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The impact of Selenium and Cobalt nanoparticles on development of Date Palm tissues C.V Barhi *In Vitro*

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Abstract. The research was carried out at the plant Technologies Laboratory in Agriculture College, Basrah University, from February 2023 to September 2024. Intending to rigorously examine the impact of incorporating nano cobalt and nano selenium in growth medium at concentrations of (0, 1, 2, and 3) Millimolar (mM) on the callus tissue and vegetative branches of the Barhi date palm growth and development. The data were statistically evaluated employing (CRD) design with 10 replicates at a probability level at 0.01%. The findings revealed that a concentration of 2 mM for both nano cobalt and nano selenium resulted in a remarkable enhancement in fresh and dry weight of callus mass (2.14, 0.24) g, (2.77, 0.28) g, respectively. Also, there is an increased number of embryos (11.3, 10.3), and robust growth of vegetative branches (12.3, 12.3). Furthermore, there were significant improvements in the anatomical characteristics of the callus (parenchyma cells, mesophyll cells, tannin cells, and vascular bundles). These compelling results underscore the vital role of nano elements and compounds in advancing biotechnology, offering innovative solutions for enhancing plant growth in field conditions and laboratory applications.

1. Introduction

Date palm (*Phoenix dactylifera* L.) It is an important plant because its fruits contain high nutritional value, as it is considered a good source of energy and It is traced to the Arecaceae family. And it is Spread in the semi-arid and arid lands [1], the climatic requirements necessary for fruit formation determine the distribution of palm trees in the region, as more than 3000 known varieties of Palm have been registered around the world [2].

Propagation using tissue culture is a modern and good way to produce many disease-free and variety-identical plants [3]. Many palm varieties have been propagated by plant tissue culture [4,5], as well as the use of technology in conducting research and studies traits related to morphological, physiological, anatomical, genetic variations traits [6-8]. Although it is not essential for plants, cobalt an element that is involved in the synthesis of certain enzymes and co-enzymatic, which affects the growth and development it [9,10]. Therefore, it is believed to be beneficial to the plant at low concentrations [11]. It plays a crucial function in nitrogen fixation in legumes and the manufacture of vitamin B12 [12].

Some studies have indicated the great importance of cobalt in improving the growth qualities of some plants grown by plant tissue cultures, such as increasing the production plant of chlorophyll pigments to enhance the photosynthesis process for growth level of Brassica rapa [13], palms [14], and pineapple plants [15].

Selenium is a trace element and plays to increasing the enzymatic antioxidant compounds which converts the toxic hydrogen peroxide compound into water molecules H_2O. [16]. Also, its association with amino acids and the formation of so-called Selenium proteins Seleno proteins [17] with ability to cell membranes and prevent the destruction of Denaturation of plant protein metabolism compounds [18]. This mechanism is one of the biological means through which the plant works to withstand the stress of selenium [17]. The use of selenium and its concentration is related to strengthening the diet and defense of plants, as low concentrations are a catalyst for the activation of antioxidants as well as their role in stimulating plants to development [19].

The production and development of the callus tissue is one of the main methods of propagation [20] and depends on the improvement of the quality of the callus is the nutrient levels of macro-and micro-inorganic elements used as Plant Tissue Culture Media as well as reinforcing and growth assistance elements [21,22]. Microelements are important in plant tissues development, especially in the technique of growing plant tissue, as they need low concentration. Therefore, this study addressed the role of nano-Cobalt and nano-Selenium assessment to development of date palms callus, embryos, and vegetative growth.

