SINAI Journal of Applied Sciences 13 (5) 2024 547-554

Available online at www.sinjas.journals.ekb.eg

SCREENED BY SINAI Journal of Applied Sciences

Print ISSN 2314-6079
Online ISSN 2682-3527

OPTIMIZING STERILIZATION AND CYTOKININS STRATEGIES FOR ENHANCEING *In vitro* PROPAGATION OF TURMERIC (*Curcuma longa*)

Sara I.H. Foda*; H.M.S. Hassan; M.A.M. Ali and Sonia A. Shehata

Plant Prod. Dept., Fac. Environ. Agric. Sci., Arish Univ., Egypt

ARTICLE INFO

Article history:
Received: 15/08/2024
Revised: 23/09/2024
Accepted: 14/10/2024

Keywords:
Turmeric (*Curcuma longa*),
Tissue culture,
Sterilization, Cytokinins,
TDZ (Thidiazuron),
In vitro propagation.

ABSTRACT

This research investigated optimization of sterilization techniques and cytokinin concentrations to improve the *in vitro* propagation of turmeric (*Curcuma longa*). Given the limitations of conventional cultivation methods, such as susceptibility to diseases, slow growth and more area requirements, tissue culture techniques provide a more efficient alternative for controlling propagation. The study conducted in the Tissue Culture Laboratory' Faculty of Environmental Agricultural Sciences, Arish University, Egypt. During the initial establishment phase, rhizome buds were sterilized using commercial Clorox at concentrations of 10%, 15%, and 20% for varying periods (5, 10 and 15 min.). The most successful sterilization approach was achieved with 20% Clorox applied for 10 minutes, resulting in a 90% survival rate and minimal contamination. For shoot multiplication, different cytokinins such as thidiazuron (TDZ), benzyl adenine benzyl adenine (BA), and Kinetin were evaluated separately at different concentrations. The best shoot proliferation was observed with 1.0 mg l^{-1} TDZ. In the rooting phase, growth media fortified with Naphthalene Acetic Acid (NAA) at concentrations ranging from 0.5 to 2.0 mg l^{-1} significantly influenced the root growth' Moreover, rooting medium supplemented with 1.5 mg l^{-1} NAA produced the longest roots. For acclimatization, a mixture of peat moss, vermiculite, and washed sand in a 1:1:1 ratio (*V*/*V*/*V*) was used, resulting in an 80% survival rate for the plants. The findings highlight the importance of optimized sterilization methods and growth regulator concentrations in improving the tissue culture propagation of turmeric, offering promising potential for the large-scale production of this medicinally important plant.

INTRODUCTION

Turmeric (*Curcuma longa*) is recognized as one of the most significant medicinal plants, extensively utilized in traditional medicine and the food industry. Its widespread use is attributed to its potent anti-inflammatory and antioxidant properties **(Aggarwal *et al*., 2007).** With the increasing demand for turmeric products in global markets, it has become essential to develop new techniques that contribute to efficient and effective production of this important medicinal plant turmeric. Traditional methods of propagating turmeric, such as using rhizomes, are limited in terms of speed and yield. These methods are also susceptible to various diseases and pests, adversely affecting the quality and quantity of production **(Suman *et al*., 2011).**

Cultivating turmeric in agricultural soil faces many challenges, including the spread of fungal and bacterial diseases that negatively affect plant growth and productivity (**Neenu *et al*., 2013**). Agricultural soil may also contain nematodes and other root diseases, leading to a decrease in the quality and quantity of the rhizomes **(Singh**

* **Corresponding author: E-mail address:** sarafoda1989@gmail.com
https://doi.org/10.21608/sinjas.2025.328694.1294

***et al*., 2010).** Furthermore, turmeric cultivation in soil requires large areas and long periods for plants to mature, which poses a challenge in light of increasing demand and the pursuit of sustainable and rapid production **(Gantait *et al*., 2011).** Soil degradation and nutrient deficiency are also factors that reduce the effectiveness of traditional turmeric farming **(Bhattacharyya *et al*., 2009).**

As a result of these challenges, tissue culture technology has emerged as an ideal option for the sustainable propagation of turmeric. This technique allows for the production of large numbers of plants in a short time while ensuring the purity and quality of the resulting plants. It also reduces the risk of soil-borne diseases **(Sundaram *et al*., 2012).** Additionally, tissue culture enables the production of plants in a completely controlled environment, enhancing the prospects for improving agricultural traits such as disease resistance and tolerance to harsh environmental conditions **(Pattnaik and Chand, 1996).**

Moreover, tissue propagation of turmeric provides an opportunity to study the genetic and chemical changes in the plant more accurately, paving the way for the development of new varieties with improved traits **(Gupta *et al*., 2010).** Based on these justifications, this study aims to highlight the benefits of using tissue culture techniques for turmeric propagation and to present tangible results regarding the effectiveness of this technique in improving productivity of *Curcuma longa* plant.

