The efficiency of interaction between cytokines and Auxins in Micropropagation of Chrysanthemum plant (Chrysanthemum indicum L.)

Abdulwadood S.M. Alsoufi¹, Ziyad Shihab Ahmed² and Aysar M. Salim³

¹Department of Biology, College of Science, University of Tikrit, Iraq
²Department of Plant Protection College of Agriculture, University of Tikrit, Iraq
³Department of Horticulture and Landscape Planning College of Agriculture and forestry, University of Mosul, Iraq

Email: zayidsh@tu.edu.iq

Abstract

This study was conducted in the Tissue Culture Laboratory at the College of Science / Tikrit University, with the aim of micropropagation of the Chrysanthemum plant and determining the optimal concentration of the growth regulators used. In this study, a single node cutting from the shoots of Chrysanthemum indicum L. was used and the growth regulator (kinetin) Kin with concentrations (0.0-4.0) mg.L⁻¹ time alone and again with the interaction (Indol-3-butyric acid) IBA at concentrations (0.0-0.6) mg.L⁻¹. The results showed that the interaction between the concentrations of Kin and IBA had a higher significant effect than the use of Kin alone. Where the average of vegetative growth of Chrysanthemum plant increased as the average number of shoots, shoot length and number of leaves amounted to 7.2 shoot.plant⁻¹, 5.27 cm and 17.0 leaf.plant⁻¹ when added 4 mg.L⁻¹ of Kin with 0.6 mg.L⁻¹ of IBA to Murashige and skoog medium (MS) after being 3.3 shoot.explant⁻¹, 3.81 cm and 5.3 leaf.shoot⁻¹ respectively when 4 mg L⁻¹ of Kin has added alone to MS medium. As for the rooting experiments, in which the average MS was used with the full strength of the salts and half the strength of the salts containing different concentrations of IBA growth regulator (0.0-2.0) mg.L⁻¹ for rooting the shoots resulting from the multiplication experiments. The MS medium half the salt strength excelled and giving the highest average of rooting percentage, average number of roots and average root length of 90%, 12.5 root.explant⁻¹ and 3.5 cm respectively when adding 1 mg.L⁻¹ IBA to the medium.

Keywords: Chrysanthemum indicum L., cytokines and Auxins, kinetin, Indol-3-butyric acid.

1. Introduction

Chrysanthemum plant (Chrysanthemum indicum L.) is a perennial herbaceous plant, a short-day plant that Its cultivation is renewed annually. It belongs to the Asteraceae family [1], and is produced as both cutting flowers and flowering pot plants. [2] Chrysanthemum plant is a special breeding plant that can be propagated by traditional methods, as well as plant tissue and organ culture methods are also used to propagate it. This does have advantages such as producing large numbers of plants throughout the year in a short time and effort [3]. In addition to avoiding the problem of sexual incompatibility which hinders the internal breeding of Chrysanthemum plants (some cultivars), and thus producing plants similar to the parents [4] [5] observed that the cultivation of the single nodes of the Chrysanthemum plant on [6] MS medium equipped with six concentrations of BA, Kin, and IAA each separately. That the nutrient medium prepared with a concentration of 1.0 mg.L⁻¹ of BA gave the best averages in all the studied (percentage of response to branch formation, number of shoots and heights) after four weeks of culture, [7] he was also recommended to add NAA auxin at a concentration of 0.5-1.0 mg.L⁻¹, interaction with BA or Kin at a concentration of 1.0-4.0 mg.L⁻¹, for the culture of single nodes and branch of Chrysanthemum plant (Ch. Palndosum) in vitro on MS medium, [8] concluded that adding 0.5 mg.L⁻¹ of NAA with 2.0 mg.L⁻¹ of BA to MS medium gave the best results as a percentage of the response with 86.6%, the average number of shoots with 3.2 shoot.explant⁻¹ and the average height of the shoots is 3.7 cm from single node culture of Chrysanthemum plant. The study aim: To Micropropagation of Chrysanthemum indicum L. and this is
done by using single nodes and culturing them on MS medium equipped with the appropriate combination of plant growth regulators (Auxins, Cytokinins).

2. Materials and methods

This study was conducted in the Tissue Culture Laboratory in the College of Science / Tikrit University.

