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UTILIZING TISSUE CULTURE TECHNIQUE AND ZNO NANOPARTICLE STIMULATION TO PROPAGATE *Asparagus aphyallus* L. PLANT

Ghada M.H.Gaballa[1*]; E.R. Marzouk[2] and S.A.S. Abdullah[1]

1. Dept. Plant Prod. Fac. Environ. Agric. Sci. Arish Univ., North Sinai, Egypt.
2. Dept. Soil and water, Fac. Environ. Agric. Sci. Arish Univ., North Sinai, Egypt.

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ABSTRACT

This research was conducted in the Faculty of Environmental Agricultural Sciences' Plant Tissue Culture Laboratory, Arish University, North Sinai, Egypt during 2019 to 2022. This study developed an efficient micropropagation protocol for the endangered medicinal plant *Asparagus aphyllus* L. using tissue culture techniques and zinc oxide nanoparticles (ZnO NPs). A toxicity experiment determined the EC_{50} values for root growth inhibition by bulk ZnO (60.2 mgL^{-1}) and ZnO NPs (48.2 mgL^{-1}). Seeds were germinated on Murashige and Skoog medium with different ZnO NP concentrations (0-20 mgL^{-1}) where 10 mg/L ZnO NPs improved seed germination. This range of ZnO NPs were selected according to the toxicity results. Nodal explants from 10 mgL^{-1} ZnO NP-treated seedlings were cultured on MS medium with 1.5 mgL^{-1} benzyladenine for shoot multiplication. In vitro shoots were rooted on MS medium with 2 mgL^{-1} indole-3-butyric acid before successful ex vitro acclimatization. The optimized protocol allows rapid micropropagation of genetically uniform *A. aphyllus* plantlets. ZnO NPs at 10 mgL^{-1} enhanced seed germination while higher levels were inhibitory. The efficient propagation and incorporation of ZnO NPs could assist conservation efforts and development of elite genotypes with enhanced medicinal properties. Plantlets were successfully acclimated with 70% survival. Further studies on secondary metabolite production in micropropagated plants are recommended.

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INTRODUCTION

Medicinal plants play a prominent role in both agricultural and industrial sectors. They are often regarded as the principal authority in the field of natural medicine or the primary reservoir of efficacious compounds. In Egypt, medicinal and aromatic crops are widely acknowledged as essential non-traditional agricultural products that lay the groundwork for the growth of Egypt's national economy. Due to its favorable environmental conditions for growth, Egypt is well known as a significant contributor to the production of aromatic and medicinal crops in the Middle East **(Elsabawy, 2012).**

The plant species aphyllus is indigenous to southern Europe, North Africa (particularly Egypt), Jordan, Lebanon, Syria, and Turkey in western Asia. The monocotyledonous plants of the order Asparagales are categorized according to taxonomic principles **(Sulava *et al.*, 2020)**. The *Asparagus aphyallus* species of asparagus, which is frequently grown for its culinary qualities, served as the source of the family name. This particular family includes a number of often encountered indoor and garden plants **(Mustafa *et al.*, 2021)**. Over 2,900 species are included in the 114 genera that make up the family Asparagaceae **(WWW. Wikipedia. Org.)**. Egypt grows the native *Aasparagus aphyllus*

*** Corresponding author: E-mail address:** ghada14791@gmail.com
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variety in its natural setting. Egypt cultivates the indigenous *Asparagus aphyllus* variety in its natural environment. This chemical is used in traditional medicine to treat cancer and liver conditions.

Asparagus is thought to have a secondary genetic relationship with *A. aphyllus*. The plant is a strong candidate for use as a gene donor in the context of crop development due to its innate disease resistance and capacity to adapt to xerophytic conditions.

In Sinai's natural environment, the variety of medicinal and fragrant plants steadily decreased, and ultimately vanished. Therefore, there is a strong need for scientific endeavors to address this issue, perhaps by using tissue culture methodology.

Replicating crops that are difficult to grow from seeds or cuttings or other traditional methods has become an important skill. A highly significant technique for reaching this goal is tissue culture micropropagation, which is widely acknowledged. In a short amount of time and in a relatively small growth area, micropropagation makes it possible to produce a significant number of plants quickly. Additionally, micropropagation enables the quick and abundant production of plants that are genetically identical to those of their progenitors. Tissue culture technologies for a variety of crops are now commercially available, which has sped up their development **(Bhoite and Palshikar 2014).**

Nanotechnology is a burgeoning field of research in the present era. Nanoparticles (NPs) are employed in several fields due to their distinctive properties. Zinc oxide nanoparticles are of considerable significance within the realm of metal nanoparticles owing to their wide-ranging utilization in several domains, including biomedical research, gas sensing technologies, drug delivery systems, biosensors, cosmetics, and agriculture, among others. Recent research has elucidated the application of nanoparticles in diverse sectors, encompassing wastewater management, textile manufacturing, and healthcare.

The main objectives of this research were to: (i) determine the optimal zinc oxide nanoparticle (ZnO NP) concentration for growth of *Asparagus aphyllus* using a toxicity experiment, (ii) develop an efficient tissue culture protocol for rapid micropropagation of *A. aphyllus* using different plant growth regulators, (iii) evaluate the incorporation of ZnO NPs at optimal levels into the tissue culture media for improving micropropagation, (iv) establish a suitable propagation method for ex situ conservation of the endangered native Egyptian *A. aphyllus* species, and (v) produce true-to-type, genetically uniform *A. aphyllus* plantlets from elite mother plants using the optimized tissue culture technique.

MATERIALS AND METHODS

Between 2019 and 2022, this study was conducted at the plant tissue culture laboratory of Prof. Dr. Abd El-Fatah Helmy Belal at Arish University North Sinai, Egypt's Faculty of Environmental Agricultural Sciences. This research sought to micropropagate the Sinai Peninsula's native wild asparagus plant, *Asparagus aphyallus* L.