MATERIALS AND METHODS

This study was conducted at the Tissue Culture Laboratory, Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Governorate, Egypt.

Establishment Stage

Plant material

Fresh rhizomes of *Curcuma longa* were obtained from the Faculty of Agriculture, Cairo University. They were planted on December in Styrofoam trays at Experimental Farm, Fac. of Environ. Agric. Sci., Arish Univ', El-Arish, North Sinai, Egypt. After one week of planting, the buds began to sprout.

Rhizome bud surface sterilization

Sprouted rhizomes were collected from Styrofoam trays. They were carefully washed under running tap water to remove medium. The buds emerging from the rhizomes were excised with a sharp blade. Surface sterilization was conducted using commercial Clorox at different concentrations (10%, 15% and 20%) for varying durations (5, 10 and 15 minutes). After the sterilization treatments, the buds were washed five times with sterile double distilled water to remove any traces of the disinfectant.

Following the initial sterilization, the buds were treated with 0.1% mercuric chloride for 10 minutes, followed by another five washes with sterile double distilled water to eliminate any residual mercuric chloride under a laminar flow cabinet. Finally, the buds were treated with 70% ethanol for one minute and washed several times with sterile double distilled water for ten minutes each.

Survival percentage =

$$\frac{\text{No.of cultures survived}}{\text{Total number of cultures inoculated}} \%100$$

Culture Media

Following the surface sterilization process, the rhizome buds were carefully trimmed to a height of 2–3 mm, dried using sterile blotting paper, and then transferred to a full-strength Murashige and Skoog (MS) medium (**Murashige, Skoog, 1962**) to promote shoot and root formation.

Media preparation

The nutrient medium used in this study was based on MS media, which was enriched with 3% (*W*/*V*) sucrose and 0.75% agar. Additionally, different combinations

of growth regulators, including auxins and cytokinins, were incorporated into the media. Before autoclaving, the pH of the medium was carefully adjusted to a range of 5.6–5.8. The medium was then distributed in 15-ml portions into culture tubes measuring 25 × 150 mm. Sterilization was achieved by autoclaving at 121°C and a pressure of 1.1 kg cm^{-2} for 15 minutes.

Multiplication stage

The objective of this stage was to enhance shoot proliferation. Explants derived from the growth achieved in the establishment phase were utilized for multiplication experiments. These experiments focused on evaluating various factors, particularly the effects of different concentrations of cytokinins, including BA, Kinetin, and TDZ, at levels of 1, 2, 3, and 4 mg l^{-1}. The goal was to identify the most effective cytokinin for promoting the highest rate of shoot multiplication.

Rooting Stage

The proliferated turmeric shoots were employed as explants and cultivated on MS medium enriched with 1.0 mg l^{-1} TDZ and varying concentrations of the auxin NAA (Naphthaleneacetic acid) at 0.5, 1.0, 1.5, and 2.0 mg l^{-1}. The medium also contained 0.1 g l^{-1} myo-inositol, 30 g/L sucrose, and 7.00 g/L agar. The impact of the different NAA concentrations on root formation was carefully observed and documented.

Statistical Analysis

The experiments were arranged in a Completely Randomized Design (CRD), incorporating four replicates. Each replicate included three jars, with four explants placed in each jar. The collected data were statistically analyzed using the analysis of variance (ANOVA) method through the SAS statistical software package (**SAS, 2004**). To compare the differences between means, Duncan's multiple range test (**Duncan, 1955**) was applied at a significance level of 0.05.

RESULTS AND DISCUSSION

Establishment Stage

Sterilization

The initial Table 1 outlines the effect of varying commercial Clorox concentrations and sterilization durations on the percentage of contamination-free buds and their survival rates. The findings revealed that the most effective treatment was 20% Clorox applied for 10 minutes, which resulted in a contamination rate of only 10% and a survival rate of 90%. This success can be linked to the higher Clorox concentration, which effectively eradicated surface microorganisms capable of causing contamination in the cultured tissues. As a result, this method significantly enhanced the efficiency of *in vitro* propagation.