2.1. The Cultures initiation stage.

Preparing explant: In this study, use stem cuttings containing 2-3 nodes of *Chrysanthemum indicum* L. Where these shoots were transferred to the laboratory and left under running water for 20-30 minutes, followed by washing with water and liquid soap to get rid of dust and materials attached to them. Then it was placed in a glass beaker containing an antioxidant solution consisting of citric acid 150 mg.L\(^{-1}\) and ascorbic acid 100 mg.L\(^{-1}\) for 24 hours to prevent browning of the explant.

Sterilization: The sterilization process was conducted in all different stages of work, both with regard to the explant taken in addition to the medium, as well as the sterilization of work tools.

a. Surface sterilization of explant:

After removing explant from the anti-oxidant solution, the leaves were removed and the individual nodes 1 cm long were taken from them, and it was placed in a 200ml glass beaker and sodium hypochlorate solution was added to it at a concentration of 2.4% (active substance) with 3-2 drops of the diffuser and left for 6-5 minutes with continuous stirring, where this process was conducted inside a pre-sterilized Laminar air floor.

B. Preparing the culture medium:

The prepared culture medium consisting of salts [6] (MS) was used in propagation stages and vitamins were added to it. Sucrose was added to it at an average of 30 g.L\(^{-1}\), then Myo-inositol at a concentration of 100 mg.L\(^{-1}\). Plant growth regulators, such as Auxin and Cytokinins, were added to the medium according to the required concentrations and the study aim and after the pH was set at (5.70), This was followed by adding the Agar by 7 g.L\(^{-1}\), and For the purpose of ensuring the dissolving of Agare and mixing the components of the medium well, the culture medium was placed on the Hot Plat Magnetic Stirrer device, after which the culture medium was distributed over the culture bottles and was sterilized by the Auto Clave device for 15 minutes.

2.2. growth regulators used in the stage of initiation and multiplication

The KIN growth regulator was used with concentrations (0.0, 0.5, 1.0, 2.0, 3.0, 4.0) mg.L\(^{-1}\) alone, after which the best concentration of the KIN growth regulator was determined in order to adding it to the MS medium containing different concentrations of IBA (0.0, 0.2, 0.4, 0.6 mg) L\(^{-1}\). The following were measured for each experiment separately: average number of shoots formed, average length of shoots formed and average number of leaves.

2.3. The Rooting Stage

Different concentrations of IBA growth regulator(0.0, 0.5, 1.0, 1.5, 2.0) mg.L\(^{-1}\) were added to the MS culture medium with full strength of salts and half the strength of salts and the following were measured: the percentage of rooting, the average number of roots and the average length Root.

2.4. Statistical Analysis:

The experiments were conducted using Complete Randomized Design and with two factor experiments. The results were analyzed and the arithmetic averages were compared according to the least significant difference test (L.S.D.) at a probability level of 0.05 [9].
3. Results and discussion:–

3.1. Effect of KIN concentrations (mg.L⁻¹) on the Multiplication of C. indicum L. Eight weeks after culture

The results in Table (1) showed that the concentrations of KIN used had a significant effect on the average of the number of shoots formed on the individual nodes of Chrysanthemum plant. If the average of this trait increased to reach 3.3 shoots.explant⁻¹ when using 4 mg.L⁻¹ of KIN, compared to the control treatment that gave 1 shoots.explant⁻¹. Also, we find that the average shoots length and average number of leaves increased significantly by increasing the concentrations of KIN used to reach 3.81 cm and 5.3 leaf.explant⁻¹, respectively, when using KIN at a concentration of 4 mg.L⁻¹, compared to the control treatment, in which the average number of shoots, shoots length and the average number of leaves decreased to reach 1.00 shoots explant⁻¹, 1.74 cm and 2.8 leaves.shoot⁻¹. The reason for this may be due to the fact that KIN is one of the cytokinins that one of its physiological effects is to eliminate apical dominance and stimulate the growth of lateral shoots and the formation of vegetative shoots[10]. It also works to reveal and widen the vessels carrying both the xylem and the phloem, prevent the degradation of chlorophyll, stimulate cell division, and increase the production of nucleic acids [13] [12] [11].