Establishment Stage

Plant materials

The seeds of *Asparagus aphyallus* L. were harvested from mature wild plants in the North Sinai governorate's Arish. The explants were divided into tiny cuttings with shoot-tip and nodal segments that ranged in size from 0.50 to 1.00 cm.

Explants sterilization

After being cultured in the media, the explants underwent surface sterilization under strictly aseptic conditions in the Laminar Air Flow Hood to prevent bacterial or fungal contamination. The seeds underwent a sterilizing process.

The seeds were washed with sterile distilled water three to five times following this treatment.

The seeds were rinsed for five minutes with a few drops of liquid soap, rinsed once more for sixty minutes under running water to eliminate any leftover detergent, and then washed three times for five minutes with sterilized distilled water. The seeds were then thoroughly cleaned with sterilized distilled water three to five times to get rid of any disinfectant residue by soaking them for five minutes in 15% Clorox, which contains 5.25% sodium hypochlorite, with two drops of Tween-20. Sterilized tools were used during the whole sterilizing process, which was carried out in the culture cabinet under aseptic circumstances. This process was used to clean the plant tissue of fungi, bacteria, and other pollutants without damaging the seeds' ability to regenerate.

Seeds were germinated in a variety of ZnO concentrations (0.0, 5.0, 10.0, 15.0 and 20.0 mgL^{-1}) as nanoparticles on MS medium for one month to find the better ZnO concentration for the subsequent stage during the establishment stage. The mean germination time (MGT), germination energy (GE), germination percentage (GP), and germination of seedling seeds (GRI) were all recorded at 7, 11, 13, 16, and 20 days. According to **Kader (2005**), the previous terms were approximations using the equation as describe below. MGT as (n d) / N, where 'n' is the total number of seeds that germinated at the end of the experiment and 'd' is the number of days since the test began,

$$GE = \frac{\text{No of Seeds Germinated on Day 16}}{\text{Total Number of Seeds Tested}} \times 100$$

$$GP = \frac{\text{No of Seeds Germinated}}{\text{Total Number of Seeds Tested}} \times 100$$

$$GRI = \frac{\text{No.of Germinated Seeds}}{\text{No.of Days}}$$

Culture media

Throughout this work, **White (1963)**, **Gamborg B5 (GB, 1968)** medium and MS, B5 media were utilized. According to **Murashige and Skoog (1962)**, these media contain macro and micro components as well as vitamins (Table 1). After that 3% sucrose and 100 mgl^{-1} myo-inositol were added to the media as supplements. Prior to the addition of 7.00 gl^{-1} agar, all media were adjusted to a pH range of 5.7–5.8 using either 0.10 N NaOH or 0.10 N HCl.

Each glass tissue culture jar and tube held 15 ml of culture medium when the media were dispensed into them. All media were autoclaved for 20 minutes at 121°C and 1.06 kg/cm^2. Transferred to the culture cabinet, the jars and slant tubes were kept refrigerated until they were needed.

Effect of explants type

To choose the optimum explant type for development, shoot tips or single nodes (0.5–1.00 cm) were removed and cultured on MS, B5, and White medium.

Nanoparticles of Zn oxide

Zinc oxide nanoparticles (ZnO NPs) were made using a modified method (**Gnanasangeetha and Thambavani, 2013**). It was broken down into 0.22 g of zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) in 50 ml of double-distilled water. The pH of the solution was then raised to 12 by adding 2M NaOH and shaking it very hard (rpm) for two hours at room temperature. After several washes and centrifugations to get rid of the liquid, the white powder was recovered. After that, the precipitate was dried in an oven at 60 °C for 24 hours to get dried ZnO NPs.

An Image taken by Hitachi-S-3400N, SEM, and JEOL JEM1000, TEM were used to determine nanoparticle size, shape and surface morphology. Included an EDX Energy-dispersive X-ray spectroscopy confirmed the elemental configuration as

zinc and oxygen. Powder X-ray diffraction (XRD) analysis verified the crystal structure of ZnO nanoparticles and inductively coupled plasma-OES (Agilent Instruments, Japan). Images of SEM with TEM indicated that the diameter of ZnO NPs ranged from 16-38 nm with a rough-surface and non-spherical shape. EDX results confirmed the presence of Zn and O peaks (Fig 1). The total concentrations of Zn in 5% HNO_3 acidified sample was 9200 ± 85.8 mg L^{-1} (mean ± Standard deviation).

Conditions of culture

The explants were cultured on the medium inside a Laminar air flow cabinet, ensuring adherence to rigorous aseptic protocols. The various cultural groups were subjected to a controlled environment inside the growth chamber for a duration of four weeks, during which meticulous monitoring was conducted. Controlled by a "Power" air conditioner, the incubation temperature was 252°C. An automatically controlled 16-hour day and 8-hour night made up the photoperiod. For all studies, there was a 3000 lux light intensity, and after four weeks of culture, the explants' survival rate was noted.

Rate of root growth and lethal amount of zinc forms

A plastic cup was used to start the seeds of the plants with vertically stacked rolls of paper towel soaked with tap water for three days. Small aperture Perspex strips supported four seedlings on the upper 600 ml glass pots. The glass containers were filled with a room-temperature solution of 1.0 mM $CaCl_2$ with 5.0 mM H_3BO_3. A base solution was used to grow the plants for 24 hours before being exposed to nanoparticle-containing solutions for 24-hs. The experiment was done as demonstrated by **Kopittke *et al.* (2011)**. From 0 mgl^{-1} to 1000 mgl^{-1}, in steps of 5, 10, 25, 50, 75, 100, 200, 500, and 1000 mgL^{-1}, ZnO NPs and bulk form were used. The amounts were picked so that they cover a range of zinc values. Japanese Sony DLSAR A2 digital pictures were used to measure the length of the roots. ImageJ, which can be found at https://imagej.net/Welcome, was used for the study. The rise rate was then found.