These results align with earlier research highlighting the critical role of proper sterilization agents in minimizing microbial contamination in tissue culture. For example, **Sundaram *et al.* (2012)** demonstrated that the use of effective surface sterilant at suitable concentrations significantly reduces contamination and enhances survival rates in tissue culture. Additionally, **Gurav *et al.* (2020)** supported the concept that higher concentrations of Clorox can significantly reduce contamination while maintaining tissue viability. **Bandara *et al.* (2023)** pointed out that either 30% or 40% Clorox for 15 or 20 minutes can be utilized for the sterilization of *Curcuma longa* rhizome buds before inoculation.

In contrast, lower concentrations of Clorox (10% and 15%) and varying durations resulted in higher contamination rates and lower survival percentages. This suggests that lower concentrations are insufficient to eliminate all potential contaminants, while longer exposure times may damage the plant tissues due to the harsh effects of Clorox on plant cells.

Table 1. Effect of rhizome bud surface sterilization by using commercial Clorox at different concentrations and durations on free contamination and survival percentage of rhizome bud

Clorox Concentration %	Duration (min)		
	5	10	15
10	50 de	55 d	45 e
15	75 c	96 ab	93 b
20	90 b	100 a	95 ab

Based on these results, it can be concluded that using 20% Clorox for 10 minutes is optimal for achieving effective sterilization of turmeric rhizome buds while ensuring high survival rates. These findings highlight the importance of selecting the appropriate concentration and duration of sterilization to ensure the success of tissue culture propagation.

Multiplication Stage

Effect of cytokinin type

In the multiplication stage, various types of tested cytokinins (BA, Kin and TDZ) were added with different concentration (1.0, 2.0, 3.0, 4.0 mg L^{-1}) to evaluate their effect on shoot proliferation. The results indicated that the optimal medium for shoot multiplication was the MS medium supplemented with 1.0 mg L^{-1} TDZ. Under these conditions, the explants exhibited the highest rate of shoot proliferation, averaging 5.67 shoots per explant showed in Table 2 and Fig. 1.

These findings are in agreement with previous research that highlights the effectiveness of TDZ in promoting shoot regeneration and multiplication due to its unique mechanism of action that stimulates cell division and differentiation. For instance, **Arafeh *et al*. (2003)** reported that TDZ significantly enhanced shoot regeneration rates in various plant species, demonstrating its potential as a powerful growth regulator in tissue culture. In addition, **Lincy, Sasikumar (2010)** reported that using TDZ at 1 mg L^{-1} gave the maximum number of shoots of *Zingiber officinale* varieties.

In contrast, the other cytokinins tested, BA and Kinetin, showed lower shoot proliferation rates. Specifically, the average number of shoots per explant for BA at 1 mg L^{-1} was 2.33, at 2 mg L^{-1} was 3.00, at 3 mg L^{-1} was 4.00, and at 4 mg L^{-1} was 3.33. For Kinetin at 1 mg L^{-1}, the average was 3.00, at 2 mg L^{-1} was 2.67, at 3 mg L^{-1} was 3.67, and at 4 mg L^{-1} was 2.67. This suggests that while BA and Kinetin can support growth, TDZ appears to be the most effective for achieving high multiplication rates in turmeric.

Rooting Stage

Effect of NAA concentrations on roots proflation during *C. longa*

The results from the rooting stage demonstrate the effects of different concentrations of NAA (Naphthalene acetic acid) on root development in turmeric. The results in Table 3 and Fig. 2 indicate that the optimal concentration for promoting root growth was 1.5 mg l^{-1}, which resulted in the highest number for each of main roots and root length. Specifically, at this concentration, the average number of main roots was 43.00, and the average root length was 9.77 cm.

Table 2. Effect of cytokinin types on number of multiple shoots/explant, shoot length, and number of leaves of *C. longa*

Cytokinin		No. multiple shoots/ explant	Shoot length (cm)	No. leaves/ plant	No. leaves/ shoot
Type	**Concentration (mg l^{-1})**				
	1	3.00 b	2.50 d	8.66 b	2.67 ab
	2	2.33 b	2.50 d	6.00 b	2.67 ab
BA	3	3.00 b	4.00 bc	9.67 b	3.00 ab
	4	4.00 ab	4.83 ab	17.00 ab	4.33 ab
	1	3.33 ab	5.00 bc	12.00 b	3.67 ab
Kin	2	3.00 b	6.83 cd	6.33 b	2.00 b
	3	2.67 b	3.50 b-d	7.33 b	2.67 ab
	4	3.67 ab	3.00 cd	12.33 b	3.33 ab
	1	2.67 b	2.83 cd	7.67 b	3.00 ab
TDZ	2	5.67 a	5.67 a	26.67 a	4.67 a
	3	4.33 ab	4.00 bc	18.67 ab	4.33 ab
	4	3.67 ab	3.67 b-d	13.00 ab	3.33 ab
		3.00 b	3.16 cd	10.33 b	3.33 ab

