill] Genotypes for Drought Tolerance. American J. Pl. Sci., 7: 1741-1758.

[25] Mohamed , M.A.H.;Harris , P.J.C. And Henderson , J. (2000). In Vitro Selection And Characterisation Of A Drought Tolerant Clone Of Tagetes Minuta .Plant Sci ., 159(2) ; 213_ 222.

[26] Muñoz, M.; Díaz, O.; Reinún, W.; Winkler, A. and Quevedo R. (2019). Slow growth In vitro culture for conservation of Chilotanum potato germplasm. Chilean, J. Agri.cult.uRes. 79(1) : 26-35.

[27] Murashige, T. And Skoog, F. (1962). A Revised Medium For Rapid Growth And Bioassays With Tobacco Tissue Cultures. Physiol. Plant, 15: 473-492.

[28] Pradhan, N., Singh, P., Dwivedi, P., Pandey, D. K. (2020). Evaluation of sodium nitroprusside and putrescine on polyethylene glycol induced drought stress in Stevia rebaudiana Bertoni under in vitro con- dition. Industrial Crops and Products, 154, 112754. https://doi.org/10.1016/j.indcrop.2020.112754

[29] Seo, J., Lee, J., Yang, H. Y. and Ju, J. (2020). Antirrhinum majus L. flower extract inhibits cell growth and metastatic properties in human colon and lung cancer cell lines. Food Science & Nutrition. 8(11): 6259–6268. https:// doi.org/10.1002/fsn3.1924

[30] Sharma, K., Gupta, S., Thokchom, S. D., Jangir, P., Kapoor, R. (2021). Arbuscular mycorrhiza-mediat- ed regulation of polyamines and aquaporins during abiotic stress: Deep insights on the recondite play- ers. Frontiers in Plant Science, 12, 642101. https:// doi.org/10.3389/fpls.2021.642101

[31] Sikuku P. A.; Netondo G. W.; Onyango J. C. and Musyimi D. M. (2010). Effect of water deficient on physiological and morphological of three varieties of rainfed rice Oryza sativa L. Journal of Agricultural and Biological Science, 5(1): 1990- 6145 .

[32] Soleimanzadeh , H.; Habibi, D.; Ardakani, M.R.; Paknejad, F. and Rejali, F. (2010). Response of sunflower (Helianthus annuus L.) to drought stress under different potassium levels.

[33] Suprasanna, P. (2010). Biotechnological Interventions In Sugarcane Improvements Strategies Methods And Progress.Protoplasma, 248.

[34] Zidane, M. M. and Ibrahim, A. H.(2014). Effect of drought on microtuber production and peroxidase activity Ex Vivo, Iraqi Agricultural Sciences Journal, Vol 45(2):143-150.