The toxicity model was changed to a log-logistic model by the solution tool in Excel 2016. We used the residual standard deviation (RSD) and correlation coefficient to test how well the model worked for root elongation rate (RER) when NP and bulk Zn amounts were changed. The EC50 number was used by **Ritz *et al.* (2015)** to figure out the lethal amount. The dose-response models **(Van der Vliet and Ritz, 2013)** that are most often used to find the EC50 are log-logistic models:

$$\frac{Y_o}{1+\exp(e\,(\log(x)-\log(m)))} \quad (1)$$

In the above equation, Y_o, e, and m represent the coefficient values, with m specifically denoting EC_{50}. The variable x represents the concentrations of Zn delivered to the plant, measured in mgl^{-1}, originating from both bulk Zn and ZnO NPs.

The root elongation rate (RER, mmh^{-1}) was determined using the following calculation:

$$RER = (R_t \_ R_0)/ T \quad (2)$$

In this context, R_0 and R_t represent the length of individual roots at the beginning of the growth period and after a specified growth time of generally 24 hours. T denotes the duration of the growth period, also set at 24 hours.

Multiplication stage

The goal of this stage was to enhance the quantity of shoots. During trials involving multiplication, the growth obtained from the establishment stage was employed as explants. To determine whether combination of BA, Kin, and 2ip at 0.00, 0.50, 1.00, 1.50, and 2.00 mgl^{-1} with 0.1 mgl^{-1} NAA would provide the greatest multiplication, several concentrations were tested.

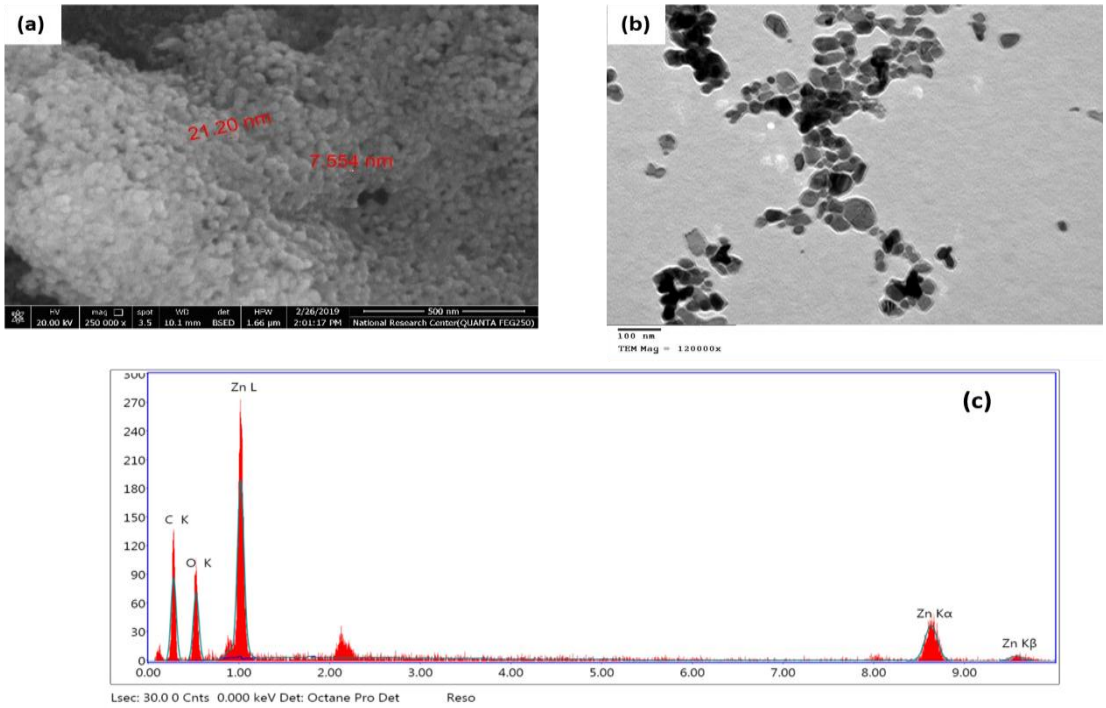

Fig. 1. Image of SEM (a), image of TEM (b), and EDX (c) of the zinc oxide nanoparticles. These images and spectrum provide evidence of the nano-scale dimensions and elemental composition of the synthesized ZnO-NPs

Rooting stage

Asparagus aphyallus L. shoots that had multiplied were utilized as explants and cultivated on MS with the addition of 100 mgl^{-1} of myo-inositol, 30.00 g/l of sucrose, and 7.00 g/l of agar. Additionally, individual shoots (5–6 cm) were separated and cultured on free MS for a week in order to remove any potential root-inhibiting or -reducing effects of plant growth regulators. The number of main roots, the length of the roots, and the number of shoots were all noted. To determine the best concentration that promoted the most root formation, shoots were grown on MS solid media with varying concentrations of IBA (0.00, 0.50, 1.00, 1.50, and 2.00 mgl^{-1}).

Acclimatization stage

Plantlets were placed in black polypropylene pots 8 cm in diameter filled with peat moss, vermiculite and, washed sterilized sand(1:1:1 *V/V/V*), peat-moss and vermiculite after being surface sterilized by soaking in a fungicide solution of Rizolex (1 mgL^{-1}) for 3 to 5 minutes. When the plantlet was ready to be transferred to the larger pots (30 cm), the polypropylene pots were then covered with white translucent bags with small holes that were made after one week and gradually became larger each week for four weeks. Plantlets were finally moved from greenhouse conditions to field conditions after they began to grow new leaves.

Statistical Analysis

The experimental design employed in this study was completely randomized, with the utilization of a factorial design layout in specific circumstances, based on the parameters under investigation in each experiment. The data underwent analysis of variance (ANOVA) using the General Linear Models (GLMs) techniques in SAS **(SAS, 2004).** Statistically significant variations were noticed in the measured value, and the means were separated using Duncan's multiple range test (DMRT) **(Duncan, 1955)** at a significance level of 0.05%.