2. Materials and methods

This study was carried out in plant Technologies Laboratory of Agriculture College, Basrah University, from February 2023 to September 2024, to find out the impact of Cobalt and selenium nanoparticles as antioxidants at a concentration of (0,1,2 and 3)Millimolar (mM) on the vegetative growth, biomass, and anatomical qualities of callus and vegetative branches Barhi variety produced *in vitro* [23]. The medium was contained Murashige and skoog salts [24], from (phyto-lab), sucrose 30 g L^{-1}, adeneni Sulfite 0,170 g L^{-1}, activated charcoal 1 g L^{-1}, Sodium Hydrogen Orthophosphate 0.04 g L^{-1}, plant growth regulator (NAA,30mg. $L^{-1}$and 2IP 5mg. L^{-1}), agar 7 g L^{-1} and heated at 90 C° by the heat source. The medium was sterilized in an autoclave for 20 minutes. Callus at the age of four months was used and distributed to the jars at a weight of 100 mg per jar. The cultures were grown in a growth chamber and a subculture was carried out every 4-5 weeks. The experiments for callus were carried out four months after culture based on [5], and the experiments for embryos and vegetative branches were carried out six months after culture using a special medium for multiplying embryos and vegetative buds based on [8].

2.1 Effect of treatments on the Callus

2.1.1 Fresh weight estimate (g)

After 60 days of experimentation, the fresh weight of callus mass was determined for each treatment. The callus was removed from the culture medium and washed thoroughly with sterile distilled water to remove any agar residue attached to it. It was then dried with sterile paper to remove any excess moisture. Then, it was measured using a sensitive balance [25].

2.1.2. Dry weight estimate of callus (g)

The callus mass was dried to estimate the dry weight using an electronic oven at 65 C° for 48 hours while weight stability than measured by a Sensitive digital balance.

2.1.3. Embryos Number and vegetative branches

Embryos number and vegetative branches formed after 60 days of the experiment was calculated.

2.1.4. Anatomical parameter

The [26] method was adopted in the preparation of anatomical sections and a Japanese-made Olympus type microscope with a camera, the characteristics of the number of parenchymal cells, mesophyll cells, tannin cells, and the diameter of the vascular bundle were studied.

2.2. Statistical analysis

The study was carried out by tow factors experiment during the callus induction stage, The data were statistically evaluated employing (CRD) Design with 10 replicates at level 0.01%, using SPSS programs 22.

3. The results and Discussion

3.1. Fresh, dry weight of callus

Figure 1 showed that adding nano Cobalt to the MS medium improved the fresh weight , it was noted that the concentration 2 and 1 mM attributed the high weight attained 2.14g and 1.78 g, while the focus 3mM did not led to a significantiy from comparative.its same effect was also observed for the weight of callus of treatment with cobalt element (Plate 1).

The results of Figure 2 showed that treatment with nano Selenium helped to improve the growth rate of callus tissue, as it was noted that the focus of 2 mM gave the high weight of 2.77 g , so there are no significant difference was observed between concentration 3mM and the comparison treatment. The same effect was also observed for dry weight (Plate 2).

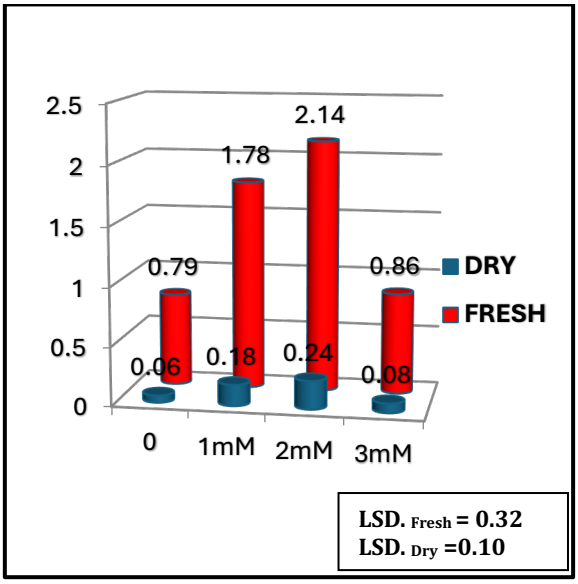

Figure 1. The impact of nano Cobalt in fresh and dry weight of callus.