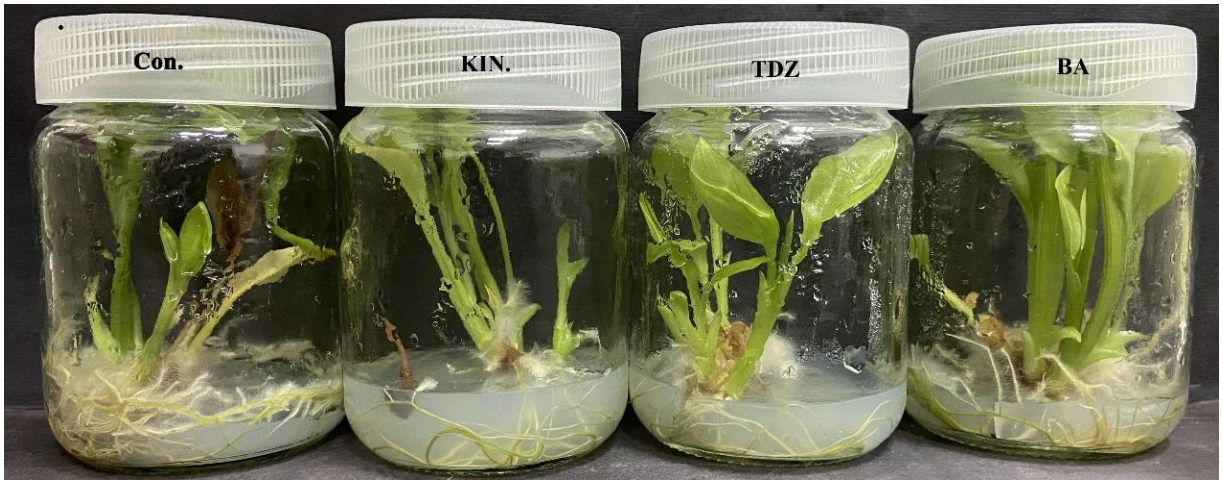

Fig.1. Effect of cytokinin types on number of multiple shoots/explant, shoot length, and number of leaves of *C. longa*

Table 3. Effect of NAA concentrations on number of multiple shoots/explant, shoot length, number of main roots, and root length of *C. longa*

NAA Conc. mg l^{-1}	No. main roots	Root length (cm)
Control	14.33 e	4.50 c
0.50	21.00 d	5.67 bc
1.00	27.00 c	6.67 bc
1.50	43.00 a	9.77 a
2.00	34.00 b	7.50 ab

These findings are supported by prior studies that have demonstrated the effectiveness of NAA in promoting root growth in various plant species. For example, **Murashige and Skoog (1962)** highlighted the key role of auxins, particularly NAA, in stimulating root formation in tissue culture, which plays a vital role in developing healthy and vigorous plantlets.

On the other hand, lower concentrations of NAA (0.5 mg l^{-1} and 1.0 mg l^{-1}) resulted in fewer roots and shorter root lengths, with averages of 21.00 roots and 5.67 cm, and 27.00 roots and 6.67 cm, respectively. Higher concentrations, such as 2.0 mg l^{-1}, produced a moderate number of roots (34.00) and root length (7.50 cm), suggesting that while NAA can still promote root growth at these levels, the optimal concentration for maximum root development is 1.5 mg l^{-1}.

Acclimatization Stage

The well-rooted plantlets of *C. longa* underwent *In vitro* treatments as part of the acclimatization process. The selected plantlets were carefully removed from the culture jars, and their roots were rinsed thoroughly under running water to remove any residual medium. Afterward, the roots were washed with sterilized distilled water, and any dead leaves or dry shoots were trimmed. To ensure surface sterilization, the plantlets were soaked in a fungicide solution of rizolex (1.00 mg l^{-1}) for 3–5 minutes.