RESULTS AND DISCUSSION

Zinc Oxide Nanoparticles Toxicity EC_{50}

The results indicated that the addition of Zn in both nanoparticle (NP) and bulk forms resulted in a significant reduction in root development when compared to the control group. Nevertheless, there was a discrepancy in the effective hazardous dosage, also known as the EC50, between the Zn nano and bulk forms. Figs. 2-4 illustrate the relationship between the respiratory exchange ratio (RER) and the concentrations of bulk and nano-Zinc (Zn). The log-logistic model yielded EC_{50} values of 60.2 and 48.2 mg Zn L^{-1} for the bulk and Nano forms, respectively, as seen in Figs. 2-4. The results of this investigation were inconsistent with the research conducted by **Marzouk *et al.* (2016)**. The researchers conducted a study comparing the toxicity of bulk Cu and CuO NPs. They found that the EC_{50} value, which was predicted using a log-logistic model, for CuO NPs was four times higher than that of bulk Cu. This indicates that bulk Cu exhibited more toxicity than CuO NPs. The researchers also provided data on the EC_{50} values for bulk copper (Cu) and copper oxide nanoparticles (CuO NPs) in soybean, which were reported as 0.22 mg l^{-1} and 0.90 mg l^{-1}, respectively. The enhanced availability of soluble Zn in bulk form compared to nanoparticles is widely acknowledged.

It seems from Fig. 4 that the RER was decreased with increasing both bulk and nano-sized ZnO forms. However, the general average, comparable to control was favoured to nano ZnO size confirming higher toxicity of nanoparticles form of ZnO. According to the results the toxicity of Zn NPs (49 mg/l) is higher than that of bulk Zn (60 mg/l). Therefore, the tissue culture experiment could be used the concentration between 0 to 30 mg/l to be in a safe side. This range is withing the permissible limits according to the toxicity experiment.

Establishment Stage

Effect of ZnO nanoparticle in seed germination on MS media

The information in Table 1 showed that adding ZnO nanoparticles to the MS media in which seeds were germinated had a significant impact. Germinated had significant ($P \leq 0.05$) a noticeable impact. The results showed that, in comparison to the other treatments, the seed that germinated in MS media supplemented with 10 mgl^{-1} of ZnO had vigor germination ($P \leq 0.05$) GP, GE, MGT and GRI values of 92.50%, 90.00, 17.93 and 5.79. However, the seeds germinated in MS with 20 mgl^{-1} of ZnO without any significant difference compared to 0. 5 and 10 $mgl^{-1}$of ZnO, and these seeds had the lowest GP, GE, MGT, and GRI values.

Effect of media type:

According to data in Table 2, MS medium outperformed ($P \leq 0.05$) the other two tested media, Gamborg (B5) and White, in terms of the number of shoots, shoot length, and number of leaves produced by the *Asparagus aphyallus* plant (2.17, 2.62 cm, and 3.17, respectively). These outcomes are consistent with **Salman (2021).** Who discovered that distinct *Asparagus officinalis* had the largest number of shoots, shoot length, and leaves when grown in MS medium. Also, **Maslanka *et al.* (2022)** observed that the *Lachenalia viridiflora* plant yielded the greatest value for the number of shoots and shoot length in MS medium. As opposed to that, **Waly *et al.* (2018)** proved that utilizing B5 medium on *Ponytail Palm* plants that had been in vitro propagated was more successful than using MS and WPM media.

Effect of explant type

One-node cutting significantly ($P \leq 0.05$) produced the most shoots, shoot length, and leaves (1.89, 2.26 cm, and 2.56, respectively) as compared to the shoot tip of the *Asparagus aphllus* plant, according to data shown in Table 3.

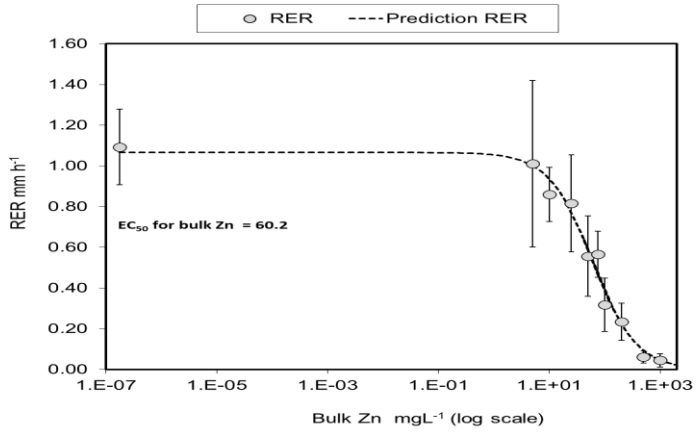

Fig. 2. RER (mm h^{-1}) of plant roots as a function of bulk Zn concentration. The dash fitting line represents the fitting of log-logistic mode. The error bar represents the SD of the four replicates

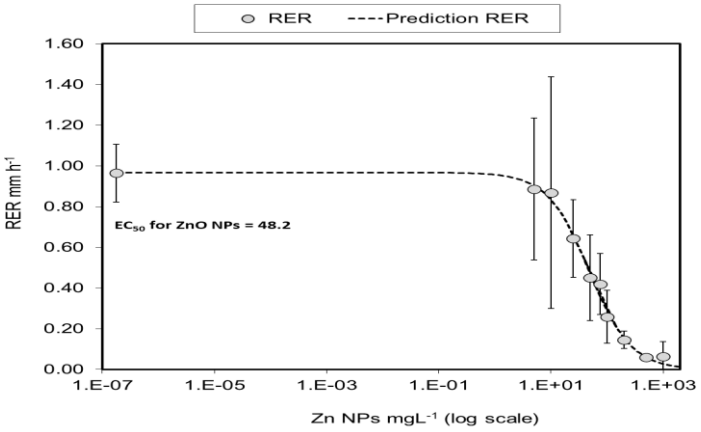

Fig. 3. RER (mm h^{-1}) of plant roots as a function of Zn NPs concentration. The dash fitting line represents the fitting of log-logistic mode. The error bar represents the SD of the four replicates