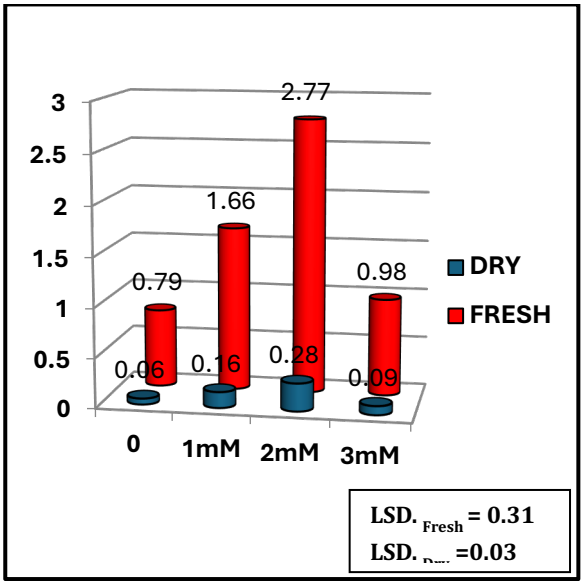

Figure 2. The impact of nano Selenium in fresh and dry weight of callus**.**

3.2. Embryos and vegetative branches

Figure 3 illustrates the impact of adding nano Cobalt to the nutrient medium (MS) on embryos growth and the formation of vegetative branches. Treatment with a concentration of 2 mM yielded the best results, with an average of 11.3 embryos and 12.3 branches per sample, showing a significant difference compared to other treatments (Plate 1).

Similarly, Figure 4 emphasizes the beneficial effects of nano Selenium addition to the nutrient medium (MS) on embryo formation and vegetative branching. At a concentration of 2 mM, selenium recorded the highest average of 10.6 embryos, while the branch count reached an outstanding 13.4. It is important to increasing the selenium to 3 mM led to detrimental effects, inhibiting embryo development (Plate 2).

These observations underscore the critical role that trace elements like Cobalt and Selenium play in optimizing plant growth and development, making them vital components for enhancing embryo and vegetative branch formation.

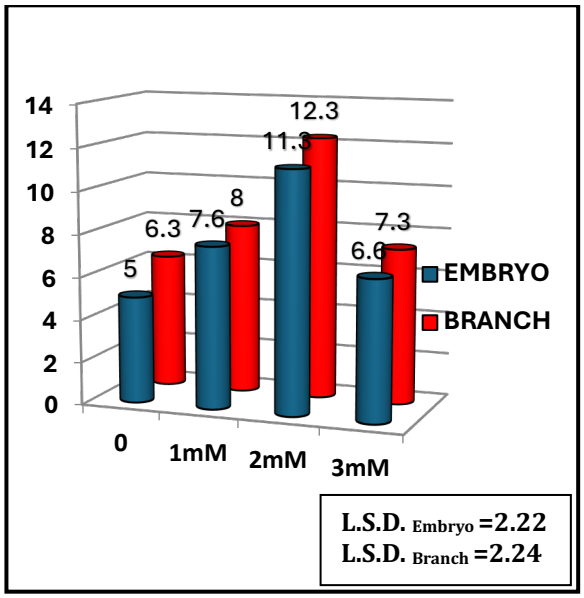

Figure 3. The impact of nano Cobalt in the Embryos and branches.