The plantlets were then transplanted into black polyethylene pots, 8 cm in diameter, filled with a mixture of peat moss, vermiculite, and washed sand in a 1:1:1 ratio (*V*/*V*/*V*). The pots were covered with white transparent bags containing small holes, which were gradually enlarged each week over a period of four weeks. This gradual exposure helped the plantlets adapt to external conditions. Once the plantlets developed new leaves, they were transferred to larger pots (30 cm in diameter) in a process referred to as "recycling." Finally, the acclimatized plantlets were moved from the greenhouse to field conditions, as described by **Abdallah *et al*. (2018).**

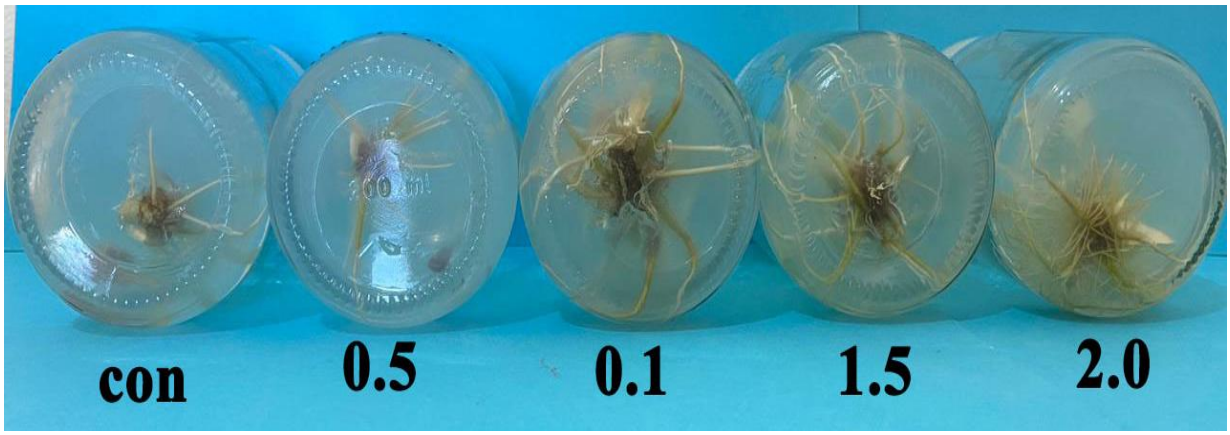

Fig. 3. Effect of NAA concentrations on number of multiple shoots/explant, shoot length, number of main roots, and root length of *C. longa*

Acknowledgement

"This paper is based upon work supported by Science. Technology & Innovation Funding Authority (STDF) under grant Post Graduate Support Grant (PGSG)".

REFERENCES

Abdallah, S.A.S.; Belal, A.H.; Ali, M.A.M. and Foda, S.I.H. (2018). Some treatments affecting propagation of *Cleome Droserifolia* (FORSSK.) plant *In vitro.* Egypt. J. Appl. Sci., 3 3(7): 254-268.

Aggarwal, B.B.; Sundaram, C.; Malani, N. and Ichikawa, H. (2007). Curcumin: the Indian solid gold. Advances in Experimental Med. and Biol., 595: 1-75.

Arafeh, R.M.; Al-Khaldi, R.M. and Al-Dhaheri, A. (2003). *In vitro* shoot regeneration of curcumin and its potential applications in agriculture. Plant Cell Reports, 22(10): 812-817.

Bandara, M.; Dahanayake, N.; Perera, P. and Subasinghe, S. (2023). Development of *In vitro* protocol to enhance mass production of turmeric (*Curcuma longa* L.). Tropical Agric. Res. and Ext., 26 (1): 44-51.

Bhattacharyya, P.; Chakraborty, A. and Chakrabarti, K. (2009). Microbial biomass and enzyme activities in submerged rice soil amended with municipal solid waste compost and decomposed cow manure. Chemosphere, 77 (4): 936-941.

Duncan, D.B (1955). Multiple Range and Multiple F-Test. Biometrics, 11: 1-42.

Gantait, S.; Mukherjee, E. and Das, P.K. (2011). Advances in micropropagation of selected aromatic plants: a review on vanilla and turmeric. Acta Physiologiae Plantarum, 33 (3): 567-577.

Gupta, S.; Sharma, N.; Sood, A. and Sharma, S. (2010). *In vitro* propagation of *Curcuma longa* and its field evaluation. Plant Cell, Tissue and Organ Culture, 100 (1): 25-35.

Gurav, P.S.; Khedkar, G. D. andThakur, M.S. (2020). Surface sterilization techniques for the establishment of *In vitro* cultures of turmeric (*Curcuma longa* L.). J. Med. Plants Res., 14 (5): 67 - 75.