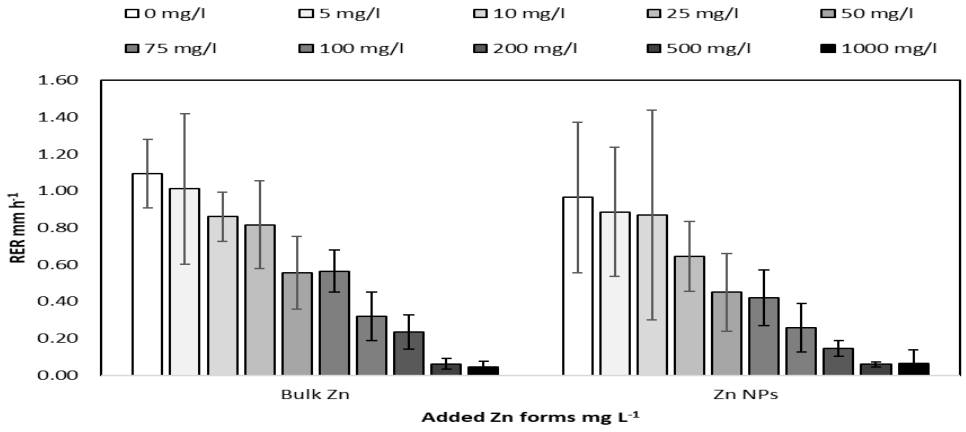

Fig. 4. RER (mm h^{-1}) of xxxxx plant soft root as a function of different concentrations of different Zn forms (bulk and Nano sized Zn, mg L^{-1}). The error bar represents the SD of the four replicates

Table 1. Effect of ZnO nanoparticle in seed germination on MS media of *Asparagus aphyallus* plant

Parameters Conc. ZnO, mgL^{-1}	Germination % (GP)	Germination energy (GE)	Mean Germination Time (MGT)	Germination Rate index (GRI)
0.00	67.50^{b}±3.66	65.00^{b} ±5.00	14.94^{b} ±0.66	4.52^{b} ±0.47
5.00	62.50^{b} ±5.90	57.50^{b} ±7.96	14.42^{b} ±1.02	4.37^{b} ±0.46
10.0	92.50^{a}±3.66	90.00^{a}±5.35	17.93^{a}±1.03	5.79^{a}±0.30
15.0	65.00^{b} ± 5.00	60.00^{b} ±6.55	14.80^{b} ±0.93	4.45^{b} ±0.35
20	60.00^{b} ±5.34	55.00^{b} ±6.27	12.92^{b} ±1.30	4.48^{b} ±0.40

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability.

Table 2. Effect of media type on studied traits of *A. aphyallus* plant

Parameters medium Type	No. Shoots	Shoot Length (cm)	No. Leaves
Murashige and Skoog (MS)	2.17^{a}±0.54	2.62^{a}±0.31	3.17^{a}±1.14
Gamborg (B5)	0.50^{b}±0.22	1.62^{b}±0.24	1.00^{b}±0.26
White	0.83^{b}±0.31	1.20^{b}±0.28	0.67^{b}±0.21

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability.

Similarly, **Mehata and Subramanian (2005), Sharan *et al.* (2011) and Pant and Joshi (2016)** they discovered that nodal explants, on *Asparagus adscendens* and *Asparagus racemosus*, respectively, recorded the great value for number of shoots and shoot length. In opposite side, **Waly *et al.* (2018). Mustafa (2021) and Maslanka *et al.* (2022)** mentioned that the highest values for shoot number, shoot lengths, and leaf number, respectively, were produced from the shoot-tips of the Ponytail palm and *Asparagus aphyallus* explants, respectively.

Effect of interaction between media and explant type

Data in Table 4 and Fig. 4 show significant ($P \leq 0.05$) effects for all studied treatments. The use of MS with one-node cutting recorded the highest values for all studied traits compared with other treatments of *Asparagus aphyallus* plant. However, the shoot-tips which were cultured on B5 and White media produced the lowest number of shoots, shoot length, and number of leaves. The similar results obtained by **(Mehata and Subramanian, 2005; Sharan *et al.*, 2011; Pant and Joshi, 2016)** they found that nodal explants recorded the great value for number of shoots and shoot length on *Asparagus adscendens* and *Asparagus racemosus*, respectively. In addition, **Sharan *et al.* (2011),** indicated that nodal segments achieved the highest explant development for different *Asparagus racemosus*. Also, **Sallam (2019)** found that the MS media produced the greatest mean number of proliferating shoots and nodes on *Asparagus officinalis* plants.

Table 3. Effect of explant type on studied traits of *A. aphyallus* plant

Parameters explant Types	No. Shoots	Shoot Length (cm)	No. Leaves
Shoot-tip	0.44^{b}±0.17	1.37^{b}±0.24	0.67^{b}±0.17
One-node cutting	1.89^{a}±0.39	2.26^{a}±0.28	2.56^{a}±0.80

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability**.**

Table 4. Effect of interaction between media and explant types on studied traits of *A. aphyallus* plant

Parameters Medium& Explant Type		No. Shoots	Shoot Length (cm)	No. Leaves
Murashige and Skoog (MS)	Shoot tip	1.00bc±0.33	2.07^{b}±0.29	0.77^{b}±0.34
	One-node cutting	3.33a±0.33	3.17^{a}±0.33	5.67^{a}±0.32
White	Shoot tip	0.33cd±0.33	0.87^{c}±0.33	0.59b±0.29
	One-node cutting	1.33^{b}±0.31	1.53bc±0.32	0.59b±0.43
Gamborg (B5)	Shoot tip	0.12d±0.22	1.17± bc 0.18	0.89b±0.33
	One-node cutting	1.00bc± 0.21	2.07^{b}±0.23	1.33± b 0.33

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability.