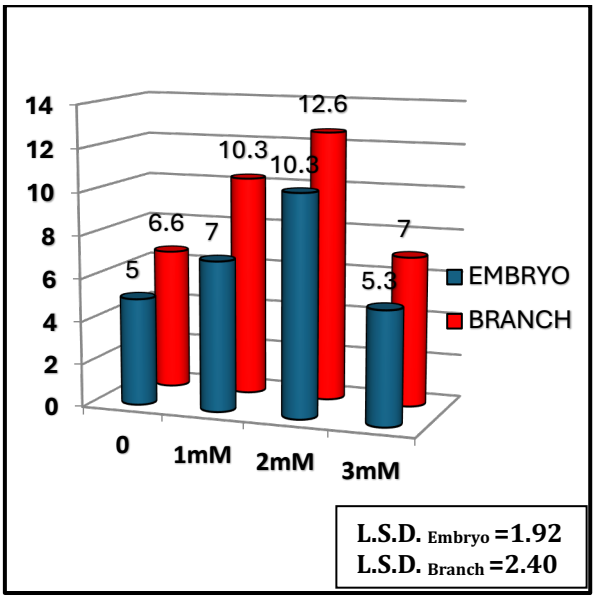

Figure 4 The impact of nano Selenium in the Embryos and branches.

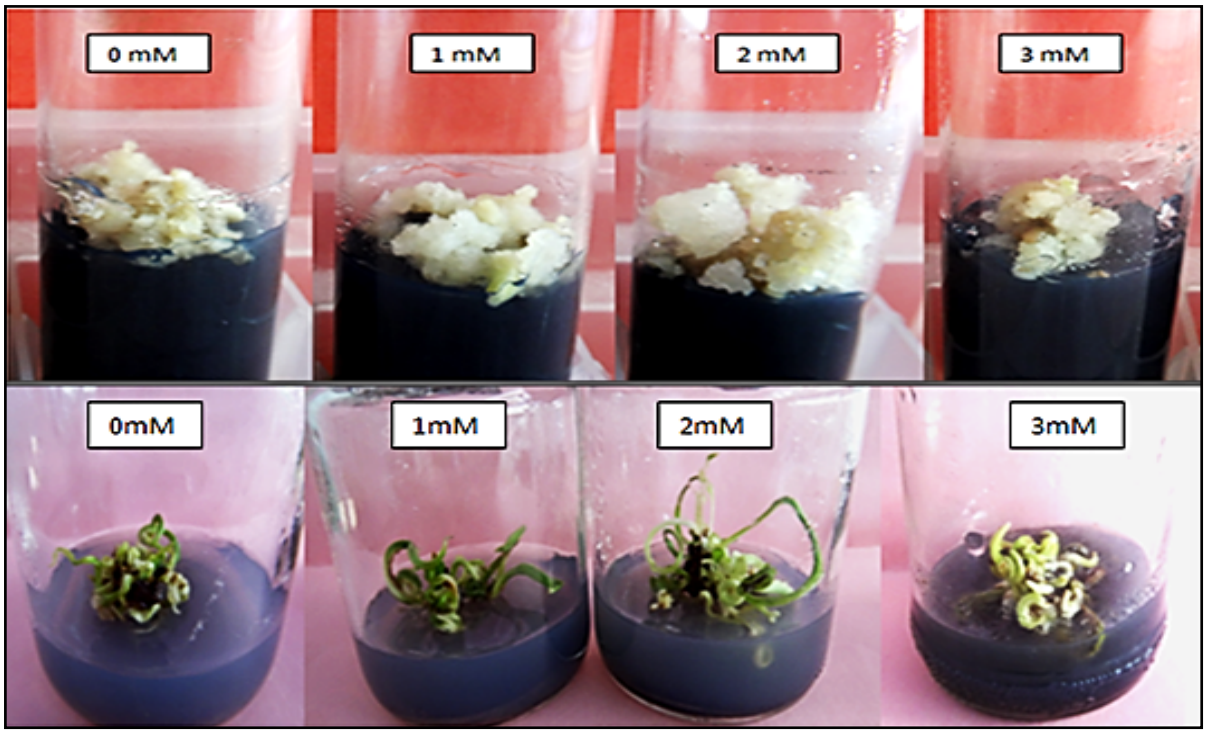

Plate 1. Showed impact of nano Cobalt on callus mass and formation embryos and vegetative branches.