Table 1. Effect of KIN concentrations (mg.L⁻¹) on the multiplication of Chrysanthemum plant (Ch. indicum L.) Eight weeks after culture.

| Type Growth regulators | Conc. KIN Mg L⁻¹ | The average number of shoots explant⁻¹ | Average shoot length (cm) | The average number of leaves.shoot⁻¹ |
|------------------------|------------------|----------------------------------------|---------------------------|-----------------------------------|
|                        | 0.0              | 1.0                                    | 1.74                      | 2.8                               |
|                        | 0.5              | 1.2                                    | 2.00                      | 3.1                               |
|                        | 1.0              | 1.5                                    | 2.04                      | 3.8                               |
|                        | 2.0              | 2.1                                    | 2.98                      | 4.2                               |
|                        | 3.0              | 2.7                                    | 3.31                      | 4.6                               |
|                        | 4.0              | 3.3                                    | 3.81                      | 5.3                               |
|                        | L.S.D 0.05       | 0.68                                   | 0.49                      | 1.16                              |

3.2. The effect of the interaction between the concentrations of KIN and IBA (mg.L⁻¹) on the mean number of shoots (shoots.explant⁻¹) of the Chrysanthemum plant Ch. indicum L. Eight weeks after culture

Table (2) showed that the interaction between Kin and IBA had a significant effect on the average number of shoots formed on the individual nodes of Chrysanthemum plant. The average of this trait increased to reach 7.2 shoots.explant⁻¹ when using 4 mg.L⁻¹ Kin adding to it 0.6 mg.L⁻¹ IBA, which in turn excelled on the rest of the other interaction treatments. By referring to the results of the same table, we find that the average number of shoots. It increased by increasing the concentrations of IBA added to the medium to reach 4.04 shoot.explant⁻¹ when treating 0.6 mg.L⁻¹ compared to 0.2 mg.L⁻¹ treatment, in which the average number of shoots was 2.56 shoot.explant⁻¹. The results of the interaction show the importance of Auxins and Cytokinins and their role in cell division and the emergence of shoots on the cultured explant [14].

Table 2. The effect of the interaction between the concentration of KIN and IBA (mg.L⁻¹) on the average number of shoots (shoot.explant⁻¹) of Chrysanthemum plant (Ch. indicum L.) Eight weeks after culture.

| Conc. KIN Mg L⁻¹ | Conc. IBA Mg L⁻¹ |
|------------------|------------------|
| 0.2              | 0.4              | 0.6              |
| 0.5              | 1.6              | 1.8              | 2.0              |
| 1.0              | 1.8              | 2.2              | 2.6              |
| 2.0              | 2.3              | 2.5              | 3.3              |
| 3.0              | 2.9              | 3.8              | 5.1              |
| 4.0              | 4.2              | 6.3              | 7.2              |
| L.S.D 0.05       | 0.77             |
| average           | 2.56             | 3.32             | 4.04             |
| L.S.D 0.05       | 0.34             |
3.3. The effect of the interaction between the concentrations of KIN and IBA (mg.L⁻¹) on the average of shoot length (cm) of Chrysanthemum plant _Ch. indicum_ L. Eight weeks after culture

Table (3) shows that the effect of the interaction between the Kin and IBA on the average shoot length of the Chrysanthemum plant. Where the table shows that the highest average shoot length of 5.27 cm was obtained from the treatment of 4 mg.L⁻¹ Kin adding to 0.6 mg.L⁻¹ IBA, which in turn excelled on the rest of the interaction treatments. As for the effect of the concentrations of IBA adding to the MS medium on the average trait of shoot length, we find the concentration 0.6 mg.L⁻¹ was significantly excelled on the other concentrations (0.4, 0.2 mg.L⁻¹). Where it gave the highest average amounted to 3.19 cm, the positive effect of Kin and IBA in increasing the number of the shoot and their lengths to their role in impeding protein and chlorophyll catabolism as well as stimulating photosynthesis enzymes, whose effects are reflected in the increase in cell size and encourage the process of division and formal differentiation, especially when the ideal state of balance between what was added to the culture medium with what is found in the explant [15].

Table 3. The effect of the interaction between the concentration of KIN and IBA (mg.L⁻¹) on the average shoots length (cm) of Chrysanthemum plant (_Ch. indicum_ L.) Eight weeks after culture.

| Conc. KIN Mg L⁻¹ | Conc. IBA Mg L⁻¹ | L.S.D 0.05 |
|-----------------|-----------------|------------|
| 0.5             | 0.2             | 0.18       |
| 1.0             | 0.4             | 0.39       |
| 2.0             | 0.6             | 2.08       |
| 3.0             | 2.09            | 2.16       |
| 4.0             | 2.16            | 2.58       |
| 0.5             | 2.23            | 2.34       |
| 3.0             | 3.16            | 3.38       |
| 4.0             | 3.38            | 3.52       |