Multiplication Stage

Effect of growth regulators

According to data in Table 5, cytokinin types plus 0.10 NAA mgL^{-1}have a significant ($P\leq0.05$) influence on all of the characteristics of the *Asparagus aphyallus* plant. The largest numbers of shoots, shoot length, and number of leaves (at 4.00, 4.78 and 7.00, respectively) were measured in the plants grown in media supplemented with 1.5 BA mgl^{-1}. These results are in agreement **Sharan *et al.* (2011)**. They found that BA at 8.9µM gave the best shoot proliferation values of *Asparagus racemosus.* Also, **Palee (2018)** indicated that the MS medium with 3.0 mgl^{-1} BA stimulated shoot production on the *Tupistra albiflora* plant with the largest number of shoots per explant compared to the control. In addition. **Sallam (2019)** who found that MS medium supplemented with BAP at 1.0 mgl^{-1} and NAA at 0.1 mgl^{-1} gave the maximum values of shoot number, but the shoot length at 2.0 mgL^{-1} gave the maximum values. Furthmore, **Mustafa (2021)** shown that the *Asparagus aphyallus* plant multiplied in medium containing 1.0 mgl^{-1} BA and 0.2 mgl^{-1} NAA, with the greatest mean length of the shoot measuring 6.60 cm with 1.0 mgl^{-1} BA and 0.4 mgl^{-1} NAA. On the other side. **Pandey *et al.* (2016)** discovered that the best shooting occurred on an *Asparagus racemosus* plant in MS media supplemented with 2.0 mgl^{-1} Kin then 4.0 mgl^{-1}BAP.

Rooting Stage

Effect of auxins type on number of roots, root length, plant length and number of shoots on *Asparagus aphyallus* plant

Data in Table 6 presented that all examined traits were significantly ($P\leq0.05$) affected by auxin types. The top records for *Asparagus aphyallus's* shoot number per

Table 5. Effect of growth regulators on studied traits of *A. aphyallus* plant

Parameters		No. shoot	Plant length	No. leaves
Control		1.00^{b}±0.58	2.10^{cb}±0.21	3.33^{cb}±1.76
BA	**0.5**	2.33^{b}±0.33	2.08^{cb}±0.08	4.00^{b}±0.58
	1	1.00^{b}±0.00	2.20^{cb}±0.65	1.67^{cb}±0.33
	1.5	4.00^{a}±1.53	4.78^{a}±0.17	7.00^{a}±0.58
	2	1.67^{b}±0.67	3.37^{b}±0.32	1.67^{cb}±0.33
KIN	**0.5**	1.00^{b}±0.00	1.93^{c}±1.18	0.67^{c}±0.67
	1	1.33^{b}±0.33	2.63^{cb}±0.24	2.67^{cb}±1.20
	1.5	1.22^{b}±0.00	1.99^{cb}±0.24	1.77^{cb}±0.67
	2	0.67^{b}±0.33	2.59^{cb}±0.34	3.00^{cb}±1.00
2IP	**0.5**	1.33^{b}±0.33	2.20^{cb}±0.20	3.67^{b}±0.33
	1	0.67^{b}±0.33	1.60^{c}±0.15	2.00^{cb}±1.00
	1.5	1.00^{b}±0.00	1.50^{c}±0.06	0.77^{c}±0.33
	2	1.67^{b}±0.33	1.60^{c}±0.13	2.67^{cb}±0.67

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability.

Table 6. Effect of auxins type on studied traits of *A. aphyallus* plant

Parameters auxin Types	No. root	Root length (cm)	Plant Length (cm)	No. shoot
Con.	0.33^{b}±0.33	0.53^{b}±0.03	1.53^{b}±0.03	1.00^{ab}±0.58
IBA	2.00^{a}±0.58	1.90^{a}±0.15	2.67^{a}±0.09	2.00^{a}±0.58
NAA	0.33^{b}±0.33	0.70^{b}±0.06	0.53^{c}±0.03	0.33^{b}±0.33
IAA	0.67^{b}±0.33	0.67^{b}±0.03	$0.20d^{\pm b}$0.06	0.67^{ab}±0.33

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability

explant, shoot length, number of main roots, and root length (2.00, 1.90, 2.67 and 2.00, respectively) were with IBA compared with the NAA and IAA auxin types. These results are in agreement with the findings of **Sallam (2019).**Who obtained that MS Basal Medium Plus IBA at 1.0 $mgl^{-1}$was the most ardent supporter of *Asparagus officinalis* in the media. While **Palee (2018)** demonstrated that the *Tupistra albiflora* plant produced the largest number of roots per explant when grown in MS medium containing 1.0 mgl^{-1} NAA.

Effect of IBA concentrations

Data in Table 7 demonstrate a considerable impact ($P \leq 0.05$) of the MS medium and auxin type on the characteristics of *Asparagus aphyallus* that were investigated. The application of MS medium with IBA at 2.00 mgl^{-1} resulted in the greatest values for shoot number, shoot length, and leaf number (2.67, 8.45, and 9.3, respectively). Similarly. **Toma (2012)** pointed out that adding 1.25 mgl^{-1} IBA to *Asparagus densiflorus* L. produced the most roots (12.4 roots/ explant) and the longest roots (4.98 cm). On the other side, **Mustafa (2021)** discovered that *Asparagus aphyallus* plants grown in half-strength MS medium with IBA at 2.0 mg/l had the maximum rooting percentage and root length.