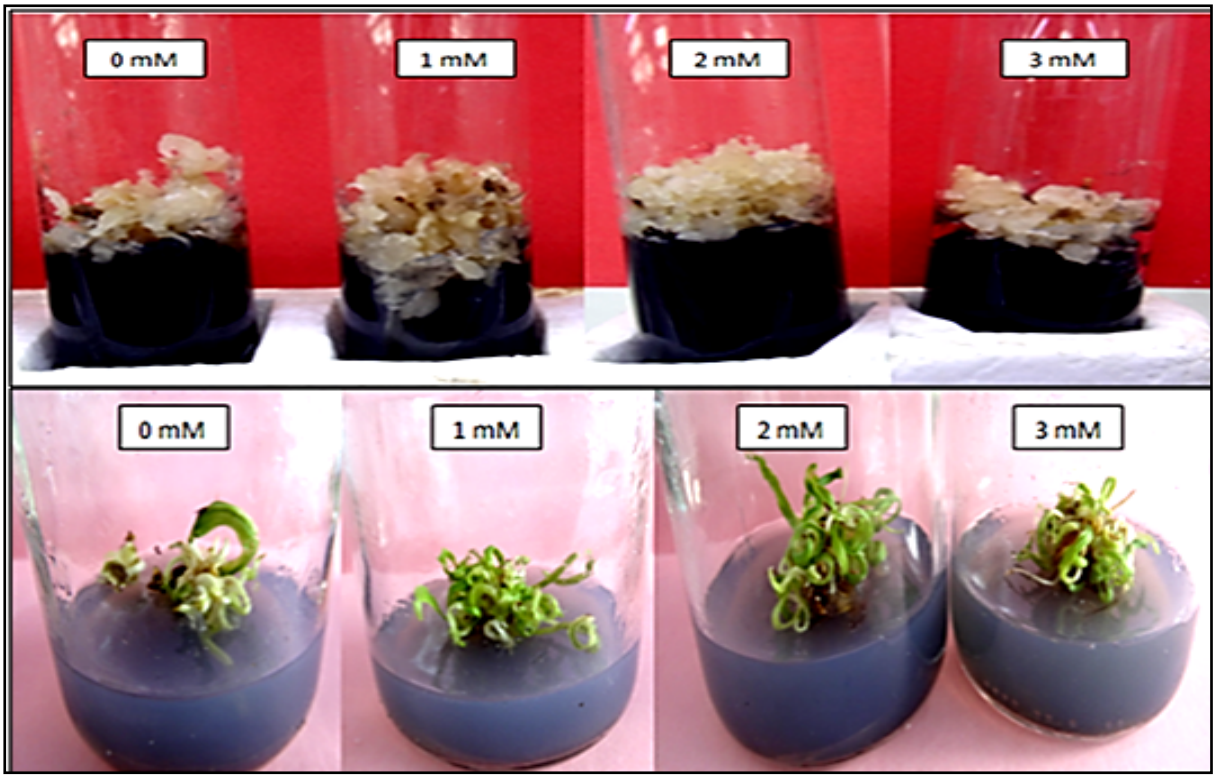

Plate 2. Showed impact of nano Selenium on callus mass and formation embryos and vegetative branches.

3.3. Anatomical parameter

The results shown in Table 1 effect of adding Cobalt and Selenium Nanoparticles to the nutrient medium (MS) in the formation of callus of the Barhi variety, noting the differences in the number of parenchyma cells, mesophyll cells, tannin cells and the diameter of vascular bundles between different treatments.

Anatomical parameters showed that the treatment at 2 mM of nano Cobalt led to increase in the studied qualities (30.7, 11.3, 153.3, 314.4µm), respectively, compared to other treatments. This was positively reflected in the growth rate, development, and formation of embryos and vegetative branches.