Lincy, A. and Sasikumar, B. (2010). Enhanced adventitious shoot regeneration from aerial stem explants of ginger using TDZ and its histological studies. Turk. J. Bot., 34 (1): 21-29.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15 (3):473-497.

Neenu, S.; Anjana, D.T. and Anju, R. (2013). Fungal diseases of turmeric in Kerala and their management. Res. J. Agric. and Forestry Sci., 1(6): 7-11.

Pattnaik, S. and Chand, P.K. (1996). *In vitro* propagation of the medicinal herbs Ocimum americanum L. syn. *Ocimum canum* Sims. (hoary basil) and *Ocimum sanctum* L. (holy basil). Plant Cell Reports, 15 (11): 846-850.

SAS (2004). SAS/STAT User's Guide. SAS Institute Inc., Cary, N.C.

Singh, R.; Dwivedi, R. and Pathak, K. (2010). Pathogenicity of nematodes on medicinal crops and their management. Indian J. Nematol., 40(2):91-95.

Suman, P.; Ankita, M. and Manasi, S. (2011). Biotechnology of medicinal plants: recent advances and potential. J. Med. Plants Res., 5(10):2021-2033.

Sundaram, S.; Manivannan, S. and Krishnan, M. (2012). *In vitro* propagation of *Curcuma longa* L. through induction of multiple shoots. Int. J. Biol. And Pharm. Res., 3 (8): 1025-1030.

الملخص العربي

تحسين استراتيجيات التعقيم والسيتوكينينات لتعزيز التكاثر الدقيق لنبات الكركم

سارة إبراهيم حسين فوده، هاني محمد سامي حسن، محمد أحمد محمود علي، سونيا عطيه شحاته

قسم الإنتاج النباتي، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.

تتناول هذه الدراسة تحسين تقنيات التعقيم وتركيزات السيتوكينينات لتعزيز إكثار نبات الكركم، نظرًا للقيود التي تفرضها طرق الزراعة التقليدية، مثل تعرضها للأمراض، والبطء في النمو، ومتطلبات المساحة الكبيرة، فإن تقنيات زراعة الأنسجة توفر بديلًا أكثر كفاءة للتحكم في التكاثر. تم إجراء الدراسة في مختبر زراعة الأنسجة بكلية العلوم الزراعية والبيئية بجامعة العريش، مصر. في مرحلة التأسيس، تم تعقيم براعم الريزومات باستخدام الكلوركس بتركيزات مختلفة (١٠٪، ١٥٪، و٢٠٪) لفترات زمنية متفاوتة (٥، ١٠ و١٥ دقيقة). وكانت الطريقة الأكثر فعالية هي استخدام الكلوركس بتركيز ٢٠٪ لمدة ١٠ دقائق، حيث حققت نسبة بقاء وصلت إلى 100% مع أقل معدل تلوث. في مرحلة التضاعف، تم اختبار أنواع وتركيزات مختلفة من السيتوكينينات (الثيديازرون، البنزيل أدنين والكينتين)، حيث أظهرت النتائج أن أفضل النتائج كانت مع ١,٠ مليجرام/لتر من الثيديازرون، مما أدى إلى أعلى معدل لتكوين الافرع. أما في مرحلة التجذير، فقد أظهرت الإضافات من نيفثالين حمض الخليك تأثيرات كبيرة على نمو الجذور، حيث كانت التركيزات المثلى هي ١,٥ مليجرام/لتر، مما أدى إلى أكبر عدد من الجذور الرئيسية وأطول طول للجذر. تُظهر هذه النتائج فعالية تقنيات التعقيم واختيار تركيزات منظمات النمو في تحسين التكاثر النسيجي للكركم، مما قد يساهم بشكل كبير في الإنتاج الكمي لهذه النبات الطبي القيم، وكانت أفضل أقلمة لنبات الكركم باستخدام مخلوط من البيتموس والفيرميكوليت والرمل بنسبة ١:١:١ حيث حققت نسبة نجاح ٨٠٪.

الكلمات الاسترشادية: الكركم، زراعة الأنسجة، التعقيم، سيتوكاينن، هرمون الثيديازرون، الإكثار الدقيق.

REVIEWERS:
Dr. Mohammed A. Abdelkader | mohammedahmed1980@yahoo.com
Dept. Hort., Fac. Agric., Zagazig Univ., Zagazig, Egypt.
Dr. Mahmoud Kassem | mmk@mans.edu.eg
Dept. Vegetables and Floriculture, Fac. Agric., Mansoura Univ., Egypt.