Acclimatization stage

The *Asparagus Aphyallus* plantlets were successfully brought to maturity in the greenhouse by being gradually acclimated using a media mixture of peat moss, vermiculite, and sterilized sand (1:1:1) at equal volume. In many plant species, newly produced leaves replace any in vitro leaves that are unable to continue to develop in *ex vitro* circumstances the same trend were obtained by **Patel and Patel (2015)** found that, under controlled conditions, covering the plants with clear polyethylene bags allowed for the maintenance of a high relative humidity. The *Asparagus Aphyallus* plants were transplanted to the soil after 4 weeks, and 85% survival was noted. However, **Mustafa (2021)** revealed that healthy rooted clusters could be effectively transplanted into a sterilized 1:1:1 *V/V/V* sand: peat: perlit combination for three weeks in a growth chamber. After two months of gradual acclimatization in the greenhouse, the percentage of survival reached about 65%.

Conclusion

In this work, an appropriate method for *Asparagus aphyallus* L. in vitro micropropagation was developed. Shoot tip explants were first utilized to produce in vitro shoots on MS media supplemented with various concentrations of ZnO. The shoots should then be doubled on MS medium. Additionally, the shoots were planted in MS medium with 2.00 mgl^{-1} IBA. After that, the rooted shoots are hardened in pots containing a 1:1:1 mixture of peatmoss, vermiculite, and sterilized sand (Fig. 4).

Table 7. Effect of IBA concentrations on studied traits of *A. aphyallus* plant

Parameters auxin Types	No. Root	Root length (cm)	Plant Length (cm)
Control	1.00b±0.00	0.50c±0.00	7.50bc±0.00
0.50	1.67b±0.33	4.93b±1.26	6.87d±0.18
1.00	1.33b±0.33	3.17b±0.44	7.67b±0.23
1.50	1.0b±0.00	0.33c±0.03	7.03cd±0.12
2.00	2.67a±0.33	8.45a±0.37	9.30a±0.11

According to Duncan's multiple range test, means in each column that are followed by the same letter are not statistically different at the 0.05 level of probability.

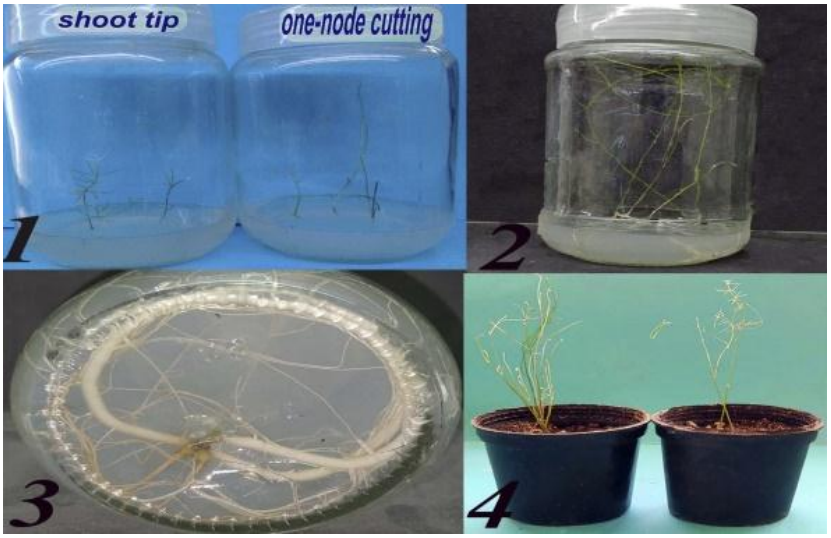

Fig. 4. Micropropgation of *A. Aphyallus* from mature plant. (1) Shoot establishment from nodal explants on MS medium in vitro, followed by (2) shoot multiplication on MS medium plus 1.50 mgl^{-1} BA and 0.05 mgl^{-1} NAA. (3) Root formation on MS + 2.00 mgl^{-1} IBA (4) Plant acclimatization

REFERENCE

Bhoite, H.A. and Palshikar, G.S. (2014). Plant Tissue Culture: A Review. World J. Pharm. Sci., 565-572.

Duncan, D.B. (1995). Multiple range and multiple F- test. Biomet., 11: 1- 42.

Elsabawy, M.N. (2012). Medicinal and aromatic crops in Egypt: A study in medical geography. Arpn J. Sci. and Tech., (2): 207-221.

Gamborg, O.L. (1968). Cells, Protoplasts, and Plant Regeneration in Culture. In Manual of Industrial Microbiology and Biotechnology. A. L Demain and N. A. Solomon, Ed., Dept. Nutr. and Food Sci., Mass. Inst. Tech., Camb., Mass. pp.

Gnanasangeetha, D. and Thambavani, D. S. (2013). Biogenic production of zinc oxide nanoparticles using Acalypha indica. J. Chem., Biol. and Physical Sci., 4: 238.

Kader, M.A.A. (2005). Comparison of seed germination calculation formulae and the associated interpretation of resulting data. J. Proc. R. Soc. Wales, 138:65–75.

Kopittke, P.M.; Kinraide, T.B.; Wang, P.; Blamey, F.P.C.; Reichman, S.M. and MEnzies, N.W. (2011). Alleviation of Cu and Pb Rhizotoxicities in Cowpea (*Vigna unguiculata*) as Related to Ion Activities at Root-Cell Plasma Membrane Surface. Environ. Sci. and Technol., 45: 4966 - 4973.

Kumar Kar, D. and Sen, S. (1985). Propagation of Asparagus racemosus through tissue culture. Plant Cell, Tissue and Organ Culture, 5: 89-95.

Marzouk, E.R.; Sally, A. and Hamza, (2016). A Toxicity of synthesized copper nanoparticles to soybean: Deferential effects of nano and bulk-size particles on root elongation rate. In: DIREK, M., 3rd Ed. Int. Conf. on Sustainable Agric. and Environ. (3rd ICSAE), September 26-28: 111-118, 2016 Warsaw, Poland. Konya, Turkey, 111-118.

Maślanka, M.; Mazur, J. and Kapczyńska, A. (2022). In Vitro Organogenesis of Critically Endangered Lachenalia viridiflora. Agron., 12 (2): 475.