The same effect is also observed for callus cells as a result of treated the nano Selenium at 2 mM increased the rate of the number of parenchyma cells, mesophyll cells and tannin cells qualities which give (9.3, 186.3, 380.3) respectively, except for the characteristic of diameter of vascular bundles qualitie which give the highest value (31.0µm) at concentration (1 mM). which led to an improvement in the qualities of cells compared to their qualities without treatment (plate 3).

Table 1. Impact of nano Cobalt and nano Selenium in the Anatomical parameter.

Treatments	Con.mM	number of parenchyma cells. mlm²	number of mesophyll cells mlm²	number of tannin cells mlm²	Diameter of vascular bundles µm
Cobalt	0	210.6 ± 2.8*	136.0 ± 2.1	16.3± 1.5	18.7± 0.8
	1	237.0 ± 3.6	142.6 ± 2.1	13.6± 1.0	25.0± 1.1
	2	314.4 ± 5.8	153.3 ± 2.5	11.3 ± 0.5	30.7 ± 2.1
	3	218.0 ± 2.2	117.3 ± 4.5	13.6± 0.5	21.6± 0.5
L. S. D		10.2	8.1	2.7	3.3
Selenium	0	210.6 ± 2.8	136.0 ± 2.1	16.3± 1.5	18.7± 0.8
	1	303.6 ± 2.6	172.6 ± 5.1	10.4 ± 0.5	31.0 ± 1.0
	2	380.3 ± 1.8	186.3 ± 2.2	9.3 ± 0.6	28.8 ± 1.0
	3	228.6 ± 7.0	141.3 ± 1.4	12.6 ± 0.6	23.8 ± 1.1
L.S. D		10.8	7.9	2.5	2.5