3.4. The effect of the interaction between KIN and IBA concentrations (mg.L⁻¹) on an average number of leaves (leaf.plant⁻¹) of Chrysanthemum L. Eight weeks after culture

The results of the statistical analysis in Table (4) indicate the presence of significant differences in the average trait the number of leaves formed on the shoot of the Chrysanthemum plant according to the different concentrations used for each of Kin and IBA and the interaction between them. This trait was increased to reach 17.0 leaf.plant⁻¹ when culture single nodes of Chrysanthemum plant on MS medium prepared with 4 mg L⁻¹ Kin with 0.6 mg.L⁻¹ IBA. While this trait decreased to reach 3.5 leaf.plant⁻¹ when treated 0.5 mg.L⁻¹ Kin with 0.2 mg.L⁻¹ IBA. We also find that the average number of leaves increased significantly by increasing the concentrations of IBA, where treatment of 0.6 mg liter⁻¹ gave the highest average number of leaves 11.04 leaf.plant⁻¹ excelled on the rest treatments, which reached 5.70 and 9.44 leaf.plant⁻¹. The reason for this may be that these treatments were the length of its shoots was longer than the remaining treatments, so it would have more leaves.

Table 4. The effect of the interaction between the concentration of KIN and IBA (mg.L⁻¹) on the average number of leaves (leaf.plant⁻¹) of Chrysanthemum plant (_Ch. indicum_ L.) Eight weeks after culture.

| Conc. KIN Mg L⁻¹ | Conc. IBA Mg L⁻¹ | L.S.D 0.05 |
|-----------------|-----------------|------------|
| 0.5             | 0.2             | 1.75       |
| 1.0             | 0.4             | 5.0        |
| 2.0             | 0.6             | 6.3        |
| 3.0             | 7.7             | 8.6        |
| 4.0             | 10.7            | 12.9       |
| 0.5             | 8.8             | 13.9       |
| 3.0             | 10.7            | 12.9       |
| 4.0             | 13.9            | 17.0       |
| 0.5             | 17.0            | 21.0       |
| 3.0             | 9.44            | 11.04      |
| 4.0             | 11.04           | 14.06      |
3.5. **Effect of culture medium type and IBA concentrations (mg.L⁻¹) on the rooting of Ch. indicum L. Eight weeks after culture.**

From Table (5), we note the presence of significant differences in the rooting of all the shoots of the Chrysanthemum plant according to the type of nutrient medium and the IBA concentrations used. MS medium excelled half the salt strength in giving the highest average of rooting percentage, average number of roots and average root length by 68%, 7.94 root.explant⁻¹ and 2.69 cm compared to MS medium with full salt strength, which gave the lowest rooting average. The average number of roots and average root length of 52%, 5.74 root.explant⁻¹ and 2.14 cm. Through the results of the same table, we find that the concentrations of IBA added to MS medium of half the strength of the salts significantly excelled most of the concentrations of IBA added to MS medium of the full strength of salts in giving the highest average of rooting. The average of rooting percentage, the average number of roots and average root length increased to reach 90%, 12.5 root.explant⁻¹ and 3.5 cm when 1 mg.L⁻¹ of IBA was added to MS medium of half the strength of salts. While most of these decreased significantly when adding 2.0, 1.5, 0.5, 0.0 mg.L⁻¹ of IBA to MS medium of full strength salts, The reason for this may be on the basis that IBA is one of the Auxins that encourage cell division and elongation, as well as its role in stimulating the formation of roots and the addition of growth regulators leads to an increase in the averages of the number of roots and their lengths to reach the optimal concentration and that increasing the concentration of growth regulators leads to adverse effects and that the treatments half the concentration of salts was excelled, perhaps the reason is due to the increase in the percentage of carbohydrates to nitrogen, It is known that increasing the percentage of sugars to nitrogen improves rooting [16].