Mehta, S.R. and Subramanian, R.B. (2005). Direct in vitro propagation of Asparagus adscendens Roxb. Plant Tissue Culture, 15(1):25-32.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tabacco tissue cultures. Phsiol. Plant, 15: 473-497.

Mustafa, S.S.S. (2021). *In vitro* propagation and secondary metabolites production in the wild rare *Asparagus aphyllus* L. plant. Egypt. J. Des. Res., 71 (2): 149-161 .

Palee, J. (2018). In vitro Shoot Cultures of Tupistra albiflora K. Larsen. Walailak J. Sci. and Tech. 17(5): 405-411.

Pandey, P.; Shukla, P.K.; Misra, P. and Ramteke, P.W. (2016). In-vitro clonal propagation of Asparagus racemosus by nodal explants. Ann. Pharm. and Pharma Sci., 17(1):14-19.

Pant, K.K. and Joshi, S.D. (2016). In vitro bud induction from the nodes of Asparagus racemosus wild and their germination behaviours, 3: 47- 50.

Patel, L.S. and Patel, R.S. (2015). Rapid In vitro Micro Propagation of Asparagus racemosus Willd. from nodal explants. Int. J. Curr. Microbiol. App. Sci., 4(5): 607-617.

Ritz, C.; Baty, F.; Streibig, J.C. and Gerhard, D. (2015). Dose-response analysis using R. PLoS One, 10: e0146021

Sallam, M.R. (2019). in vitro propagation of f1 male hybrid lines in asparagus officinalis. Egypt. J. Desert Res., 69 (1): 67-86.

Salman, S. (2021). Invitro propagation and secondary metabolites production in the wild rare *Asparagus aphyllus* L. plant. Egypt. J. Desert Res., 71 (2): 149-161.

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

Sharan, M.; Nene, C. and Sharon, M. (2011). Regeneration of Asparagus racemosus by shoot apex and nodal explants. Asian J. Plant Sci. Res., 1 (2): 49-56.

Sulava, S.; Andia, S.P.; Bhol, R. and Jena, S. (2020). *In vitro* micropropagation of Asparagus racemosus by using of nodal explants. J. Cell and Tissue Res., 20 (1): 6883-6888.

Toma, R.S. and Rasheed, K.A. (2012). The response of *Asparagus densiflorus* L. *in vitro* propagation to different potassium and ammonium nitrate levels in culture media. Int. J. Pure and Appl. Sci. and Technol., 17(2): 21.

Van Der Vliet, L. and Ritz, C. (2013). Statistics for analyzing ecotoxicity test data. in: ferard, j.-f. and blaise, C. (eds.) Encyclopedia of Aquatic Ecotoxicology. Dordrecht: Springer Netherlands.

Waly, A.; Abdelfattah, Y.M. and Shoman, A.A. (2018). Effect of media type and BAP concentrations on micropropagation during multiplication stage on Ponytail Palm (*Beaucarnea recurvata* Lem.). Hortsci. J. Suez Canal Univ., 7 (1): 41-45.

White, P.R. (1963). A Handbook of Plant Tissue Culture. Jacque Cattell Press, Inc., Tempe, Ariz. pp.

الملخص العربي

استخدام تقنية زراعة الأنسجة وتحفيز جسيمات أكسيد الزنك النانوية في إكثار نبات الاسبرجس البري

غاده محمد حامد جاب الله[1] ، عزت رشاد مرزوق[2]، سونيا عطية شحاته عبدالله[1]

1- قسم الإنتاج النباتي، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.
2- قسم الاراضي والمياة، كلية العلوم الزراعية البيئية، جامعة العريش، مصر.

أجريت هذه الدراسة في معمل زراعة الأنسجة النباتية – كلية العلوم الزراعية البيئية بالعريش – جامعة العريش وذلك خلال الفترة من 2019 – 2022. كان الهدف من هذه الدراسة هو استخدام تقنية زراعة الأنسجة لإكثار نبات الاسبرجس البرى. حيث تم استخدام تركيزات مختلفة من جسيمات الزنك النانويه. وقد تم إنبات البذور على بيئة موراشيج وسكوج مع أفضل تركيز وهو (10ملجم/لتر). وقد تم زراعة كل من القمة النامية والسلامية ذات العقدة الواحدة على ثلاث أنواع من البيئات وهى بيئة موراشيج وسكوج (MS) ، وبيئة جامبورج (B5)،بيئة وايت(white) مضافا اليها 10ملجم/لتر من جسيمات الزنك النانويه. وقد أظهرت النتائج ان زراعة السلامية ذات العقدة الواحدة على بيئة موراشيج وسكوج + 10 ملجم/لتر أعطت أفضل نمو فى مرحلة البداية. وكان أفضل تضاعف للسلامية ذات العقدة الواحدة بإستخدام بيئة موراشيج وسكوج مضافاً اليها بنزيل ادينين بمعدل 1.5 ملجم/لتر مع نفثالين حمض الخليك بمعدل 0.10 ملجم/لتر. كان أفضل تجذير مع استخدام 2.00 ملجم/لتر اندول حامض البيوتيريك . وأجريت الأقلمة فى الصوبة بإستخدام مخلوط مكون من البيتموس والفيرميكيوليت والرمل بنسبه 1 : 1 :1 حيث حققت نسبة نجاح 70% .

الكلمات الاسترشادية: *Asparagus aphyallus* L، التكاثر الدقيق، الوسط البيئي والتجذير.

REVIEWERS:
Dr. Mahmoud Abdo | mahmoud.abdo@mu.edu.eg
Dept. Horticul., Fac. Agric., Minia Univ., Egypt.
Dr. Ibrahim A.Maqsoud Ibrahim |ibrahim.ibrahim@gebri.usc.edu.eg
Dept. Plant Biotechno. Genetic Engineering and Biotechnology Res. Inst., Sadat City Univ., Egypt.