* Values represent the mean of triplicate per treatment ±SD

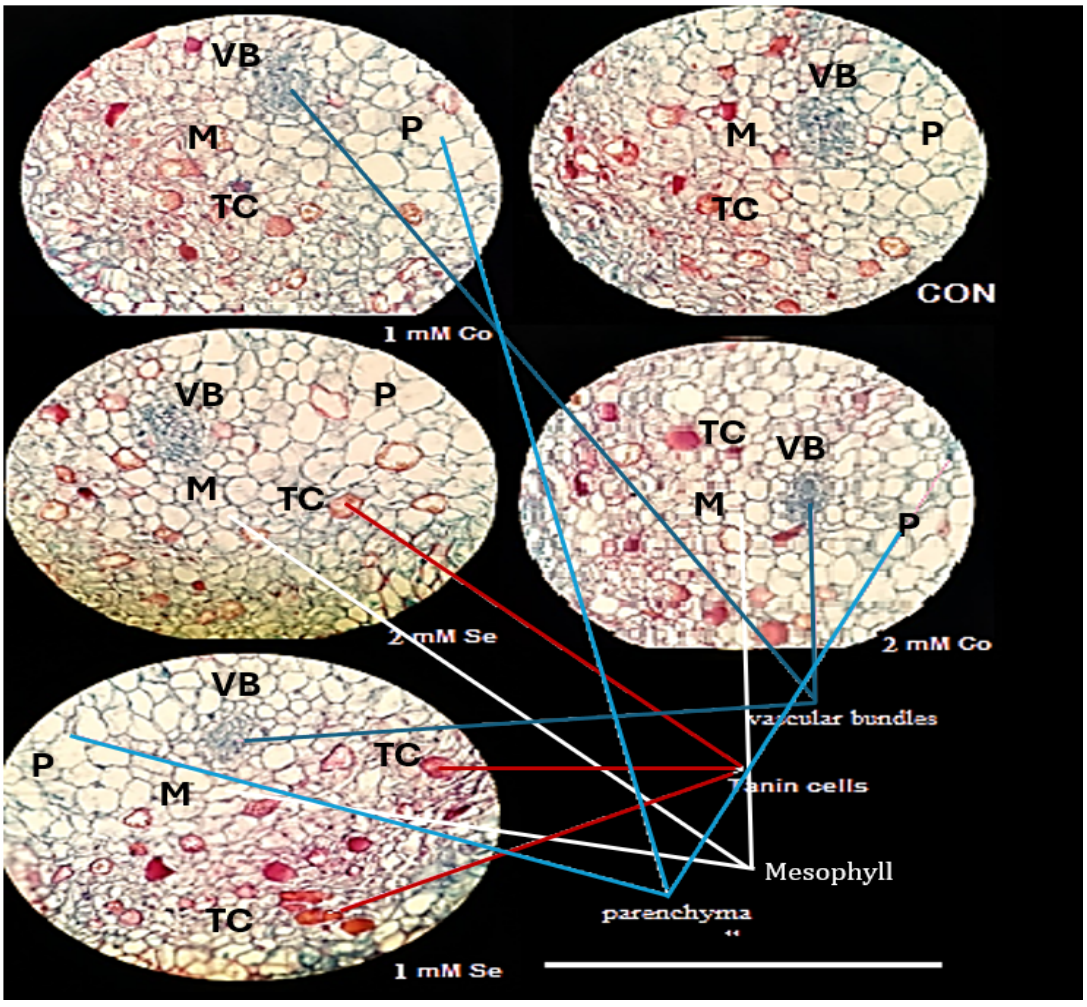

Plate 3. Effect of nano Co and nano Se treatments on anatomical characteristic, Vascular bundles (VB), Tannin cells (TC), mesophyll cells (M), Parenchyma cells (P). (X 40).

Microelements in nutrient media play an important role by assessing their various effects on the growth of callus, the level of elements is determined by the response of the explants, which differs in the supply of essential nutrients to plants *in vivo* cultivation [27]. Callus was a mass of undifferentiated, andunspecialized cells from the tissue of explant [28]. Vegetative(somatic) embryos can be defined as having evolved from cells that have undergone a series of biochemical changes, as it includes a series of stages starting from the formation of formation of embryos, their development and maturation [29].

Cobalt is a microelement that has beneficial on plant moderate levels [30]. High concentrations of cobalt lead to toxicity of plants, so cobalt is one of the elements that are interested in controlling the synthesis of ethylene [31]. [15] explained that Cobalt in nutrient medium actively contributed to the buds formation from the callus of date palm and promoted to development of callus cells in addition to increasing of buds obtained and the result was obtained when adding Cobalt at 2 micromole.

Selenium is one of the microelements in the plant that works in low concentrations participates in antioxidant processes and increases the plant's tolerance to abnormal conditions that lead to oxidative stress, such as exposure to ultraviolet rays [32]. The level of selenium in plants is considered the determining factor of its role, There are three vital actions of selenium, low concentrations are important for development processes, medium concentrations maintain genetic structures, stability, and resistance to genetic mutations, while High levels lead to damage that may reach wider areas of the plant [33]. We obtained the best results in callus biomass parameters at a 2 mM selenium. This may be due to selenium enhancing RNA activity. Selenium enters into the formation of some amino acids, especially methionine and cysteine. These compounds can bind to other amino acids, thereby enhancing RNA activity [34]. This increase promotes cell capacity, growth, and division [31,35].

4. Conclusions

The morphological growth of callus and vegetative shoots of Barhi date palms was positively affected by the addition of both nanoparticles of selenium and cobalt at (2mM level. Biomass of callus (dry and fresh weight), the number of vegetative embryos and vegetative branches increased, and a significant improvement in anatomical characteristics was observed at the same level and for both nano elements. While the high levels of both nano elements (3mM) showed a lesser response to the studied growth parameters. The study provides additional support for the importanc inclusion of the nutrient medium of some nanocompounds stimulates the response as well as in improving development of laboratory-propagated plants and their effective role at specific concentrations at the plant tissue and cellular levels, and it supports their future use in commercial propagation programs.

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