### Table 5. The effect of the interaction between the concentration of KIN and IBA (mg.L⁻¹) on the rooting of Chrysanthemum plant (Ch. indicum L.) Eight weeks after culture.

| Culture medium type | Conc. IBA Mg L⁻¹ | Rooting percentage% | Average | Average number of roots (root.explant⁻¹) | Average | Average root length (cm) | Average |
|---------------------|------------------|---------------------|---------|-----------------------------------------|---------|-------------------------|---------|
| MS                  | 0.0              | 20                  | 0.5     | 1.1                                     |         |                         |         |
|                     | 0.5              | 50                  | 5.7     | 2.04                                    |         |                         |         |
|                     | 1.0              | 80                  | 9.8     | 3.22                                    |         |                         |         |
|                     | 1.5              | 60                  | 7.7     | 5.74                                    | 2.18    | 2.14                    |         |
|                     | 2.0              | 50                  | 5.0     | 2.15                                    |         |                         |         |
|                     | 0.0              | 40                  | 2.1     | 1.85                                    |         |                         |         |
|                     | 0.5              | 70                  | 8.9     | 2.67                                    |         |                         |         |
| 1/2MS               | 1.0              | 90                  | 12.5    | 3.50                                    |         |                         |         |
|                     | 1.5              | 80                  | 9.8     | 7.94                                    | 2.92    | 2.69                    |         |
|                     | 2.0              | 60                  | 6.4     | 2.51                                    |         |                         |         |
| L.S.D 0.05          | 26.5             | 12                  | 3.19    | 1.43                                    | 1.21    | 0.54                    |         |

### References

[1] Song, A., Zhu, X., Chen, F., Gao, H., Jiang, J., and Chen, S. A. 2014. Chrysanthemum heat shock protein confers to lerance to abiotic stress. Int. J. Mol.Sci. (15):5063-5078.

[2] Van Der Ploeg, A., and Heuvelink, E. 2006. The influence of temperature on growth and development of Chrysanthemum cultivars: a review. J. Hort. Sci. Biotechnol., 81(2): 174-182.

[3] Smith, R. H. 2013. Plant Tissue Culture: Techniques and Experiments. Academic Press, pp:188.

[4] AA, S., AJ, S., BA, A., & EF, S. (2019). Optical Properties of Polyvinyl Alcohol Membrane with n-HAp for Bio-Medical Applications. La Prensa Medica Argentina, 105(6). doi: 10.47275/0032-7455x-163.

[5] Yesmin, S., Hashem, A., Das, K. C., Hasan, M. M., and Islam, M. S. 2014. Efficient in vitro regeneration of Chrysanthemum (Chrysanthemum morifolium Ramat.)through nodal explant culture. Nuclear Sci. and Appli., 23(1 and 2): 47-50.

[6] Murashige, T., and skoog, f. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3): 473-497.
[7] Asija, N. 2014. In Vitro cloning of Chrysanthemum paludosum an important ornamental plant. (masters dissertation). Department of Biotechnology. University of Thapar. India.

[8] Nalini, R., Anjana, M. J., Arathi, C. S., Aswathy, M., Ayana, B., and Bhuvaneswari, R. 2016. Effect of growth regulators on Micropropagation of Chrysanthemum (Dendranthema grandiflora Ramat.). Scru. I. R. J. of Agric., 3(4):7-9.

[9] Al-Sahuki, M., and Wahib, K. A. 1990. Applications in design and analysis of experiments. Ministry of Higher Education and Scientific Research. Iraq.

[10] Wasfi, E. E. H. 1995. Growth and flower regulators and their use in agriculture. academy. Cairo, Egypt.

[11] Ahmed H. A. Al-Jobouri. (2020). Studying Some The Functional Properties of Tamarind Tamarindus indica L. Mucilage. Al-Qadisiyah Journal For Agriculture Sciences, 10(2), 304-307.

[12] Haberer, G., and Kieber, J. J. 2002. Cytokinins. New Insights Into a Classic Phytohormone. Plant Physiology. 128: 354-362.

[13] Schmülling, T. 2004. Cytokinins In Encyclopedia of Biological Chemistry. Academic Press/Elsevier Science.

[14] Skoog, F. and Miller, C. O. 1957. Chemical regulation of growth and organ formation in plant tissue culture in vitro. Symp. Soc. Exp. Biol. 11: 118 – 148.

[15] Al-Bayati, Y. A. 2002. A comparative study of the behavior of Chrysanthemum morfolium Var. MoonLight Spoon Textile and Conventional Vegetable Cultivation. PhD thesis, College of Agriculture and Forestry / University of Mosul - Iraq.

[16] Hartmann, H. T., Kester, D. E., Davies, F. T., and Geneve, R. L. 2002. Plant Propagation Principles and Practices. 7th. ed. Perntice Hall. Inc : New Jersey. USA.